BIOLOGICAL PSYCHIATRY

Edited by

Hugo A. H. D'haenen
Johan A. den Boer
Paul Willner

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Biological Psychiatry

Volume I

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Volume II

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Preface

This textbook aims to review the current state of biological psychiatry, a field that has expanded tremendously over the past few decades. The book is aimed at clinical, postgraduate and research audiences, within psychiatry, neurology, psychopharmacology, and psychology.

After a few introductory chapters dealing with conceptual and measurement issues in biological psychiatry, the first part of the book deals with basic principles relating to animal models, transmitter systems (aminergic, amino acid, peptidergic), neuroendocrinology, neuroimmunology, psychophysiology, neuropsychology, brain imaging, neurogenetics, gene-environment interactions, and gender issues. These chapters are intended to provide the necessary basic information that would enable the reader unfamiliar with each of the fields addressed to understand the later chapters applying this knowledge to specific psychiatric disorders.

Although it has frequently been argued that the diagnostic categories of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders — IVth edition (DSM-IV) are not immediately relevant to biological psychiatric insights, we have used the DSM-IV classification as an organizing principle, for didactic reasons.

Thus, the second part deals with most of the major diagnostic categories of DSM-IV: cognitive disorders, substance-related disorders, schizophrenia, mood disorders, anxiety disorders, somatoform disorders, dissociative disorders, sexual and gender identity disorders, eating disorders, sleep disorders, impulse control disorders, and personality disorders.

With the exception of four comprehensive chapters on "the psychobiology of" somatoform disorders, dissociative disorders, sexual and gender identity disorders, and impulse control disorders, all other sections (cognitive disorders, substance-related disorders. . . .) aim to recapitulate each of the topics introduced in part 1 of the book, as well as including for each disorder, a chapter on the current therapeutic armamentarium.

Although the editors are European, authors from all over the world, indeed from almost all continents, have participated in this endeavour. In editing the book, we have tried as much as possible to avoid overlap between chapters, but the autonomy of each chapter had precedence. Nevertheless, we have found the contributing authors extremely receptive to our editorial suggestions, and take the opportunity to acknowledge their collaboration.

It has also been a real pleasure working with the staff from Wiley, who from the beginning has supported this undertaking. More particularly so, the cooperation with Charlotte Brabants and her assistant Layla Paggetti has been fruitful, encouraging, stimulating and exceptionally pleasant.

Everyday, new biological psychiatric insights in psychopathological disorders are to be found in the professional literature. It is inherent to the requirements of a book such as this, for some time to elapse between the delivery of a manuscript and the publication of the book. All those concerned have made a great effort to limit this interval as much as reasonably possible.

The editors express the hope that the information gathered in this book will provide a basic reference source upon which further knowledge can be built.

Hugo A.H. D'haenen
Johan A. den Boer
Paul Willner

Part A

BASIC PRINCIPLES

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I

Conceptual Issues

German E. Berrios and Ivana S. Marková

Born as a by-product of the nineteenth-century movement to organize society on a scientific basis, psychiatry was charged with the construction and enactment of normative views of madness. (Literature on social and clinical history of psychiatry is large; for scholarly papers on both, see the journal *History of Psychiatry* (started 1990).) Under the protection of medicine and the economic practices following the Industrial Revolution, psychiatrists developed representations of mental disease together with the professional and institutional apparatus to enjoin them.[1] Since its construction, the modalities of psychiatry have been many. For example, there is the group called biological psychiatry,[2] organic psychiatry, neuropsychiatry and behavioural neurology, which seem to share the foundational claims (FCcs) that: (1) mental disorder is a disorder of the brain; (2) reasons are not good enough as causes of mental disorder; and (3) biological psychiatry and its congeners have the patrimony of scientific truth. These four congeners are man-made and there is nothing in nature to suggest real differences between them; indeed, any differences that might be suggested are bound to be historical in origin. To avoid confusion, the term 'biological psychiatry' will be used in this chapter as a proxy for the other three congeners. Since the seventeenth century, versions of what might be called biological psychiatry have come in and out of fashion;[3] the reasons for these cycles are unclear.[4]

FOUNDATIONAL CLAIMS AND THE 'TECHNOLOGY ALIBI'

FCs are unproven and unprovable propositions used to start off the narrative of science. Chosen by 'experts', FCs escape audit and are unchallengeable. The view that mental disorders are 'caused' by changes in the physical conformation of the brain is a typical foundational claim; that 'reasons' cannot constitute efficient causes for the generation of mental disorder is another.

Since the nineteenth century, and every time it has failed to deliver, biological psychiatry has used the excuse that ongoing technologies are not sensitive enough to identify the organic causes of all mental disorders and reassured the expecting public that future techniques will do so: we call this the 'technology alibi'.

Foundational Claim 1: Mental Disorders as Brain Disorders

Meanings for this FC range from the broad assertion that all psychological activity must have brain representation to the narrow assertion that specific brain lesion *l* is a necessary and sufficient cause for presence of specific mental disorder *m* (more on this later). Similar claims were made during the middle of the seventeenth century, e.g. by Thomas Willis in 1685 (see Vinchon and Vie, 1928).[5]

The claims that the body (brain) houses the human soul (or mind) and that changes (lesions) in the conformation or constitution of the body (brain) cause mental disorder (i.e. FC 1) are historically independent, and their relationship is asymmetrical; indeed, the

[1] A representation is an image, model, view, concept or other definitional form that helps someone to think of something. Control includes social practices, such as therapy, prophylaxis, support, management, rehabilitation, policing, punishment, incarceration and the death penalty. A social practice is a rule-governed form of social behaviour that runs on habit, is passed from generation to generation, and contributes to the stability of society; the stability of the concept has been questioned (Turner, 1994).

[2] At this stage, a clarification needs to be made. In this chapter, we distinguish clearly between (1) biological psychiatry as a methodology and research *doctrine* and (2) the *clinical* practice of those who call themselves 'biological psychiatrists'. This is because it is highly likely that they will, like non-biological psychiatrists, talk, understand, support, counsel, and resort to whatever psychological therapies are needed to help their patients. This chapter is entirely about the limitations affecting the *doctrine* and *methodology* of biological psychiatry. Indeed, given its narrowness, we believe that it would be very difficult, even for rigid biological psychiatrists, to guide their clinical practice by doctrine alone. On the other hand, it would be wrong to invoke the flexibility and variety of approaches that biological psychiatrists have to use in their clinical practice as *evidence* that the *doctrine* itself is flexible and comprehensive, for it is not. For earlier work from our group on the methodology of biological psychiatry, see Berrios, 1995; Berrios and Dening, 1990.

[3] Neither cross-culturally nor trans-historically can biological psychiatry be considered as a *unitary* activity: according to social and economic need, different types and practices predominate in different contexts. For example, biological psychiatry during the second half of the nineteenth century was characterized by very specific definitions of disease, internecine disputes concerning the professionalization of neurologists and alienists, an increasing use of the light microscope, and early studies of brain chemistry. Differences were, on occasions, exaggerated by international rivalries and jingoism; for example, this is the case during and after both the Franco-Prussian War, and the Great War, when even writers like Chaslin and Kraepelin made silly remarks about 'enemy' countries. (On the history of biological psychiatry, see Berrios and Marková, 2002a.)

[4] An interesting issue here concerns the relationship between the historical versions of biological psychiatry. Are they manifestations of an ongoing idea existing *sub specie aeternitatis*? Is their similarity skin deep and not worth bothering about? When Thomas Willis stated that mental illness is a 'disorder of the brain', was he being a precursor of similar current claims?

[5] These claims are intelligible to us only because they were made within the new epistemological frame (dualism) created by Descartes. Similar-sounding claims made in earlier periods (e.g. by Galen) are much harder to understand. In pre-Cartesian times, explaining the *existence* of a form of behaviour (e.g. madness) in terms of a *link* to the soma (whether heart, hypochondrium or brain) made little sense because there was no differentiation between anatomy and function in the way that we understand it today. In this sense, it is anachronistic to believe that the history of biological psychiatry starts with the Greeks. Indeed, current concepts, meanings and categories in psychiatry were constructed during the nineteenth century (see Berrios, 1996).

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belief that brain disease causes mind disease is not logically dependent on the belief that the mind resides in the brain (because the former could be plausibly held without the second being true).[6]

The claim that the body (brain) must house the human soul (or mind) follows from the materialist assumption that at the time the world came into being, there was only matter (Lange, 1957). Thinkers of this persuasion need to explain, however, where 'mind phenomena' come from, particularly if a highly configured model of the latter is entertained. Materialist thinkers make use of two types of matter: *Plain* matter is constituted by homogeneous units of analysis (atoms, fields, strings) and cannot explain mind configurations; these then are assumed to come from outside (cultural context). *Baroque* matter may be constituted by atoms of different shapes and sizes moving at different rates, or may include dynamic and hidden qualities (Cappelletti, 1979). For example, Cabanis (1802) (although overtly a materialist) held a baroque view of matter and hence was able to account for complex aspects of the mind without resorting to any external agencies.

Foundational Claim 2: Causes and Reasons

Causes and reasons are forms of accounting for the existence and/or origin of objects, tokens, episodes, processes and behaviours. Although etymologically different, they have overlapping semantic fields (i.e. are used as synonyms) and similar epistemic power (capacity to explain actions). Causes are used in the natural sciences; reasons are used in the social sciences (which, in the past, included psychology). What about psychiatry?[7]

Causes

What we have said above suggests that to ask for the cause of a mental disorder is already to opt for a particular model of explanation (Berrios, 2000a), as equally cogent would be to ask for a reason, based on the view that people may do things, including 'talking and behaving crazy', for reasons better known to themselves. Reason in this sense needs not be construed in material or mechanical terms but may remain expressed in the language of folk psychology. Even more radically, it could be said that mental disorders are not *effects* at all, that they do not follow either causes or reasons, and that they are just 'givens', i.e. they have been there from the beginning of time. In a world keen on looking for the causes of all things, this latter view may sound strange, but it is not unintelligible and it simply reflects the ontology of preformationism, a view of biology predominant in the eighteenth century.

It could be argued that reasons are just a subtype of cause. This claim muddles the issue further. It is true that the Latin *causa* meant reason, motive, inducement, but it also meant occasion and opportunity. It translated the Greek *aitia* and *aition* (stems of the medical term aetiology), which meant origin and ground but also the occasion of something bad. In practical terms, cause has been used mostly as a *relational* concept, i.e. as one without which another thing (effect) cannot be. Based on his own model of the Universe, Aristotle (1991; p. 1600) defined it thus:

> We call a cause (1) that from which (as immanent material) a thing comes into being, e.g. the bronze of the statue and the silver of the saucer, and the classes which include these. (2) The form or pattern, i.e. the formula of the essence, and the classes which include this (e.g. the ratio 2 : 1 and numbers in general are causes of the octave) and the parts of the formula. (3) That from which the change or the freedom from change first begins, e.g. the man who has deliberated is a cause, and the father a cause of the child, and in general the maker a cause of the thing made and the change-producing of the change. (4) The end, i.e. that for the sake of which a thing is, e.g. health is the cause of walking. For why does one walk? We say 'in order that one may be healthy', and in speaking thus we think we have given the cause. The same is true of all the means that intervene before the end, when something else has put the process in motion (as e.g. thinning or purging or drugs or instruments intervene before health is reached); for all these are for the sake of the end, though they differ from one another in that some are instruments and others are actions ...

Of the four Aristotelian types of cause (material, formal, efficient and final), only the efficient one has survived in our day.[8] In the first half of the seventeenth century, Galileo interpreted efficient as resulting from general physical laws. During the second half of that century, Newton redefined cause in terms of a physical metaphor ('collision between two objects, such as billiard balls') (Doney, 1968). This made the concept of cause quantifiable, predicting and reversible but excluded human agency and rendered it soulless.[9] In spite of Hume's opposition to the Newtonian model of cause, the nineteenth century accepted the Newtonian view and soon enough this was incorporated into medicine and alienism.

Since the time of Georget (1820), biological psychiatry has hung on to the thin hope that the demonstration of a brain lesion in a patient with mental illness may be sufficient to generate a cause–effect inference that can be applied to all. During the nineteenth century, the existence of such a link was liberally assumed; for example, post-mortem lesions were considered, *simpliciter*, to be the cause of a disease, irrespective of the nature and age of the lesion and of the period in the patient's life when the disease occurred; that is, the need for contemporaneity was not acknowledged. In current biological psychiatry, this principle of guilty by association is still present. It is unclear what type of contemporaneity is required by the ascertainment of reasons (e.g. discursive accounts of behaviour) and of causes (e.g. statistical correlation) (for the latter, see p. 18).

Reasons

Although in common parlance it is accepted widely that reasons provide adequate accounts for a range of human behaviours, the semantic engine that makes these explanations possible is unclear. Cicero used *ratio* to translate the Greek for account,

[6] For example, at the beginning of the nineteenth century there was a debate as to whether the mind or soul was located in the cerebrospinal fluid or the cortex (Hagner, 1992). Likewise, the view was popular in the same century that because the soul (mind) was a marker of the divine, it could become neither divided or diseased. Its putative location on the cortex also caused difficulties in regard to its functional (or phrenological) parcelling (e.g. Jackson; see p. 8). The issue of whether organs other than the brain might contribute to the formatting of the mind and its diseases was discussed up to the nineteenth century; prime candidates remained the heart (Willis, 1685), hypochondrium (Whytt, 1768) and stomach (Broussais, 1828).

[7] Biological psychiatry has not yet taken on board the importance of the distinction between reasons and causes. This may be due to the fact that explaining human action or symptoms by means of reasons does not fit into the rather crude mechanistic and deterministic frame of neurobiology.

[8] From Greek times, the concept of cause has been used to refer to both aspects of human agency (as per Aristotle) and the belief that effects result from the action of universal laws (as per Plato) (Berrios, 2000a).

[9] This came back to haunt the studies of man during the nineteenth and twentieth centuries when the human sciences tried to ape the natural sciences and abandoned hermeneutics. Without a machinery to deal with meaning, reasons, subjectivity, and the understanding of the individual, the human sciences (and psychiatry with them) became vacuous. The Freudian movement attempted unsuccessfully to provide nineteenth-century psychiatry with a semantic model.

calculations, etc., and soon enough, it also translated the Greek for cognition, thinking, discourse, understanding, etc. This combination of meanings has plagued the term ever since. In most European vernacular languages, reason refers to the *illative* rather than the creative or imaginative aspects of the intellect. Since ancient times, reasons have been used to justify actions (*post facto*) or explain their origin. To say that I did x because of y remains a good way of accounting for my behaviour as long as: (1) there is a cultural or semantic frame within which y mostly leads to x; and (2) there is no reason to believe that I am cheating.

To some, however, accounts of behaviour by means of reasons are considered as after the event and hence do not constitute proper causal accounts. Two factors (*inter alia*) can explain this disenfranchisement: (1) causality is currently understood in terms of general laws (not idiosyncratic explanations); and (2) the neurobiology of reasons remains obscure, and hence there is no material or organic handle with which to convert reasons into real physical causes. There is also the question of whether reasons (as it is mostly believed in medicine, jurisprudence and moral theory) entail intentionality[10] and conscious awareness; in other words, whether in order for person P to claim that he had reason x for behaviour y, he must have been aware *pre facto* of x and then taken a decision that y is the best course of action. This view is considered by others as narrow; for example, according to psychoanalysis, it would be sensical to claim that John gives so many presents to Mary because he is in love with her although he is not aware of it (and also sensical for John to say likewise *post facto* as an explanation for his generous behaviour).

The application of the language of reasons to psychopathology makes things complex. However, a claim by a clinician that a patient has a reason to report 'I am hearing voices when there is no one around' should not be dismissed *tout court*: at a superficial level, it may mean that the patient is believed to be 'entertaining an image' (i.e. experiencing something in terms of the old, passive recipient model), or it may mean that the clinician believes that the patient is actually participating (consciously or unconsciously) in the construction of his experience and that this participation is far more important to the fact that he is having such an experience than whatever signal might be flashing in his brain. This claim by the clinician would be based on a model of symptom formation in which formatting codes are essential to the constitution of the experience itself and its report. Let us say that a patient experiences an inchoate bundle of sensations (elsewhere called 'primordial soup' by us) (Berrios *et al.*, 1995). In order to experience it, handle it cognitively, remember it, and talk about it, he/she needs to configure the primordial soup. He/she does this by means of formats (personal, familiar, cultural) and in negotiation with the clinician (all these factors are extrinsic to the primordial soup). Once this process has been completed, the formatted product will be reported as a voice, a strange belief, sadness, anxiety, a physical sensation, etc. It follows that the fact that the primordial soup is always the result of a brain signal (related to a lesion or dysfunction) is not sufficient (and on occasions not necessary) to explain the utterance. This is because the utterance is about not the primordial soup as such (or it is, but in the remotest of ways) but a final product that is the result of a formatting cascade integrated by many reasons and decisions. In other words, the conceptual distance between brain signal, primordial soup and (reported) mental symptom is so wide that to say that the brain signal is the cause of the mental symptom (for it might correlate with it) has little explanatory meaning.

This claim about the depth of the formatting process must not be confused with the conventional view (which most biological psychiatrists would be happy to accept) that the form of the symptom is determined by the brain site from which it originates (e.g. symptoms related to the occipital cortex are visual, or those related to Broca's area are linguistic). The model of symptom formation explicated above states that not only the content but also the form of the mental symptom is determined by the formatting process. (There is no space in this chapter to discuss the confused and confusing distinction between form and content in relation to mental symptoms.) This has interesting consequences, for if the formatting process can override the influence of the site of origin, then correlations between blood flow (as a proxy for site) and mental symptoms have little meaning.

To recapitulate: (1) brain site and lesion can provide only a jejune and nonspecific causality; (2) the fact that a proxy marker of a brain state (e.g. change in blood flow) correlates with a mental symptom cannot be considered as meaningful; (3) the formatting (i.e. construction) of the mental symptom (i.e. the cause of its form and content) has been governed by reasons; (4) personal factors seem essential to the formation of mental symptoms; and (5) what makes tokens of symptoms (say, hallucinations as reported by different patients) the same symptom is not that all those hallucinations originate from the same location in the brain but that the patients in question have formatted a variety of primordial soups (probably originating in different parts of the brain) by means of the same formatting routines.[11]

The Technology Alibi

As far as we know, the first person to use the technology alibi was Georget (1820) to explain the failure of biological psychiatry to find any sustainable and replicable connections between brain and madness. Then, as now, it was only a 'matter of months' before the pathology and genetics of dementia praecox were sorted out; then, as now, the expectation was kept alive by the release of premature data; and then, as now, there was never the scientific honesty of publishing a denial saying that what had been claimed earlier was nonsense.

As popular now as it was a century ago, the technology alibi is not a hypothesis but the tail end of a foundational syllogism: the mind is represented in the brain → the mind is tantamount to a set of behaviours → hence, all behaviours are represented in the brain → abnormal behaviours are still behaviours → hence, all abnormal behaviours have brain representation → hence, the fact that no brain representation can yet be found for mental disorders must be due to faulty techniques. It is important to realize, however, that finding something in the brain that might be related to the disorder under investigation would not prove the truth of the foundational claim placed at the beginning of the syllogism (i.e. of the antecedent). To believe that it does is to commit the fallacy of affirmation of the consequent, because reasons other than the foundational claim can account for the same finding. Indeed, the same fallacy is committed by biological psychiatrists who believe that rejecting the null hypothesis actually proves that their own explanation is correct.

General Conceptual Issues in Biological Psychiatry: First Pass

The relationship between neuronal activity and mental symptoms can be examined from three perspectives, namely (1) the nature

[10] Intentionality has at least two meanings. According to the psychological one, as per Brentano (1973), all mental acts, *ex definitione*, have a content and are addressed at something. According to the causal (narrower meaning), intention to initiate (or inhibit) action is a requisite component of the very definition of behaviour (some may want to include here unconscious behaviour).

[11] Biological psychiatry has dealt with this by creating a mosaic model of specialized brain sites according to which the form of mental symptoms is determined by its putative site of origin. Evidence for this is surprisingly lacking as current methodology seems tautological.

of the mental symptoms or syndromes, (2) the nature of the neurological dysfunction/lesion, and (3) the empirical determination of the relationship between mental and neurological dysfunction. The division here is simply for ease of analysis and is not meant to imply any actual conceptual separation between the different aspects of the psychiatric/neurological relationship. Thus, attempting to clarify the issues around the empirical determination of the mental/neurological relationship is crucially dependent on some understanding of the conceptual problems, assumptions and constraints affecting the nature of the variables on either side of the relationship. Here, we are concentrating primarily on the mental symptom/syndrome side for it is here that the main conceptual problems and lack of clarity arise.

Before addressing some of the specific points in relation to the mental/neurological relationship, and that will be the main focus of this chapter, it is necessary to raise, at least briefly, a few general issues. Exploration of the above relationship, i.e. between psychiatry and neurology, is dependent on defining the conceptual framework within which such work takes place. In turn, such a conceptual framework is underpinned by not only different sorts and levels of concepts but also assumptions relating to the degree to which such concepts can be understood and held valid. Here, three levels or perspectives are raised in order to highlight the difficulties that need to be acknowledged and the assumptions that must be made in attempting such work.

The Mind–Body Problem

The first, and perhaps most obvious, issue relates to the mind–body problem. In other words, the issue here revolves around the conceptualization of mind and body as distinct categories of substances (various forms of dualism) or conceptualization of them as belonging to a single substance (various forms of monism). Depending on the sort of view one has, different sorts of conceptions will be held in relation to 'psychiatric' and 'neurological' events and the relationship between them.

Taking a position vis-á-vis the mind–body problem is relevant to the biological psychiatrist from both the conceptual and historical perspectives. Historically, it makes him/her aware of the fact that in regard to FCs 1 and 2, there are fundamental differences in meaning between pre- and post-Cartesian writers, and that for current thinkers it is almost impossible to get into the mindset that must have been natural to pre-Cartesian thinkers. Indeed, half-seriously it could be said that the solution to the mind–body problem is to return to a pre-Cartesian time when man was conceived as a substantial unity. Conceptually, the biological psychiatrist must try to become aware of the views he/she really holds in regards to the mind–body problem. Saying 'I am an empirical researcher and hence these philosophical issues are irrelevant to me' is not an option, for it is the case that the very language of neurobiology is soaked in foundational claims. Furthermore, a significant statistical correlation between a mental event (e.g. hallucination) and a brain event (e.g. change in blood flow as per neuroimaging) cannot be understood unless it is given at some level some sort of metaphysical interpretation, i.e. unless some sort of ontological allocation is given to each — and this means taking a position in regards to Cartesian dualism. (On the conceptual basis of correlation, see p. 16.)

Mental Events and Brain Events

Second, we must consider the relationship between 'normal' mental events and brain events. This refers back to the old question of localization of mental states to brain states. The issue is not one of whether mental states are realized in brain states — most would agree that mental states are necessarily determined by brain neuronal activity. The issue concerns the level of *specificity* that can be assumed. In other words, questions have to be asked whether, firstly, mental states can be localized to specific brain systems (and, if so, on what basis), and secondly, at what level or specificity can (a) mental states (moods, thoughts, fears, urges, etc.) and (b) brain states (molar/molecular, etc.) thus be considered.

For example, if it is argued that mental states such as moods, fears and intentions can, in theory, be localized to specific brain states (e.g. particular brain sites or neuronal systems), then by extension it will make sense to look for dysfunction in such areas or systems in relation to abnormal mental states, such as pathological fears, abnormal moods, etc.[12] It needs to be understood, however, that the above claim is, itself, dependent on assumptions. Foremost amongst these is the assumption that our conventional taxonomy of mental states actually corresponds to the brain/neuronal taxonomy. In other words, is it the case that the sorts of mental states or functions as constructed, identified and differentiated by man actually reflect the sorts of divisions operating through neuronal systems? Is there evidence that thoughts, perceptions, intentions, moods, etc. as individuated by language are individuated in a similar fashion by the brain? Evidence would seem to be stronger in relation to some mental functions (e.g. perception, memory) than others (e.g. moods, worries, intentions).

Similarly, considering the level or degree of specificity assumed in relation to both mental states and brain states demands questions that remain to be answered. For example, in regards to mental states, can worries be differentiated from thoughts, memories from daydreams, etc.? And what level of neuronal activity can be expected then to 'account' for preoccupations as opposed to fleeting thoughts, or beliefs as opposed to judgements, etc.? Again, this carries implications for pathological states, for example trying to localize delusions, hallucinations, etc. Furthermore, the level of specificity can go further. What about the content of thoughts, moods, beliefs, etc.? Will fear of dying be localized elsewhere or represented differently than fear of a spider, for example?

Role of Language

Third, from a slightly different perspective is consideration of the role of language in the psychiatry/neurology relationship. Language is used to describe both mental states and brain states, but the issue here is that the language used in relation to each concept has a different epistemic value. In other words, the sort of information captured in relation to mental states (moods, fears, preoccupations, etc.) is different from the sort of information captured in relation to brain states (temporal lobe lesion, cerebral atrophy, demyelination plaque, etc.). Crudely, language of the mind is not equivalent to language of the brain, and this carries implications for the hermeneutics of data (see p. 10).

THE HISTORY OF BIOLOGICAL PSYCHIATRY

The conceptual problems of biological psychiatry can only be understood from a historical perspective; unfortunately, the definitional blurredness of biological psychiatry gets in the way. In other words, is its history like that of a *real object* (horse, orchid, chair, Rosetta stone), a *purview* (aesthetics, ethics), a *disease* (dementia,

[12] The issue of whether emotions can be related to brain sites depends on definition. The passions, an older name for the phenomenon, were conceived of as manifestations of the animal part of man; this view was temporalized by Darwinian evolutionism. Because of their relationship to old brain structures and omnipresence in the brain, emotions are likely to modulate perception, movement plans, memory and cognition. This was also Descartes' view; hence, the idea that he left emotions out of the picture by 'error' is both historically and conceptually nonsense.

anxiety disorder); a *social practice* (priesthood, cobblery, medicine, palmistry), an *ideology* (realism, idealism), or a *construct* (French Revolution, guilt, prostitution)? For the purpose of this chapter, biological psychiatry will be defined as a package of changing ideas and social practices seeking to explain and manage[13] deviant human behaviours in terms of a neurobiological discourse. This enables the historian to ask why biological psychiatry has been successful, what social needs it meets, its best selling point, and how it reconstitutes itself.[14]

Before the Nineteenth Century

A version of the foundational claim (central to biological psychiatry) that mental disorders have a somatic origin can be found in the work of Thomas Hobbes (1588–1679) for whom the causes of madness were 'motions of the blood and animal spirits as they variously expand and contract; the causes of these motions are phantasms concerning good and evil excited in the mind by objects' (Hobbes, 1991). As efficient cause, Hobbes (1968) considered both external images of evil and physical causes:

> ... and to have stronger, and more vehement Passions for any thing, than is ordinarily seen in others, is that which men call MADNESSE. Whereof there be almost as many kinds, as of the Passions themselves. Sometimes the extraordinary and extravagant Passion, proceedeth from the evill constitution of the organs of the body, or harme done them ...

As Gert (1996; p. 165) has noticed, this dual aetiological view helped Hobbes to remain consistent with his materialistic stance: 'Hobbes can be taken to say that sometimes a defective bodily part produces unusual motions, and sometimes the unusual motions of the passion injure the bodily part.'

Historians of medicine consider Thomas Willis (1621–75) to be the main English sponsor of the iatrochemical theory of disease: 'All diseases [are] perversions of natural fermentations in which the sulphurous and spirituous particles in the sanguineous mass were set in too great a motion, and the blood therefore became overheated' (Frank, 1980; p. 167) Brain diseases were explained in the same way (italics in original; Wills, 1685; p. 462):

> ... that we may deliver the *formal nature* and *causes of melancholy*, we may opine, that the liquor distilled from the blood into the brain (which filling and irrigating all the pores and passages of the brain, and its nervous appendix is both the vehicle and vinculum of the animal spirits) has degenerated from its mild, benign, and *subtle* nature, into an acetuos and corrosive disposition ...

Willis also believed that these changes could be shown if careful post-mortem studies of the brain were to be carried out (Conry, 1982). By the middle of the eighteenth century, G.B. Morgagni (1682–1771) found little of interest in such work:

> If you join these six dissections of mine [of patients with a variety of mental disorders] with that which I described to you in the first letter (h) and compared them all with those you have in the *Sepulchretum*,[15] or other books, you will immediately perceive, that among those things which others have observed, *some of them have been never found by me* ...

(our italics; Morgagni, 1769; p. 156). Indeed, Morgagni makes the point that similar forms of madness may show lesions in different parts of the brain; this caused some embarrassment to the 'specificity' claim hinted at earlier on by Willis.

Pierre Cabanis (1757–1808) will be known forever as the man who said that the 'brain secretes thought as the liver does bile'. (Taken out of context, this quotation was used by anti-materialist writers to discredit Cabanis (Chazaud, 1993).) Closer reading, however, shows the complexity of his thought. Cabanis (1802) started with a baroque definition of matter, which, as Moravia (1981; p. xxiii) remarked, 'comprised a full array of forces and properties from whose physico-chemical combination even the most complex living organisms may emerge'. Cabanis' matter could thus give rise to a complex mind (and complex mental disorders) (see p. 3). 'Body' meant brain, heart, stomach, genitalia, etc., and for Cabanis all these organs generated complex patterns of feelings and meanings; it would be anachronistic, however, to consider this view as an anticipation of James and Lange. Biological psychiatrists interested in somatizations and unexplained medical symptoms could study Cabanis with advantage as his ideas are at the basis of the concept of cœnæsthesis and 'cœnæsthopathy' (Berrios, 2001b).

The Nineteenth Century and After

Georget and Bayle

Based on Bichat's vitalism,[16] E. Georget (1795–1828) proposed an organic aetiology of mental disorder (and introduced the technology alibi). His views seemed confirmed by A.J. Bayle (1799–1858), who 'showed' that dementia, other forms of mental disorder, and general paralysis might be related. This has been hailed as the *fons et origo* of biological psychiatry, but it is historically and conceptually wrong (Berrios, 1985). Quétel (1990; p. 161) is correct in claiming:

> General paralysis thus defined might have done no more than figure in psychiatry nosography (though difficult to situate in the nosography of Pinel and Esquirol) had it not fitted in so well with the arguments of those who believed that the aetiology of madness was necessarily organic ... For the first time, then, something had been discovered in the brains of the insane! Moreover, these anatomo-pathological lesions were not only to make general paralysis the model of organic mental disease, they were also to swing the psychogenetic conception of madness.

Griesinger

The quotation that 'all mental diseases are diseases of the brain' has been attributed to Wilhelm Griesinger (1817–68). A physician (the concept of psychiatrist did not exist at the time) interested in mental disorders, his contribution to 'psychiatry' is limited to

[13] Explaining and managing are forms of accounting that can be felicitous, unsuccessful, etc. but of which cannot be said that they are 'true' or 'false' because there is no way to ascertain it.

[14] The process of self-reconstitution can only be understood if the history of terms, concepts and behaviours is considered independently. For example, the fact that the word melancholia has lasted for more than 2000 years has led some to believe that the current condition called melancholia is, in fact, the same as that known by the same word 2000 years ago. This is an anachronism based on the ontological view of disease. The same error has led some to express surprise about the fact that schizophrenia was not reported before the eighteenth century. The explanation regarding melancholia is that all that survived was the word as an empty shell. In the case of schizophrenia, the condition was not reported simply because it did not exist: it was only constructed during the late nineteenth century (see Berrios, 2000b).

[15] *Sepulchretum Sive Anatomica Practica* (Bonetus, 1679) included almost 3000 post-mortem reports; Morgagni set out to check many of its findings.

[16] Vitalism is a doctrine according to which the phenomenon of life results from a principle different from the physical and chemical principles that sustain matter in general. Bichat reacted against metaphysical vitalism, i.e. the idea that the vital principle was part of nature. For Bichat, such a principle can be understood only if it is incorporated into each tissue as sensitivity and contractibility (see Haigh, 1984; Pickstone, 1976).

a textbook, a few papers, and the foundation of the *Archiv für Psychiatrie und Nervenkrankheiten*. His views were grounded on materialist biologism, the new physiology, vitalism and romanticism (Arens, 1996). When Griesinger wrote the first edition of his *Textbook of Mental Pathology and Therapeutics*, he was 28, had little psychiatric experience, and had to borrow most of his clinical illustrations from French and German sources (Griesinger, 1861). Like Jaspers' *General Psychopathology* , published seven decades later, the book is important not so much because of its clinical content but because of its philosophical maturity and imaginative proposals. Griesinger's view that official clinical categories are (and always will be) arbitrary but that their 'elementary' units of analysis (mental symptoms) are ontologically sturdy and stable has provided the basis for descriptive psychopathology (Berrios, 1996).

Put back into its context, and corrected by what the words meant in the 1840s, the claim that 'all mental disorders are disorders of the brain' loses its perspicuity. Griesinger had reacted against German materialism (Gregory, 1977) and was keen on the new physiology of Wunderlich, Roser and Müller; his concept of lesion, therefore, was not anatomical but physiological. It was in the new (and ambiguous) space of the new physiology (Schiller, 1968) where Griesinger located mental disorders. On account of this, and due to the crude ontology to which twenty-first-century biological psychiatry has returned, it is not easy to translate Griesinger's concepts into current categories; for example, although he called mental symptoms 'organic' or 'biological' in nature, he did not expect them (necessarily) to leave an imprint on the brain (Berrios, 1984). His concept of physiological lesion led in the fullness of time to that of psychological lesion.

Meynert

Influenced by Darwinian evolution and the new physiology, Meynert (1833–92) sought to correlate development, function and form. Associationism, localizationism, top-to-bottom inhibition, regional cerebrovascular variations, and the nutritional status of neurons were the pillars upon which he based his speculations on the nature and localization of mental illness. Influenced by the representational ideas of Herbart, he believed that in the network of projection and association fibres, there inhabited a functional ego. The first volume of his *Psychiatrie* (Meynert, 1885), the only one published, is a textbook of neuroanatomy with minor references to psychological concepts (Marx, 1970). Physiological and pathological states (such as obsessions, hallucinations, mania and melancholia) Meynert explained by changes in cerebral blood supply. Nutritional changes at cellular level, resulting from hæmodynamic changes or from congenital defect he considered as the ultimate causal mechanism.

The contribution of Meynert to the aetiology of mental illness is not easy to assess (Lévy-Friesacher, 1983 and Pappenheim, 1975). He has been portrayed as a 'brain mythologizer', but this accusation neglects the fact that on occasions he seemed to be speaking metaphorically (he was also a poet), even in matters medical. He believed that brain activity depended on brain nutrition; the latter was controlled by vasomotor activity, thus, circulatory disturbance could cause mental disorder.

Wernicke

A disciple of Meynert, Karl Wernicke (1848–1905) is considered to be one of the most important psychiatrists of the late nineteenth century (Lanczik, 1988). From the perspective of biological psychiatry, three are his important contributions: (1) a model to encompass all brain-related diseases (whether so-called psychiatric or neurological); (2) the development of a pathophysiological model to mediate between the brain and behaviour, a model that had, until then, been absent from psychiatry; and (3) the introduction of the first neuropsychological approach to mental symptoms.

Central to Wernicke's model was Meynert's idea that the brain of man was endowed with a system of projection and (transcortical) association fibres, and that the latter was the organ of consciousness and high intellectual functions (Wernicke, 1906). Focal lesions to the projection system caused neurological disease; damage of the association system generated mental illness. Using modern jargon, Wernicke was more a connectionist (Stein and Ludik, 1998) than a localizationist.

Neglected Models of Biological Psychiatry

It is an interesting question as to why men like Hughlings Jackson, Von Monakow, Goldstein and Guiraud (*inter alia*) have not had more impact on biological psychiatry. Their ideas are paid lip service but somehow have not been taken up.[17] On account of lack of space, only the ideas of Jackson and Von Monakow will be mentioned here.

Jackson

J.H. Jackson (1834–1911) (Critchley and Critchley, 1998; López Piñero, 1973) spent most of his clinical life giving opinions on the nature of mental disorder. In 1894, he published the 'The factors of insanities'; a paper of relevance to biological psychiatry (Jackson, 1894).[18] Jackson proposed a hierarchical model of the nervous system, with the upper layers inhibiting the bottom ones. Evolution made brain function more complex but less stable; dissolution (Smith, 1982a and 1982b) did the opposite.[19] He defined insanity as a departure from ordinary mentation, which included physiological states such as sleep, chronic brain diseases, and temporary toxic states.[20]

Jackson proposed that insanity originated from the action of four factors. Factor 1 concerned the depth of dissolution; factor

[17] In general, the history of medicine (and psychiatry) remains a chronology of socially successful views that are presented as truthful. This type of history does not seek to understand and explain but to congratulate. It is of the 'whom to worship' variety. It assumes that diseases are like plants waiting for a discoverer. The alternative view is to see all clinical categories as constructs, and discoverers as those sponsoring views that became a social or financial success. The advantage of this view is that it also lends attention to the losers for their work, which in fact, may be far more important from the point of view of coherence, rationality, humanity and predictability than that of the victors.

[18] Why Jackson has had so little influence on British (and later) Anglo-Saxon psychiatry (compared with neurology) is an interesting and understudied question (Berrios, 1977; Dewhurst, 1982). Even the unsuccessful classification of the symptoms of schizophrenia into positive and negative has been based not on Jackson but Reynolds, although its sponsors believed it to be Jacksonian (Berrios, 1985). This was not a trivial error: the Jacksonian model demands an interaction between positive and negative symptoms that has never been shown in schizophrenia (Berrios, 1992).

[19] Jackson's conceptual model was not meant to be mapped on to the real central nervous system, and he said so. However, efforts have been made to find analogical links (e.g. Kennard and Swash, 1989; Dubrovsky, 1993).

[20] He seems to have advocated a continuity (rather than a categorical) view of mental disease, which in practice stretches from physiological states to all gradations of insanity. In this regard, he introduced the concept of mental diplopia, by which he meant definitions of the same behaviour that led to clashes and contradictions, such as using insanity for proper madness and also for a mild degree of drunkenness. Jackson wondered why post-epileptic coma (which he called acute temporary dementia) was not considered as an example of insanity by alienists while post-epileptic mania was. Jackson's concept of insanity or dissolution is only meaningful when compared against a standard, and he used the state of the same person before the disease.

2 the person who has undergone dissolution;[21] factor 3 the rate of dissolution, i.e. how slow or fast the removal of control was; and factor 4 personal variables, providing lists of putative elements and themes around which positive mental symptoms may concrescence. Factors 2 and 4 feed meaning (i.e. information about individuals) into Jackson's model (Berrios, 2001a). Interaction of the four factors gives rise to positive and negative symptoms (Berrios, 1985). The model cannot explain the generation of different types of mental symptoms (e.g. hallucinations, delusions, etc.).

Because of his dualism à outrance,[22] Jackson's stance vis-à-vis FC 1 is equivocal. It is true that he believed that all insanities were related to the brain, but his concept of insanity is so different that it is difficult to link the two.[23] Put together, his views do not tally well. Given his dualism, his concept of mind should have been ontologically independent enough to provide a platform for mental disorders; but it does not. Although not a religious man (Critchley and Critchley, 1998), at some level Jackson seems to have harboured the (nineteenth-century) belief that the mind (or soul) was indivisible and intangible and beyond the reach of disease.

Von Monakow

Constantin von Monakow (1853–1930) authored three books, founded three journals, and mentored great biological psychiatrists (Mourgue, 1931). Together with R. Mourgue, a French psychiatrist and historian of science, he also wrote one of the most important (and neglected) books on biological psychiatry in the twentieth century (von Monakow and Mourgue, 1928). Central to this work is the notion of *Horme*, i.e. the tendency of all living beings to develop all their genetic potential. In each individual, the Horme is governed by *Syneidesis*, i.e. a principle that regulates and balances all instincts in the interest of the given individual. These principles govern both function and structure, hence von Monakow and Mourgue develop a neuropsychiatric model of the type that Guiraud (1950; p. 165) called 'dynamo-morphological'.

Based on the assumption that biological psychiatry was a subfield of biology, the authors imported into it the notion of chronogenetic localization. This concept required that the variable time was built as a parameter into all neuropsychiatric phenomena. Functions (e.g. movement) are processes that, like music, unfold in time and according to a specific kinetic melody. Hence, it would be a mistake to attempt to localize processes (i.e. brain functions) in terms of specific brain sites (i.e. space alone). Now, since most mental symptoms are considered to result from disordered brain function, it follows that it would be equally erroneous to try to localize symptoms on specific brain addresses. Influenced by Jackson, von Monakow and Mourgue believed that chronogenetic localization was a late acquisition in evolutionary time, and hence regarded it as a complex but unstable mechanism.

One of the implications of the concept of chronogenetic localization is that both cross-sectional studies and conventional longitudinal studies (as collections of cross-sectional snapshots) are inadequate for the capture of neuropsychiatric symptoms. The latter, von Monakow and Mourgue insisted, have to be observed as they unfold in time according to their own kinetic melody; for example, a hallucination is understood fully only when an entire token or hallucinatory episode, which may last minutes or hours, has been studied. In addition to its conventional cross-sectional features, such an episode includes real longitudinal information, such as, for example, modulations in intensity and changes in imagery, and accompanying emotions can make sense only when integrated along a time dimension. From an aetiological viewpoint, knowledge of these longitudinal variables may in fact provide more information on the brain localization of the symptom than traditional static snapshots.

THE DEFICITS OF BIOLOGICAL PSYCHIATRY

The empirical credibility of biological psychiatry is threatened by not only its weak foundational claims and equivocal history but also the suspicious validity of its data (capture), of its descriptive and analytical methods (data processing and interpreting), and of the rhetoric of its data reporting. There is no space in this chapter to discuss all these problems in depth; to render the points clear, however, one or two examples will be discussed in each section.

Data Capture in Biological Psychiatry

In the context of biological psychiatry, and the psychiatric-neurological relationship in particular, data fall into two groups, namely psychiatric data (psychopathology — symptoms, signs, behaviours) and neurological data (neurology symptoms, signs, neuroimaging lesions, etc.). The focus in this section will be on psychiatric data, but even on superficial examination, it becomes apparent that there is a mismatch between the way in which the 'mind' or psychiatric data are captured and the way in which the 'brain' or neurological data are captured. With respect to the latter, it is evident that ever-more sophisticated tools are being developed and finer discrimination being sought in terms of visualizing brain structure (e.g. computerized tomography (CT) scanning, magnetic resonance imaging (MRI), etc.) and brain processing (e.g. single positron emission tomography (SPECT), positron emission tomography (PET), functional resonance imaging (fMRI), etc.). Pathology is described at molecular, neurotransmitter, amino acid, etc. levels. In contrast, the level of capture of 'psychiatric' data remains much the same as it was in the nineteenth century when such descriptions were developed (Berrios, 1996). In fact, in many ways descriptions of psychopathology have become narrower and less rich than they were then, constrained perhaps by the classification systems of current times. Thus, the problems with respect to psychiatric data capture in the context of biological psychiatry can be divided into two main areas: general issues pertaining to data capture in psychiatry as a whole, and specific issues relating to data capture in relation to biological psychiatry.

[21] Twenty years earlier, Jackson (1874) had described factor 2 as 'the kind of *brain* in which reduction occurs'. This rephrasing reflects maturity of thinking and a mellowing attitude towards the mentally ill. Jackson believed that variables pertaining to the individual and genetics influenced the form of the insanity but says little else. Personal factors such as age, intelligence and education showed best in states of minor dissolution; 'genetic' factors concerned not the inheritance of specific mental disorders but a tendency to 'give out' to dissolution.

[22] Jackson's views on mind, matter, their relationship, and FC 1 are difficult to map in a coherent way. He was so much of a dualist that he borrowed Clifford's concept of concomitance (Berrios, 2000c) to explain how mind and matter might interact. Jackson (see Taylor, 1931) wrote: 'The doctrine I hold is: first that states of consciousness (or, synonymously, states of mind) are utterly different from nervous states; secondly, that the two things occur together — that for every mental state there is a correlative nervous state; third, that, although the two things occur in parallelism, there is no interference of one with the other. This may be called the doctrine of Concomitance.'

[23] For Jackson, insanity was on a continuum with other states, such as drunkenness or dreaming (hence, he called them states of 'temporal insanity'); only its negative symptoms actually reflected pathology (i.e. lesions or dissolution of the higher, human, inhibiting layers of the brain); positive symptoms were the expression of evolution, of *normal* activity produced by the release of *normal* tissue.

General Problems

Some of the general problems facing data capture in biological psychiatry need to be mentioned. First of all, what sorts of data are being captured? Are psychiatric data different from other sorts of data? What kind of differences are we dealing with? How might this affect the informational value we can obtain from such data? What are the implications for use of such data as variables in correlational studies, etc.?

To answer such questions, we need first of all to define the particular psychiatric data. Broadly, these can be divided into four types: (1) subjective complaints of patients (e.g. feeling depressed, anxious, hearing voices, etc.), (2) signs and behaviours elicited by clinicians (e.g. thought disorder, psychomotor retardation, disinhibition, etc.), (3) psychiatric diagnoses (Diagnostic and statistical manual (DSM) IV categories) (APA, 1994), and (4) scores on various rating scales (e.g. Beck Depression Inventory (BDI), Hospital Anxiety and Depression Scale (HADS), Hamilton Depression (HAM-D), etc.).

Subjective Complaints as Data

The Subjective complaints made by patients (given spontaneously or elicited by questioning) form part of the data captured by clinicians and used to determine the presence of a particular psychiatric symptom, syndrome and/or disorder. It is, however, the nature of such symptoms as 'data' that raises the problems: problems that relate to the sort and quality of information that can be inferred from such data. In other words, questions have to be asked concerning the validity and reliability of subjective complaints as data. Such questions must relate to not only how validity and reliability might be determined but also the epistemological justification for the validity and reliability of the data.

By definition, subjective complaints consist of individual interpretations of some sort of perceived dysfunction or change — whether this is experienced bodily or mentally. Where, then, and what are the origins and constituents of such complaints, and how do they develop into symptoms? These are the crucial questions, since understanding the validity and epistemic value behind subjective symptoms as 'data' depends on the answers. If it is assumed that, irrespective of aetiology (brain lesion, neurological dysfunction, external event, etc.), there is a change in the internal state of an individual, then how does that particular change become transformed and translated into subjective complaints? And can it really be assumed that the 'same' (and this particular assumption carries many problems that cannot be addressed here) internal state will result in its equivalent interpretation and equivalent description by different individuals? In other words, can it be assumed that the data obtained from individual reports of internal states are of similar type to the data obtained from reports of, for example, objects in the public domain, such as sightings of trees, or, more relevantly, fracture on an X-ray, pulse rate, lesion location on CT scan or hot spot on PET? One problem here is that there is not the same system of verification in regard to internal states as there is in relation to external events. How can we know that the internal state as described by an individual is actually that particular experienced state that is matched by the description (i.e. does the description match the experience accurately — assumption of validity)?[24] Secondly, even if the match is assumed to be accurate, how do we know that that particular experience is the same as the one described by someone else (i.e. assumption of reliability)? Furthermore, there is no good model of symptom formation that might help us answer such things (for a précis of our model, see p. 4). Nevertheless, it is difficult to conceive of the process of interpreting a particular internal state and subsequently articulating this in a particular way, as a uniform one, unaffected by individual and sociocultural factors at any stage. Far more plausible would be the possibility that changes in internal states are going to be interpreted in different ways by individuals. For example, it is conceivable that a particular unpleasant internal state change might be read or interpreted as depressed mood in one individual (or at a particular time) while the 'same' or similar internal state might be interpreted by another individual (or at another time) as anxiety or irritability or pain or tiredness, and so on. The factors that might be important in determining a specific interpretation (e.g. past individual experience, cultural biases, contextual issues, language limitations, contribution from the eliciting clinician, etc.) are not the issue here. The point, however, is that it is more than likely that making sense of changes in internal states, and interpreting such changes, is going to be affected by many factors. This means that it is equally unlikely that there will then be a direct relationship between whatever the neurobiological 'signal' manifesting the internal change is and its outward manifestation as a subjective complaint. In other words, there will be a marked degree of formatting going on (noise from various sources), which will change (distort) the original signal to variable extents.

Further complications arise from the fact that subjective complaints themselves cannot be considered a homogeneous class of data. Such symptoms vary in form (moods, fears, perceptions, etc.) and content (anxiety, irritability, elation, hopelessness, grandiosity, voices, images, etc.), and the heterogeneity is likely to be reflected also in the ways in which such symptoms develop and their overall structures (Marková and Berrios, 1995a). In other words, the formatting by other factors is likely to be different in relation to different subjective symptoms. Complaints of depressed mood, for example, may involve types of interpreting factors other than complaints of hearing voices, which one could envisage as perhaps more directly representing the original signal. And when it comes to more complex symptoms or judgements made by patients, e.g. insight into their mental experiences or illness, then the sort of distortion produced by the very many different factors contributing to the construction of such a meta-concept is likely to be much greater (Marková and Berrios, 1995b).

So, what will all this mean for the validity and reliability of subjective complaints as data for biological psychiatry? Given that at present there is little evidence to indicate that a particular description of an internal state actually reflects in a direct way a specific signal or dysfunction, then we have little to justify the validity of such data. Evidence for validity can only start to be gathered when the factors important in distorting the original signal can be identified and teased out in some way. Similarly, reliability will also depend, in part, on the factors determining validity. In addition, however, the issue of whether a particular signal/dysfunction is going to be experienced (leaving aside the issue of interpretation) in the same way in different individuals is open to question. Or perhaps the question here might more usefully relate to there being sufficient similarity in internal state experiences for the state to warrant similar descriptions (again leaving aside the issue of interpretation). What is evident, however, even on this superficial analysis, is that epistemological justification for subjective data in terms of their validity and reliability remains poor. That is not to say, of course, that such data are not useful in themselves. Individuals do have to communicate about not only external objects and events but also internal states, and in general, degrees of understanding can be achieved between individuals (though clearly to various extents), which would argue for some

[24] Aspects of this debate (particularly a criticism of the epistemic value of data obtained by introspection) were first rehearsed during the second half of the nineteenth century in the nascent field of 'scientific' psychology. It was then argued by experimentalists that the quality and reliability of information obtained in the laboratory was superior to that obtained by introspection (Boring, 1953). Those who took this seriously started to train subjects into being good and attentive reporters. This created a 'professional' class of experimental, which no doubt biased findings further.

level of concordance. Thus, subjective symptoms as data used, for example, in clinical management, in terms of making some sort of sense of what and why a patient might be experiencing a particular state is likely to be of practical importance. The essential issue here, however, is not to confuse the practical validity with the epistemic validity of such data. This is of crucial importance in the context of biological psychiatry and any research exploring the psychiatric-neurological relationship, particularly in terms of inferences drawn from correlational research (see Section 3.2), where the data on either side of the correlation may not be epistemologically comparable.

Objective Signs and Behaviours as Data

Psychiatric data are also collected as signs and behaviours identified by the clinician during clinical assessment. Thus, based on a patient's presentation, behaviour, speech and responses, clinicians will determine the presence of, for example, disinhibition, psychomotor retardation, thought disorder, blunted affect, etc. These types of data are thus dependent on the clinician's interpretation of speech and behaviours manifested in the patients. The validity of such data in turn depends on (1) whether the clinician is actually identifying changes that are, in fact, present in the patient, and (2) whether such changes are correctly described and named by the clinician. (In addition, of course, there is the issue of whether the signs and behaviours that are identified by the clinician are in fact the relevant ones out of many possibilities.) Reliability, on the other hand, relates to the extent to which clinicians will agree on the nature of the data presented.

Once again, the questions here, then, are how can validity and reliability be determined, and what are their epistemological justifications? Examining the types of data in this case, it is evident that the data elicited must be dependent not only on whatever dysfunction is manifest in the patient but also on the clinician's interpretation of the patient's presentation. In turn, this will again depend on a number of factors, including clinical experience, knowledge, individual biases, context of subjective complaints, etc. For example, whether a clinician identifies a sign such as affective blunting might depend not only on the explicitness with which it was present but also on the clinician's past experience in seeing this feature, his knowledge and consequent readiness to identify this, his own concurrent mental state, e.g. level of concentration, and whether the patient was denying, for example, depression and anxiety. It can be argued, however, that given the more direct elicitation of such data, in terms of observation of particular outward manifestations, there is more objective determination of the phenomena and hence less likelihood of distortion by the individual factors related to the clinicians.[25] Thus, validity and reliability may, in this sense, be greater in relation to these types of psychiatric data than in relation to the subjective complaints as data. This may well be the case, but the issue remains that interpretation of signs and behaviours must still occur (although perhaps to a lesser extent) so that the potential for distortion is still there and there is so far little known about the factors important in the development of such 'noise'. Similarly, the issue of heterogeneity is again a factor to consider in the capture of these data. It is likely that some signs and behaviours may be more direct manifestations of dysfunction than others and thus less prone to distortion by clinician factors.

Once more, then, the question must be asked: what does all this mean in relation to the epistemic justification of signs and behaviours as data? Such data are clearly once again very different from the sort of data collected in, for example physical science, electrocardiogram (ECG) readings, measurements of height and weight, etc. The latter are, of course, subject to interpretation as well as are any external perceptions. However, the degree of interpretation involved in the elicitation of signs and behaviours, fluctuating in character, themselves dependent on often unclear or abstract theories, must be of a different and greater extent. Identifying changes in patients and naming them correctly (whatever that may mean) is dependent on more than actual dysfunction or lesion. This, together with the fact that little is known about the specific relevance of the identified in relation to the non-identified signs and behaviours, indicates again that epistemological validity is weak.

Psychiatric Diagnoses as Data

Again, in the context of biological psychiatry and the search to explain or clarify the psychiatric-neurological relationship, psychiatric diagnoses are frequently used as data against which brain abnormalities or dysfunction are assessed. In general, issues surrounding validity and reliability of such data have tended to be addressed by the use of lists of diagnostic criteria and diagnostic categories from established classification systems such as ICD-10 (WHO, 1992) or DSM-IV (APA, 1994). The question arising here, though, is what does this actually mean in relation to the use of diagnostic categories as data in biological psychiatry? Reliability here refers to the extent to which clinicians will agree on a specific diagnostic category as applying to a particular patient. Thus, reliability will depend on clinicians agreeing that a certain clinical state fulfils a number of set criteria. And, insofar as levels of agreement are reached, then the classification systems can be said to be relatively successful. But the issue is that whilst this refers to the general facility with which clinical descriptions can be applied, it does not refer to the nature of the data themselves. In other words, it is again the validity of these data that needs to be questioned. The validity of psychiatric diagnoses refers to the extent to which the diagnostic categories actually reflect specific diseases or conditions. Looking more closely at this brings the epistemological problems to the surface. Making a diagnosis will depend, in theory at least (and arguments have been forward against this assumption; see Berrios and Chen, 1993), on identifying and eliciting the primary psychiatric data, namely history, symptoms, signs and behaviours. These will then need to be checked against the list of diagnostic criteria to determine the best fit and hence the most probable diagnosis. It is apparent, therefore, that the informational value of diagnoses (assuming that these are second-order data made on the basis of primary data [signs, symptoms, etc.]) will be affected, in the first place, by the same factors discussed above in relation to subjective complaints and signs and behaviours. In other words, the epistemic value of such data cannot be greater than that underlying the original constituent data. In addition, there is another problem here as far as validity is concerned. This concerns the actual grouping of the primary data into the diagnostic categories and the question of how far such groupings represent true disease process. And, given the so far lack of knowledge concerning aetiology of psychiatric disorders, it has to be accepted that the current groupings are determined by more arbitrary factors, with face validity based on consensus of opinions, but not by information concerning disease states themselves. Furthermore, epistemic justification of diagnostic categories as data will also be affected by heterogeneity in diagnostic categories. For example, criteria set out for the diagnosis of bipolar affective disorder are likely to have an epistemic basis of

[25] Aspects of this issue relate to the old nineteenth-century debate, inspired by the first positivist philosophy, on whether signs were more informative, stable or objective than symptoms. By the end of the century, the success of neurology over alienism seemed to confirm that this was the case (Berrios and Porter, 1995). As far as these authors know, and even within the semiological revolution that took place during this period, there has never been an adequate debate on the epistemic value of signs and symptoms and on the fact that they name continuous rather than discontinuous phenomena (Berrios, 2001c).

a different sort to the criteria set out for diagnosis of, for example, neurasthenia or dissociative disorder.

It is important to emphasize again the point applying to all these types of psychiatric data, i.e. that the issue of epistemic validity should not be confused with their practical utility. It is the former that is weak, and the limitations need acknowledging in research.

The use of specified diagnostic categories, by virtue of their specified criteria, may give rise to assumptions about their validity as data, which may be unjustified since these categories mask the actual lack of epistemic validity underpinning primary psychiatric data.

Data from Rating Scales as Data

To measure is to map in numbers features of objects or processes so that operations on the numbers might obtain patterns of relationships assumed to exist in the objects themselves.[26] As a praxis, measuring is likely to have started very early in the history of mankind, particularly in the fields of bartering, commerce, building and engineering, and astronomy. Cubit, foot, fathom, grain, etc. attest to the fact that *ab initio* parts of the human body and/or ordinary objects were used as measures. Since very early, standardization was required to prevent disagreements and cheating; the former process has not yet been completed.

The stage at which measuring changed from being a praxis to becoming an epistemological tool, i.e. a process considered as important in the acquisition of knowledge about the world, is difficult to say. Neither Plato nor Aristotle seems to have felt that measuring added to the rational analysis of reality. By the high Renaissance, however, a theoretical shift towards quantification is clearly noticeable (it is applied to painting and drawing, map making, and even the deployment of soldiers in the battlefield) culminating with the epistemological redefinition of the new seventeenth-century sciences as 'quantitative' (Crosby, 1997). This was a reflection of the belief that nature itself had a numerical structure, and hence that knowledge of such codes would make man a master of reality. Thus spoke Galileo: 'Philosophy is written in this grand book, the universe, which stands continually open to our gaze, but the book cannot be understood unless one first learns to comprehend the language and read the letters in which it is composed. It is written in the language of mathematics ...' Newton shared this view.

By the late eighteenth century, the rational, discursive analysis of quality and individuality had been abandoned by the natural sciences and had to be taken over by a small group of inchoate disciplines; a century later, these became the great group of 'sciences of the spirit', which, in the hands of Dilthey and others, developed their own epistemological structure, set 'understanding' (Verstehen) as their objective, 'Erlebnis' as their subject matter, 'hermeneutics' as their tool, and shunned quantification altogether. For a while, psychology was considered to be part of the *Geisteswissenschaften* (e.g. by Dilthey), which may explain why efforts to introduce measurement into psychology met resistance at first. In the event, Weber, Fechner, Donders and Ebbinghaus encouraged the unilateral independence of psychology, redefined concepts, and set the process of its 'naturalization' going (Boring, 1942; Boring, 1950; Boring, 1961).[27]

In the shape of rating scales, graphic representations and statistical analysis, measurement did reach psychopathology and psychiatry after the Second World War. Rating scales and graphic representations had been in use in psychology since the turn of the century, and statistical analysis had become available since before the Great War but was not used in medicine or psychiatry. Although momentous, these events have been neglected by historians.[28] All we know is that rating scales arrived furtively, sometime during the late 1950s, hidden in the Trojan horse of the incipient drug industry of the day. Early scales (such as the Hamilton Depression Scale) were designed exclusively for the evaluation of drug trials (Berrios and Bulbena, 1990). After some resistance, rating scales become popular for they seem the ideal response to the *cri de coeur* for objectivity and reliability so characteristic of biological psychiatry, and because they yield the scores required by statistical manipulation.

Given the hybrid nature of psychiatry (a discipline whose subject matter requires both the natural and human sciences), it is extraordinary that at the time (and since) it occurred to no one that the introduction of quantification needed to be justified epistemologically.[29] Of the three topics listed above, only rating scales will be treated in this section. We will mention statistical analysis later. There is no space to deal with the numerical, aesthetic, rhetoric and heuristic aspects of graph representations.

Rating Scales

Under the aegis of the first mental tests — such as the Ebbinghaus word lists to evaluate memory (Ebbinghaus, 1964), questionnaires appeared towards the end of the nineteenth century purporting to capture other phenomena such as hallucinations (Parish, 1897). (Lest it is concluded that this is the beginning of measurement in psychiatry, the point must be made that Parish and others were amateur gentlemen wanting to test beliefs in the parapsychological and psychical fields.) Encouraged by eugenics, educational policies and the war effort, 'proper' tests were first developed to measure intelligence and personality. The development of psychometry and factor analysis was, in turn, spurred on by the need to standardize and process such instruments.

When rating scales arrived in psychiatry in the 1950s, the view that mental symptoms were immeasurable was still common, and not only amongst those of a psychodynamic persuasion.[30] However, the need to measure outcome in drug trials encouraged the construction of rating instruments based on items believed to be susceptible to rapid change (in days or weeks), their incomplete item coverage making them unsuitable for diagnosis (e.g. the

[26] These may range from comparative orderings (larger than, equal to, etc.) to complex pattern-recognition techniques said to be able to extricate from data sheets meaningful numerical configurations. In either case, the assumption is that numbers are mapping configurations that exist in nature.

[27] That is, the view that because the mind is a product of natural evolution, then its study should also fall within the purview of the natural sciences. The narrower view that meaning and individuality are irrelevant to scientific psychology is based on additional assumptions adopted happily during the behaviourist era. Current 'cognitivism', particularly the one based on information technology, has not been able to regain any of the old semantic and symbolic approach to human behaviour.

[28] The arrival of quantification in psychiatry needs analysis. The historian is interested to know about the social conjuncture that allowed its adoption, particularly the groups that benefited from it.

[29] The issue here is not whether measurement should be adopted by psychiatry. The issues are: (1) what types of information or forms of meaning included in the psychiatric discourse are susceptible to measurement? and (2) how much of the semantics of qualia is lost after it is translated into the items of a rating scale? These questions cannot be answered by empirical research.

[30] Personal communication by the late Professor Max Hamilton. The immeasurability hypothesis must be taken seriously. It is based on the view that mental symptoms are semantic events, and that mensuration of features external to meaning or imposed upon their structure (such as dimensions, intensity and localization) missed out on the only element relevant to the understanding and management of mental symptoms. A claim of immeasurability can also be grounded on technical argument, such as the unreliability and low epistemic value of proxying (see p. 16).

Hamilton Depression Scale) (Berrios and Bulbena, 1990; Berrios and Marková, 2002b).

The numerical and semantic meaning of the resulting global score has also caused confusion amongst biological psychiatrists. It does not take long to realize that such a score masquerades as a continuous number. In fact, it is just a totted summary of qualitative (ordinal scale) decisions taken at item level. In the case of the Hamilton Depression Scale, for example, not all items are captured by scales of the same size. The reasons why some researchers believe that a continuous number can issue out of the arithmetic addition of qualitative decisions is one of the mysteries of psychiatric psychometry. There are, however, hundreds of papers where the Hamilton global score has been entered into statistical analyses that are known to demand numerical and parametric features of the figures involved which the global scores cannot hope to meet.[31] The curious thing here is that biological psychiatrists, in general demanding of reliability and objectivity, are happy to accept global scores as numerical proxies of that kind. In fact, the best approach is to understand global scores as summary narratives, as forms of mapping a phenomenon by means of the capture of its features (items).

Something must be said on the noble and mysterious concept of 'item', the 'unit of analysis' of psychiatric scales. Items are claimed to stand as semantic and mathematical proxies for attributes, dimensions or expressions of a phenomenon under measurement (see *The semantic mediator of rating scales* below). The content of items ranges from questions meant to capture the presence of the phenomenon (the question 'hallucinations present/absent' is an instrument with one item; indeed, it generates the global score 1/0, which is ordinarily entered into statistical calculations), to scalar anchor points (nominal, ordinal, interval, ratio and, very rarely, absolute forms of measurement), to visual analogues. All these contents are made to yield a number. Through its content, each item is also made to fulfil a proxy function, and the success of any scale depends on the quality of the proxying. So-called standardization methods (including those addressed at evaluating reliability, validity and internal coherence such as Alpha Cronbach) do not, indeed cannot, evaluate the proxying function of each items (on the complex problem of proxying, see Section 3.2.1). In a way, the relationship of a rated psychiatric instrument to its items is (on the model of DSM-IV) like that of a made diagnosis to the mental symptoms or criteria it is based on. Much fuss has been made about the reliability of psychiatric instruments and of DSM-IV diagnostic categories, but all this work is vacuous without having a clear idea of the proxying function of individual items and the way in which mental symptoms themselves (criteria) are actually recognized.

The Semantic Mediator of Rating Scales

Whether depression, thought disorder, apathy, anxiety, alexithymia or whatever, rating instruments do not measure *directly* the target clinical state, trait or condition. They measure it indirectly, i.e. they are always mediated by a construct. A construct is a notion (such as the concept of virtue or disease) or image (painting) purporting to map or represent something else. Schizophrenia, obsessional disorder, Cotard syndrome, autism, theory of mind, etc. are not objects like stones, horses or stars; they are all 'constructs'.[32] They are semantic grids that format opaque and formless behavioural phenomena and allow their capture. On occasions, the phenomenon may be linguistic or semantic and hence devoid of neurobiology (the co-varying 'something' that is captured by neuroimaging is not necessarily the phenomenon but resources used to express the phenomenon, e.g. linguistic activity). This should not worry us, however, for as far as the patient is concerned, the phenomenon is still a bother, irrespective of whether it has representation in his/her own brain.

Constructs are thus semantic structures that select, circumscribe, impose coherence upon, interpret and create a narrative about mental symptoms. Rating scales hang off these constructs and proxy for them. Hence they have no direct contact with the phenomenon underneath, whether neurobiological or not. Constructs are used to: (1) set the boundaries and interpret the raw phenomenon in question; (2) break it up into features, attributes, dimensions and latent traits (in which latter case it will need to include probes or handles to get at them), thereby creating a 'featural pool'; (3) translate the featural pool into a language that can be understood by the average person; (4) select from the pool attributes according to criteria (pathognomonic, representative, convenient, fashionable, interesting, susceptible to change, susceptible to measurement, etc.); and (5) convert the attributes into items by tethering them to specific response categories.

At each step, distortions and biases of a hermeneutic or semantic nature are introduced into the representation that cannot be eliminated by the statistical evaluation of the scale. Calculating a reliability index (e.g. alpha Cronbach) can only ascertain whether the attributes selected cohere or relate to each other and converge on to a unitary construct; they cannot help to decide on the relationship between the construct and the behaviour. The little there is left of semantic information after a scale has been constructed is to be found in the instructions and in the history of the scale. And yet the latter two are rarely consulted and pondered over, for there is the magical belief that once treated statistically, rating instruments become autonomous entities that do not need to have a past. This is wrong, for all scales belong in cultural and clinical niches and hence wear off, loose reliability through time or mismeasure when applied to phenomena or contexts not specified by their instructions or semantic history.[33] Researchers in biological psychiatry do not seem to bother much about such semantic information; this explains why, for example, they continue using the Hamilton scale (which is, in fact, losing sharpness as the surface symptomatology of depression changes) to measure 'depression' in frontal lobe stroke (and other neurological conditions) in spite of the fact that Max Hamilton instructed specifically that patients suffering from such disorders fell outside the semantic purview of the scale (Berrios and Bulbena, 1990).

The Informational Capacity of Rating Scales

Rating scales are believed to be objective. The denotation of this term is valid and reliable; its connotation is scientific, good, above board, not subjective, sellable, better than other approaches, etc. Hence, rating scales are considered to be a form of assessment superior to the happy chat or the clinical interview. Evidence for such a notion is surprisingly limited, but it survives as a

[31] Except in the case of rating instruments based on absolute measurement, global scores cannot be considered as absolute, i.e. as numbers starting from true 0. For example, a 0 score on the Beck Depressive Inventory cannot be interpreted as absence of depression; nor can someone scoring 40 be said to be twice as depressed as someone scoring 20.

[32] Resistance to the idea that the categories of psychiatry are constructs may issue out of fear that such a view will undermine their ontology (and consequently threaten their localizability, neuroimaging and management by medication). This fear may in turn result from the (wrong) belief that constructs are not part of nature but artefacts of language or fictions. This, in fact, is not the case.

[33] This is a well-known aspect of psychometry. For example, the original Wechsler scale was found to have lost calibration because it was standardized in pre-war samples that are now believed to have been less 'bright' than post-war samples. This principle has not yet been applied to psychiatric rating scales in spite of the fact that there is evidence that the expression of mental disorders has changed on account of both neurobiological and social reasons.

counterpart to the (wrong) view that interviews are subjective. Rating scales have benefited much from comparing them with rulers and other measuring instruments. In fact, the conceptual distance between a stick and the ruler is much shorter than that between a mental symptom and its scale. By conceptual distance, we mean here the number of semantic interventions required to convert a phenomenon into an item. The more interventions, the more the loss and/or distortion of information. As long as scales are taken to be short-hand, semantic devices, or summary narratives, such distortions matter little. They cause needless fatalities, however, when rating instruments are considered to be mirrors of nature and powerful generators of knowledge, and their global scores to be true measurements (and entered into correlations).

In general, there is nothing magic about meters or scales or the other devices created by man to allocate numbers to objects and qualities. All measurements (including the simplest ones) are mediated by concepts and are dependent on aids taken from anatomical (cubit, inch, fathom) or common objects (grain, stones) that have since become abstracted and standardized. Measurements in earlier days were comparative and relational. Reliability (that users meant more or less the same by each unit of measurement) was in those early days more important than accuracy (the concept of validity is not important in a world of comparisons).

Specific Problems

So far, we have concentrated on problems associated with psychiatric data capture in general. Emphasis has been placed on the issue that psychiatric data are of a different sort to other types of data that are used in research and other situations (e.g. height measurements, pulse rate, number of plaques on MRI, etc.). Whilst some differences in validity and reliability can be identified between subgroups of psychiatric data, as a group psychiatric data are marked by weak epistemological support and hence poor epistemic validity. This carries implications for correlational research in biological psychiatry where such data are treated as hard variables (see p. 18).

In addition to conceptual problems applying to psychiatric data capture in general, there are issues specific to psychiatric data capture in biological psychiatry that need to be considered. The specificity of these issues is, to some extent, defined by the presumed neuropsychiatric relationship. This relationship is generally taken to encompass several possible perspectives. Limiting things here to a situation where a patient has a neurological condition and associated psychiatric problems, then these perspectives can include views that: (1) the neurological lesion might be causing directly the psychiatric manifestations (directly in the sense of specific brain sites or neuronal systems damaged); (2) the neurological lesion might be causing indirectly the psychiatric problems in that the latter may reflect the response of the patient to the consequences of the neurological condition/disability; (3) the neurological lesion might be both the direct and indirect cause of the psychiatric problems; and (4) the neurological lesion and psychiatric problems might be occurring independently and hence coincidentally. These perspectives, whether or not stated explicitly, seem to underpin a number of the conceptual and empirical problems facing data capture. The problems are various, and again it is not possible to cover all in this chapter.

Behavioural Phenocopies

One such problem concerns the nature of psychiatric data in the context of neurological disease and relates to the question of whether such data can be considered similar or different to those manifested in conventional psychiatric disorders. This problem is raised in a number of ways. For example, in the case of organic depression (e.g. depression in the context of stroke), questions remain as to whether this might be, in fact, the same sort of condition (i.e. same sort of structure, symptom pattern, behaviour, response to treatment, etc.) as depressive disorder occurring without a neurological condition. ICD-10 classifies organic affective disorders in a separate diagnostic category from affective disorders, but uses 'presumed organic aetiology' as the only discriminator between the two. Some evidence suggests that phenomenologically there may be differences between the two depressions, and that some types of organic depression may be behavioural phenocopies in that although appearing similar, they have different symptom profiles.

For example, a cluster of affective complaints can mimic depression, only to disintegrate after a few days of observation. It could, of course, be the case that the depression has resolved rapidly and spontaneously, but often enough what the observer has noticed is a behavioural phenocopy of depression (Berrios and Samuel, 1987). Such clusters carry implications in relation to the neuropsychiatric relationship perspective in that they may also be mediated by the neurological dysfunction either specifically or as a general response. Another example raising the issue of similarities and differences between mental states in the context of biological psychiatry is that of dementia and so-called 'pseudodementia' (or whatever other name one wants to use to name the behavioural phenocopies of dementia). Thus, dementia-like syndromes can be manifested, in terms of both symptoms and the disease, by other functional and neurological disorders (Berrios and Marková, 2001).

The question, then, is how does this problem of similarities and differences affect the psychiatric data or rather the epistemic validity of such data in this context? Again, in order to tease out some of the possible factors involved, various possibilities need to be considered. First, the psychiatric data in the context of neurological disease may, in fact, be of the same nature and structure as those manifested in conventional psychiatric disorders. In other words, the same sort of psychiatric symptoms, signs, behaviours and diagnoses would occur, and the specific neurological lesion would not be adding anything different or new to the possible psychiatric picture. In this case, the epistemic validity of the data is limited by the same factors discussed earlier in relation to psychiatric data in general.

Second, some of the psychiatric data in the context of neurological disease may appear and actually be different in nature and in structure, from those found in conventional psychiatric disorder. Patients might express different sorts of complaints, talk about strange experiences, and exhibit odd signs and behaviours, all of which might not necessarily fit current lists of conventional symptoms and diagnoses. In the context of the neuropsychiatric relationship, such differences may arise directly as a result of the neurological lesion (relating to the site and/or neuronal system affected) or be mediated by any ensuing brain damage (e.g. dysphasia interfering with abilities to articulate experiences, personality changes colouring behavioural manifestations, etc.). The problems in this situation, as far as data capture are concerned, would relate to the identification, the capture and the naming or classification of such data. For example, where 'new' and odd experiences are complained about, i.e. those not fitting into conventional categories, then it might be more difficult to identify these as data; often, such complaints are then ignored (or forced into the nearest category available). Similarly, were such experiences to be completely new to the patient, it might be particularly difficult to articulate, for they may not have the language to do the experience justice. Again, this may result in ignoring the experience or fitting it into a known category of description, thus causing difficulties in capturing the data. Both such difficulties carry implications for the 'correct' naming of data.

Third, some of the psychiatric data in the context of neurological disease may only appear to be similar to those found in conventional psychiatric disorder. This is the issue raised in relation to mimics and behavioural phenocopies. The question then follows: what does 'similar' mean? The analysis of similarity is complex, but here

we shall use a simple definition: 'two symptoms are similar when they have many but not all properties in common.' A property, in turn, is an 'attribute or quality belonging to a thing or person: in earlier use sometimes, an essential, special, or distinctive quality, a peculiarity; in later use often, a quality or characteristic in general (without reference to its essentialness or distinctiveness)' (Oxford University Press, 1992). Properties can be essential and accidental. For example, 'being related to vision' is an essential property of the current definition of a visual hallucination, and 'forgetting' is an essential feature of a memory deficit. On the other hand, properties that can change without affecting the class of symptoms involved (e.g. content) are called 'accidental'. Both types of properties can generate similarity. Following previous definitions (Berrios and Marková, 2001), the term 'mimic' is used here to refer to a clinical state that shows partial similarity to another. In the case of dementia, it may consist of one predominant symptom (e.g. disorientation). The term 'behavioural phenocopy', on the other hand, is used to refer to a mental state that resembles more completely the full clinical picture of a particular condition. For example, organic depression could be considered to be a behavioural phenocopy of depressive episode, and depressive pseudodementia might be said to be a behavioural phenocopy of dementia (Berrios and Hodges, 2000). In addition, behavioural phenocopies are met with in clinical practice that remain unnamed and hence unrecognized because they are fleeting, clinically uninteresting, or cause little subjective distress; all three, however, are still temporary coincidences of symptoms (each originating from a different mechanism) that create the impression of being something else.

The question of whether all similarities engender mimics remains to be answered. Nor is it clear whether essential or accidental properties are more likely to give the impression of similarity. For example, in a patient with psychomotor retardation and memory deficits, the presence of delusions with a content of poverty (an accidental property) will be more likely to create a copy of depression than a delusion with another content. On the other hand, in a patient with confusion and memory deficits, the presence of visual hallucinations (an essential property), regardless of content, may create a copy of Lewy body disease (Perry *et al.*, 1996).

Similarity can be predicated of both symptoms and diseases. In regards to the latter, patients are said to have a similar disease x, regardless of whether they share all the same symptoms pertaining to x. For example, if x is constituted by symptoms x_1 to x_{10}, in an extreme case one patient could suffer x_1 to x_5 and another suffer x_6 to x_{10} and they would still be considered as having the same disease in spite of their phenomenology being different. In practice, however, not all symptoms x_s have the same weight (e.g. some may be considered as pathognomonic, sine qua non) and hence decisions taken concerning similarity depend on more complex judgements relating to patterns and selection of symptoms presented. Alternatively, both patients might have x_1 to x_5 and would thus show very similar presentations of the disease.

The issue of similarity, or mimics and behavioural phenocopies, is clearly important, but what are the implications for psychiatric data capture? One way to answer this and to examine the epistemic problems around the psychiatric data capture in this context is to separate out where such similarity might occur. In other words, the question first is at what level might the similarity be generated? Following the previous line of analysis, broadly three levels can be distinguished here: (1) the level at which the actual dysfunction is being *experienced* (ontological level); (2) the level at which the internal changes are being *interpreted* and/or manifested; and (3) the level at which the psychiatric symptoms and signs (data) are being *captured* by the clinician and hence identified and named. The level at which similarity might be generated carries different implications for the epistemic value of the resultant psychiatric data, and it is useful to briefly examine each of these in turn.

Experiential Origin

Similarity may be generated at the level at which a particular dysfunction is experienced. This would mean that different sorts of lesions or dysfunctions would result in similar internal experiences. For example, similar feelings of low mood, fatigue, amotivation, etc. may be produced in response to a brain tumour, stroke, multiple sclerosis, dementia or major depression. Whether it is individual symptoms or whole clinical syndromes that are experienced as similar matters little at this point. The issue is, however, that similar experiences may be produced as a result of quite different brain insults. To make better sense of this, the notion of similarity here demands deeper analysis. The latter, however, is complex and well beyond the remit of this chapter, since we are dealing here with an ontological issue, itself dependent on the exploration of underlying problematical concepts such as identity and localization. Here, the only point to highlight is that at the level of symptom origin, internal changes might be experienced as similar and hence give rise to similar clinical pictures. This issue relating to the specificity or non-specificity of mental symptoms in relation to different brain insults is not new. During the late nineteenth century, there was a debate as to whether the phenomenology of the organic mental disorders (also called exogenous psychoses) always carried information or a mark that betrayed its underlying aetiology. For example, did the symptomatology of exogenous psychoses produced by hypothyroidism, brain tumours, toxins, meningitis and brain injury differ from each other in any way? Again, there is little space here to expand on this, which was but a remnant of the old medieval theory of 'signatures'. Kraepelin (1899) believed that, in principle, such subtle marks were embedded in the phenomenology of most exogenous psychoses but that not enough research had yet been done to identify them in all cases. By the early twentieth century, however, and mainly under the influence of evolutionary theory, views began to change. In the event, Bonhoeffer's proposal was accepted that the brain had only a limited number of stereotyped responses to insult and injury (confusion, delirium, stupor, cognitive disorganization, etc.), which were triggered by whatever aetiology. Subsequent questions asked by Bonhoeffer (1910), Redlich (1912) and others referred to whether some aetiologies preferentially chose particular stereotyped responses.

What, though, are the implications for the epistemic validity of the psychiatric data, in the context of biological psychiatry (the neuropsychiatric relationship), if similarity were to be generated at this level? If very similar internal states are being generated, irrespective of the specific cause, then any determinable clinical differences will depend not on the original signalling but on the nonspecific (in relation to the brain or other trigger) individual interpretation (formatting) of the internal change. Such interpretation would, however, represent the 'noise' in the system rather than reflect specifically the original signal. Thus, mimics or behavioural phenocopies arising at this level may have similar core structures but different subjective colouring and different aetiologies.

Epistemic justification of such psychiatric data in this situation will therefore be weakened on two main grounds. Firstly, the factors important in the interpretation of internal states, as discussed in the general section, remain to be established. Secondly, and specifically concerning the similarities issue, as far as the relationship to the neurological lesion/dysfunction is concerned, then the informational value of the psychiatric data will be limited primarily by the level at which original signals can cause different internal changes. In turn, this depends on understanding at a deeper (and as yet unknown) level the mechanisms underlying brain changes and their mental manifestations. In practical terms, clearly similarity occurring at this level would mean that attempts to develop finer clinical or phenomenological discriminators would not help to differentiate between 'true' psychiatric

states (e.g. depression, psychosis, etc.) and different mimics or behavioural phenocopies.

Interpretative Origin

Similarity, postulated to arise at the level of interpretation of an internal change, carries different implications for the nature of psychiatric data. Here, the idea is that different brain events or lesions produce different internal experiences, but similarity is generated through a commonality of interpretation. In turn, this implies that there may be limits to the way in which internal states are interpreted and named by individuals. Such limits are different from those mentioned above, since they are imposed not by the signals themselves but by the capacity of an individual to describe and discriminate between different internal states. For example, it could be envisaged that different states of sadness, emptiness, gloominess, tension, irritability, etc. could all be interpreted as feelings of depression associated perhaps with loss of interest and enjoyment. In turn, these could lead clinicians to infer the presence of depressive disorders. Nevertheless, it may be that these differences and other qualitative differences in internal states may, in fact, be real differences, in the sense that they represent different (and significant) internal changes. Similarities are thus invoked on account of the way in which such changes are interpreted and articulated. In terms of epistemic justification for the validity of psychiatric data in this situation, there are, therefore, the same general problems as mentioned before in that the factors important in determining such interpretation and articulation will need to be understood. Firstly, it may be that differences may exist between individuals in terms of sensitivity to changes in internal states. Some individuals may be able to discriminate between more subtle differences than others. Secondly, even if all could discriminate to the same level, then limits to interpretation may be imposed by language itself and the linguistic categories available to the individual. Again, it is only by determining and clarifying such factors that the validity of the psychiatric data and the neuropsychiatric relationship itself can be improved.

Observational Origin

Similarity can also be generated at the level at which psychiatric data are being elicited and captured by the clinician. In other words, there may be differences in clinical symptoms, signs and behaviours that are simply not being determined by the clinician. For example, patients may be manifesting and/or expressing symptoms and behaviours that are suggestive of a depressive disorder and hence interpreted as such by the clinician, whereas in fact the clinical states are similar only superficially. Epistemic validity of psychiatric data here is dependent not only on the previously discussed problems of validity and reliability in general but on the specific factors determining similarity. The latter were raised earlier in the discussion concerning the notion of properties both essential and accidental and will not be explored further. The main point, however, is that, as elsewhere, such factors remain largely unresolved and hence again contribute to the weakness of the validity of the psychiatric data. Practical implications follow from this since, in theory, developing finer phenomenological discriminators would help to distinguish between mimics and behavioural phenocopies arising at this level. Thus, in the example above, applying finer discriminators to the patient with a behavioural phenocopy of depression might help distinguish those clinical states likely to benefit from antidepressant medication from those needing other forms of management.

In summary, psychiatric symptoms, signs and behaviours are data of a different sort to those captured in the physical/biological sciences and, as such, present specific problems in terms of their epistemic validity that carry important implications for both the clinical and research aspects of neuropsychiatry. Given the increasing technical sophistication of techniques designed to capture infinitesimal neurological lesions or brain dysfunction, the aim in this section has been to focus on the other aspect of neuropsychiatric data collection, namely psychiatric data. It is evident that the latter, in comparison, remain relatively neglected as objects of research, and only some issues relating to their epistemic justification as data have been raised here. Nevertheless, in the context of biological psychiatry, and the neuropsychiatric relationship in particular, further work on such issues relating to psychiatric data will be crucial.

Data Processing in Biological Psychiatry

The section on data capture dealt with threats to the validity of the information on which biological psychiatry bases its conclusions. This section deals with problems affecting data processing. The availability of canned statistical packages has changed the way in which biological psychiatrists conceive (or rather do not conceive) of the meaning and conceptual basis of statistical analysis. The old art of preparatory thinking (calculations in the pre-computer age were labour intensive[34]) has been replaced by a high-speed, magical processing that in milliseconds flashes on the screen more data than the operator can ever handle. Far from releasing time for the understanding of the concepts and limitations of statistical data processing, computers have just created a mirage of objectivity. The conceptual complexities of biological psychiatry require this bewitchment to be broken. The best research starts at the level of concepts.

The slowness of progress in biological psychiatry research can be attributed to difficult subject matter, bad or inappropriate technology, lack of ideas, and excessive reliance on the epistemic power of statistical analysis. This section will focus on the latter.

The best way to understand issues concerning epistemic power (here, 'epistemic' means capacity to gain, construct, generate knowledge) is through an example: is there any epistemic difference between the claim by a biological psychiatrist that hallucinatory voice x correlates with a hot spot z in Broca's area and the claim by a physical anthropologist that in humans, there is a correlation between size and weight? Unpacking this question should illuminate the (epistemic) problems confronted by biological psychiatry. Before doing so, three issues need to be discussed: representativeness and proxying (as in proxy variables); correlations (as in statistical correlation); and generalizability (as in whether statistical techniques can be used in fields other than those in which they were originally created).

Representativeness and Proxying

It is a common assumption that the world is a complete and stable system populated by fixed objects, facts and events all regulated by the same laws of nature, and that only experimental science can identify and harness these facts for the good of humanity. The furniture of the world is organized in sets sharing similar essential and accidental features (natural kinds) that can be represented (without residuum) and measured by man-chosen variables. Members of a natural kind are so similar (except for some accidental features) that to know them all it is enough to measure some. It is, however, important that the sample selected

[34] For example, when done by hand calculator, each factor analysis might take up to one hour. This encouraged reflection on its appropriateness to the project in hand, on the variables to be included, on the type of rotation, etc. It is unclear whether similar thinking goes on in the computer age.

is representative of the group, i.e. within respective value ranges, sample and universe should share essential features.

In real life, things are not as cosy as this. There is little evidence that the world is a stable system, or that its furniture is fixed, or that the laws of nature apply in all instances. The same probabilistic notions that encouraged the development of statistics also influenced twentieth-century physics, and natural laws (and later biology) are now understood as statements of probability (Gigerenzer *et al.*, 1989). To biological psychiatrists working in the no man's land between the organic and the psychological, these new views are of importance; hence, their main worry is not sample representativeness (as there are conventional techniques to resolve such) but what we call here 'object representativeness'.[35] This refers to the nature of the relationship between the object and its qualities, and can be divided into ontological and conceptual representativeness and proxying. It should be remembered that representativeness (of whatever type) becomes an issue only when phenomena are studied scientifically with the intention of drawing generalizable conclusions.

Ontological Representativeness

This refers to the nature of the relationship between the object of inquiry and its features (essential and accidental). Whether a diamond, a dog, a virtue or a mental disorder, features must contribute to the ontology (existence) of the objects they characterize. The issue of whether an object is just the collection of its features or a substance from which all features hang is not relevant to ontological representativeness. For when it comes to diamonds or dogs (and exemplars of other natural kinds), essential features must still contribute to the objects' ontology, ideally without leaving a residuum (unless there are unreported features); the point being that the object's total ontology would be diminished if one feature were to be taken away. It remains unknown whether features are aliquots, i.e. contribute the same amount of ontology.

Things become interesting when the same question is asked of objects such as a virtue (justice, prudence, temperance, etc.) or a mental disorder (say, schizophrenia) that are not natural kinds but constructs, i.e. their ontology is dependent fully on a bet (prediction) made by a person in times past (say, Kraepelin or Bleuler) that features x_1 to x_n (which was all that was available to them and is available now) define an object s as a firm candidate for being a natural kind. In this regard, pathognomonic would name features that contribute the most to the ontology of their object. Given the way mental symptoms and disorders have been constructed historically, it is unlikely that even the pathognomonic features themselves are ontologically or informationally homogeneous (i.e. aliquots).[36]

But then who decides for how long can the bet be allowed to go on? Should that be a scientific, social or economic decision? At what stage should it be said that the people who placed it have lost in that all they produced was an empty construct, a mirage? This does not mean to say that the complaints of the many people who have been told they suffer from schizophrenia are the less real or devastating. It means that those who put together the earlier cluster of features wrongly selected, ontologically privileged, interpreted and explained some of the complaints (features) uttered by those patients. In doing, so they created a malformed concept,[37] a concept that nature, after a century of persistent interrogation, knows nothing about.[38]

It could be argued that it is nonsense to claim that schizophrenia is like a diamond or a dog or a virtue in that the very definition of disease entails that it is always expressed through someone's body and/or mind. The problem of ontological representativeness becomes more complicated in this case, for now we have object x and its x_n features (i.e. schizophrenia as an abstract disease) and object y and y_n features and object z and z_n features (y and z are, in fact, two people suffering from schizophrenia). The question here is whether the way in which the disease expresses itself through the brain resources of Messrs Y and Z will distort the distribution or pattern of ontological representativeness suggested by the abstract definition. The answer is that it should, for in each individual, personalized formatting activity will take place that would cause major distortions in the expected patterns, particularly in relation to symptoms that are (1) idiosyncratic re-elaboration and reformatting of primary experiences (which makes them escape the control of whatever lesion underlies the disease), and (2) symptoms that are atavic behaviours written into the brain by evolution and hence nonspecific, and (if we are to believe Hughlings Jackson) are regularly 'released' by the lesion to the point that some believe that they are caused by it.[39] It can be concluded that disease features relate to bearers in different ways (have different ontological representativeness), and hence the choice of a feature or features to establish correlations with other variables poses interesting problems. These cannot be handled statistically, for the values for ontological representativeness are unknown.

[35] It is important to distinguish between sample, design (ecological) and object representativeness. *Sample representativeness* refers to the assumption that the objects selected for study (the sample) show the same distribution of qualities as the parent universe. Generalizability is warranted by the degree of sameness. *Design* or *ecological representativeness* concerns the view that sampling theory must also be applied to the objects or, more generally, the stimulus or input or environmental conditions of experiments. Because subjects live in a probabilistic world, their responses to tests and stimuli will be tentative in the sense that they never have enough information or training to respond in the deterministic way that the theory dominant in a particular area will predict. In consequence, responses will also be probabilistic and often biased by cultural learning. Design representativeness relates to ecological validity. *Object representativeness* concerns the issue discussed in this section, namely how well the variable represents the object under study.

[36] We have studied symptom heterogeneity elsewhere, but for the needs of this chapter suffice it to say that mental symptoms are constructs exhibiting different inner structure, semantic load, and relationship to the brain signal that putatively originates them. These dissimilarities result from the fact that they are constructed by different mechanisms that provide them with their specific form and content. Structurally, there is very little similarity between a hallucination, delusion, sadness, fear, etc. The real difference is determined not by content or brain site but by the formatting imposed upon the symptom at the moment of its formation (Marková and Berrios, 1995a).

[37] Conceptual malformation results from semantic underspecification, poor biological anchoring, negative definition, conceptual parasitism, over-contextualization, porous boundaries, anomalous linguistic practice, etc. Malformed concepts can be identified, understood and corrected only by historical analysis (not empirical research). There is nothing terrible about psychiatry creating malformed concepts. It is in the nature of the descriptive and explanatory enterprise that this happens. What is bad is that biological psychiatry is not aware of malformed concepts and hence has no mechanism to deal with them.

[38] The problem is that since no real neurobiological marker has ever been discovered, samples of schizophrenic cases are selected in terms of conventional feature arrays, which seriously distorts the interrogation of nature exercise, e.g. if pancreatic weight were to be an important marker, how could that be discovered if that information is not collected in the first place? For a full discussion of these issues in relation to schizophrenia, see Berrios, 1995; Berrios, 2000b.

[39] If frequent (say, like auditory hallucinations), dormant features may be taken to be pathognomonic in spite of the fact that they are related to the disease neither structurally nor aetiologically. The fact that they often they show a high correlation with diagnosis is a good example of the unreliability of correlations.

Conceptual Representativeness

Given that we do not know about the ontological import of symptoms (features) in relation to specific bearers, choosing those with adequate representativeness (say, for the creation of a rating instrument or a diagnostic tick list) becomes difficult. Conceptual representativeness refers to the relationship between the chosen features and the expression of the disease (i.e. how a subset of features co-varies with a vectorial representation of the disease).[40]

Now, a statistical solution (such as 'random' selection) will not do, for disease features are structurally and empirically different from one another.[41] This is certainly the case intra-individually and some may want to argue that the same holds across individuals. For example, let us say that disease d as specified by DSM-IV consists of criteria c_1 to c_5. Although criteria are often made to include more than one feature of the disease, we shall assume that there is a $1:1$ correspondence between criterion and feature. As a rule, c_1 to c_5 are considered as different from each other in all respects (phenomenology and structure), but the manual does not specify their representational or epistemic validity. Previously, this notion was also conveyed by categories such as primary/secondary, pathognomonic/accidental, etc., but for some reason symptom hierarchies have gone out of fashion.

However, it is dangerous to assume that all criteria (features) have the same representational validity (vis-à-vis the disease) since little is known about the structure of the concept of mental disorder in general and of schizophrenia (or any other disorder) in particular. Indeed, it is even unclear whether it makes sense to claim that all mental disorders share a similar structure or, as in the case of mental symptoms, each group (psychoses, neuroses, personality disorders, etc.) will have its own internal structure, which would have different implications for the way in which features relate to each disease. At the moment, features are chosen in terms of criteria, such as ease to measure, availability, saliency, etc., but not in terms of their conceptual representativeness.

Proxying

Proxying is a form of conceptual representativeness. A proxy variable is defined as 'a variable[42] used as an *indirect measure* of another variable when that second variable is difficult to measure or to directly observe. For example, the frequency of abuse of street drugs is difficult to measure but it can be studied through the proxy variable of hospitalizations for drug overdose' (Reber, 1995). Proxying is often used in biological psychiatry, and hence it is important to know how it works.

The practice of proxying is based on the assumptions that: (1) variables can represent other variables (where 'represent' seems to mean co-vary) and (2) sensible or plausible proxy variable selection is possible. The first claim can be understood as meaning that variable p (proxy) co-varies with variable x (proxied), or that p and x co-vary with variable z (although not necessarily with each other). In order for proxying to be made to work, the biological psychiatrist needs a model that embraces p, x and z (otherwise the exercise become nonsensical). The assumption that sensible or plausible proxy variable selection is possible leads to the same conclusion, namely that selection can be justified only on the basis of a model. Only on the basis of the latter could finding a significant co-variance be reasonably taken to be a form of ascertainment.

Ex definitione, a proxy variable is further removed from the object of inquiry than the proxied one. This means that it is less under its ontological and epistemological control and that is more exposed to extraneous factors than the proxied variable. Because in most cases the distance between object and proxy variable is unknown, the latter is chosen on the basis not of science but of expediency. For example, the choice of the proxy variable 'hospitalizations for drug overdoses' to measure levels of street drug use or of using 'changes in blood flow' for changes in brain activity (and thereby subjectivity) may not sound extremely plausible, but it is all the biological psychiatrist has. However, in terms of verification (empirical ascertainableness), there is a difference between the two examples. Impractical as it may be, it should be possible to carry out a large epidemiological study to measure street drug usage and its co-variance with hospitalizations. However, no amount of empirical research will be able to ascertain the real nature of the co-variance between blood flow and subjective mental activity.[43] Unfortunately, there is no space in this chapter to develop our own model of proxying.

Correlation

One of the assumptions built into earlier metaphysical accounts of reality was that everything in the world was connected with everything else; hence the qualitative or philosophical concept of association (a mode of correlation) can be considered as ancient. The Newtonian and probabilistic revolutions introduced a new metaphor, to wit, that the book of nature is written in the code of mathematics, and that knowledge about objects and their laws and relationships has to be ascertained piecemeal.

The modern concept of correlation appeared in the biological thinking of the eighteenth century as a general explanation for observed (size) co-variations between organs in living beings. Such co-variance could be *physiological*, i.e. organs worked towards a common purpose (e.g. stomach, guts, liver, etc. in relation to digestion); *developmental*, i.e. organs grew in a synchronized fashion (e.g. secondary sexual organs) when serving a specific function; and *featural*, i.e. somatic characters developed in connection with each other. These three aspects of organ co-variance were conceptualized in terms of the old laws of association.[44] During the nineteenth century, featural co-variance became incorporated into evolutionary theory, for it was characters or traits (anatomical and physiological) that were believed to be the substratum for spontaneous variation and the target for selection.

Up to the 1870s (including the work of Darwin), the description of variation was qualitative, but Francis Galton (Darwin's cousin), Weldon, Edgeworth, Karl Pearson (Stigler, 1986) and others developed quantitative descriptions, which, in the event, became the

[40] Since biological psychiatrists believe that all mental disorders have a seat in the brain, then the presumption must be that such a feature will also correlate with a vectorial representation of a putative lesion.

[41] All diseases are constructs locked in a particular space and time. The small sample on the basis of which the original description was made tends to be fixed as prototypical. Ideology, opportunity and luck determine the success of the construct: constructors are always guided by expectations and anticipatory concepts, are opportunistic, and must have a modicum of luck.

[42] A variable is a mathematical symbol for a quantity that can take any value from a set of values called the range. A variable that can take any value between two given values is called continuous; otherwise it is discrete. A quantity that can take only one value is called a constant. This definition does not deal with the issue of how that quantity in question relates to its source, how it is measured, and how it is represented.

[43] The term qualia (singular = quale) has become standard amongst philosophers of mind to refer to properties of mental states and events that determine what it is like to have them, i.e. to subjective, experiential aspects. It is a synonym of phenomenal properties or qualitative features.

[44] According to associationism, certain principles of thought encourage human beings to assume a relationship between objects, e.g. when they are close together (contiguity), look the same (similarity) or are the opposite (contrast). This form of cognition and prediction is likely to have had its origin in the simple observation that members of the same genus have a family resemblance and march together. First formalized by Aristotle, the principles of association were to become psychological laws in Western thinking (Warren, 1921; Rapaport, 1974).

current concept of correlation. Pursuant to his overwhelming interest in eugenics, Galton wanted to show that 'good' features ran in 'good' (English) families and used for this purpose 'error theory' (originally developed to correct measurement errors in astronomy). He reintroduced the term 'correlation' to name a new idea, a numerical association (coefficient r) that was supposed to measure the strength of co-variances between objects or their properties.[45]

What data are needed to calculate r, say, between objects or features a and b? One measure of each will not do, for r needs profiles of variance, i.e. it needs to be based on a data series, i.e. a number of magnitudes, degrees of some attribute, or the like, viewed as capable of being enumerated in some sort of order. Series of pairs of data points can be obtained from either a group of people (cross-sectionally) or one individual (longitudinally). The former are single measures (from many individuals) of properties a and b at a given moment in time; the latter are repeated measures (from one individual) taken on a time series. The statistical treatment of both series may be very similar, but the conceptual treatment should be different as they are ontologically dissimilar. As far as the authors know, no mechanisms seem to exist to carry the ontological differences to the interpretation of r.

To make possible their inferences and generalizations, biological psychiatrists must make (often implicitly) a number of assumptions: (1) that if features a and b co-vary, they do so in the same way regardless of the source of the data; (2) that a and b change with time; (3) that the changes will be the same whether spontaneous or provoked by the researcher; (4) that when a and b change, they do so in a synchronized way such that the pattern of their relationship is preserved; (5) that r (as a quantity) holds a relationship of sorts with whatever is happening in the ontological substratum or natural phenomenon (this relationship has been called reflection, picture, mapping, etc.); and (6) that a very low r means that there is a weak or no relationship, and a very high r means that is a very strong relationship. All these are ontological assumptions for they pertain to the reality that the correlation is numerically meant to portray. At the moment, they remain implicit and often contradict each other. We hold that they must be connected with the scientific hypothesis that the researcher is trying to test.

In order to achieve this connection, an ontological model needs to be specified. Is the co-variance pattern the same when a and b: (1) hold a cause–effect relationship (the type of relationship that biological psychiatrists long for but that is, in fact, rarely available as the ontological basis for a correlation); (2) depend on a third factor (this type of ontological account is ambiguous as it can be interpreted as coincidental (trivial) or meaningful); (3) hold a pre-established harmony (this type of co-variance may give rise to a significant r but is empty of meaning); and (4) are truly coincidental?[46] Is the co-variance pattern the same if the changes in a and b are spontaneous or if they are provoked by the researcher?

Correlations are one of the most common forms of statistical analysis used by biological psychiatrists as they search for cause–effect relationships. Since all the number crunching is now undertaken by canned computer programs, many are no longer aware of the operations performed on the data series (or of the data themselves). This can be excused as division of labour between man and computer; less excusable, however, is ignorance of the issues discussed in this section, namely that correlations are patterns of thought based on forms of reality (ontological models) assumed to behave according to specific assumptions. These patterns must dovetail with the general model controlling the area of research in hand (whether depression, brain receptors, PET scan of verbal hallucinations, neuropathology of schizophrenia, etc.). In other words, this knowledge is essential to the biological psychiatrist both in the context of discovery (hypothesis making) and in the context of justification (relating 'evidence' to the logic of the model). Without this knowledge, the scientist is creating empty narratives, just like writing a bad novel guided by a recipe.

Generalizability

Does the fact that statistical techniques were developed to resolve problems generated by particular object arrays affect their applicability to the field of biological psychiatry constituted by different object arrays? For example, does the fact that R.A. Fisher[47] developed analysis of variance specifically to measure the effect of variable additivity on seed growth (*static* objects) mean that it should not be applied to *dynamic* arrays such as are obtained from the longitudinal measurement of human behaviour? Lest there is confusion, this problem is not about the normality of distribution of variables (and the remedial application of so-called parametric and nonparametric statistical techniques); it is about the relationship between the mapping capacities of mathematical models and the ontological features of an object array on the basis of which they were first developed. In the case of analysis of variance this means object array (seeds) → representations and concepts (choice of variables, definitions, measurements specific to seeds) → algorithms for analysis (analysis of variance). Has the original reality (seeds, static object) formatted the algorithm to the point that the latter cannot be used in object arrays other than the original or very similar one? The stock answer is that the whole point about algorithms is that they are formal and abstract and transferable and hence can be applied to any realm of reality.

To recapitulate, analysis of variance was developed to deal with static arrays of objects (seeds in bags) where simultaneity between variables data points (size, weight, etc.) was guaranteed. The question is whether analysis of variance can work equally well (here, 'work' means able to generate valid results rather than formally applied) when applied to data whose simultaneity cannot be guaranteed. Simultaneity cannot be guaranteed in much of the data collected in biological psychiatry. For example, patients may be tired, thus neuropsychological assessment needs to be undertaken on more than one occasion. (However, when data are entered in a two-dimensional matrix, the assumption is made that data are at least contemporaneous.) Surprisingly, there has been little work on the (temporal) definition of contemporaneity. Without good accounts (backed up by empirical data) of why correlations of variables measured a year apart are epistemically weaker than correlations of variables measured contemporaneously, it is difficult to carry out research in biological psychiatry.

Biological psychiatrists seem to take a common-sense view of this problem. In some situations, say PET scanning of

[45] Two objects or features are said to co-vary when changes in one 'map' changes in the other, so that the overall pattern of their relationship remains unchanged. Co-variation may be linear or sigmoid. In the former case, both variables change more or less at the same rate along the range of measurement; in the latter, they may change at a different rate. Correlations may be positive (both magnitudes increment at the unison) or negative (increases in one correspond to decreases in the either). Co-variation is a dynamic and relative concept that must be interpreted. This is done in terms of an ontology model; without the latter, correlations are empty.

[46] Claims that a coincidence has taken place (i.e. a concurrence of events or circumstances having no apparent causal connection) are meaningful only if made within a specific domain. For example, the claim 'Within this particular theory of schizophrenia, a significant correlation between intensity of formal thought disorder and length of left toe must be considered as coincidental' is intelligible; the same claim made in general about schizophrenia becomes nonsensical.

[47] When Fisher arrived at the agricultural station of Rothamsted, he found years of raw data waiting for analysis, mostly seed collections that had been cultivated in different environmental conditions (Box, 1978).

hallucinatory experiences, researchers are likely to want to define contemporaneity as simultaneity (i.e. require a time overlap between image capture and subjective experience). However, in many other studies, say analysis of association between plaque density and cognitive deficits in multiple sclerosis, researchers worry less about contemporaneity, i.e. about the event (memory impairment) occurring within a reasonable time of the development of certain other events (plaques) (Berrios and Quemada, 1990). Little is known about what 'reasonable' means in this context or about the way in which simultaneity and contemporaneity affect statistical significance, and the interpretation of results (on interpretation, see p. 20).

All this suggests that when applying statistical techniques to data in biological psychiatry, the researcher must take into account what could be called 'hermeneutics of analysis', namely the relationship between ecological time, semantics (pertaining to the data), and the way in which 'statistical significance' is interpreted. A biological psychiatrist genuinely keen to understand their subject matter should spend more time solving this crucial problem than worrying about formally meeting journal conventions.

Epistemic Value

Now to the question asked earlier as to whether there is any epistemic difference between the claim by a biological psychiatrist that hallucinatory voice x correlates with a hot spot z in Broca's area and the claim by a physical anthropologist that in humans there is a correlation between size and weight. The anthropologist's claim is redolent of the old eighteenth-century debate on correlations between physical features in biological entities; as we have seen above, the question first was given an ontological answer and later a probabilistic one. This happened because in the intervening century, probability and evolution theory had developed sufficiently to change the definitions of species and variability. The former concept reconstructed the idea of biological natural kind; the latter offered the substratum upon which evolution would operate its selection process. The epistemic value of the physical anthropologist's claim depends on three conditions: (1) compliance with representativeness and other sampling requirements; (2) theoretical warrant for their ontological claims; and (3) a factor x, namely a combination of acceptability by peer group, fashionableness and luck.[48] The anthropologist's claim seems to have met all three conditions.

Although it seems to have the same form as the anthropologist's, the claim by the biological psychiatrist differs in terms of epistemology and ontology. This means far more than the trite claim that all variables differ from all other variables. Therefore, its epistemic value depends on the resolution of these differences. The claim is very different for it reports a correlation between two *sui generis* concepts, hallucination and change in blood flow (deeply different from size and weight). Auditory hallucination is a construct based on the utterance by a subject that they are hearing voices when there is no one around. The issue is not whether the experience and phenomenon are real but that all the researcher has to go by is (1) an utterance and (2) two assumptions: that utterances always report images and that the quality of the reporting is not impaired by the presence of an acute psychotic state.

Although the two claims above are formally similar, only the hallucination/Broca's area claim is trapped in an unresolvable ambiguity for there is no way of telling whether the subject reports an auditory image or the belief that he is having one. From a therapeutic perspective, this may not matter, as the same treatment is offered. It is, however, crucially important to neuroimaging studies purporting to correlate the utterance with a marker of brain activity. The discourse of science may or may not be about truth, but it certainly is about coherence and reduction of ambiguity, and the one reported above cannot be overlooked for it affects all neuroimaging studies of mental symptoms. In practice, this means that it drastically reduces their epistemic value.

[48] In fashionable areas of research, e.g. neuroimaging, statistical rules may be violated on the excuse that it is early days, and that the mathematics will be sorted out eventually. Manuscripts violating statistical rules (e.g. with no correction for $>150\,000$ t tests) may therefore be published and their results accepted by the throng; this constitutes factor X.

Data Interpreting and Reporting in Biological Psychiatry

How does biological psychiatry fare in relation to data interpretation? It is often believed that in modern research, methodological and statistical algorithms can even indicate what conclusions to draw. This may be so superficially; in practice, however, efforts are made by biological psychiatrists to select and interpret. How do they fare? Due to the nature of its research questions and variables, problems that are standard in other fields of inquiry become magnified in biological psychiatry.

Interpretation and Evidence

To interpret means to expound the meaning of (something abstruse or mysterious); to render (words, writings, an author, etc.) clear or explicit; to elucidate and to explain. The sort of data obtained in biological research are of the type that require careful interpretation in all the senses listed above. An interesting feature of interpretations is that they cannot be said to be true, exact or apodictic; they can only be fair, imaginative, beautiful, audacious, speculative or silly.

Evidence

In biological psychiatry, much is made of the claim that clinical narratives and decision making are now based on evidence. Members of the public may rightly wonder as to how medical and psychiatric decisions were made before the concept of evidence was invented in the Oxford of the 1980s. In fact, the concept of evidence (Latin, *evidens*: clear, distinct, plain, visible[49]) is ancient, complex, kaleidoscopic and changeable, and there is no reason to believe that the current (blurred) meaning will last for long.

One problem with the historical analysis of evidence is that its origins go back to conceptual contexts and worlds that are no more. Another problem is that because it is an epistemological notion (one that talks about how we know the world), its study causes tautologies and self-reflexive contortions. The notion of evidence was first conceived in a world in which, in addition to sense perception, humans were believed to acquire knowledge through a variety of other mechanisms. Thus, between the fourth and second centuries BC, *enargeia* was used to mean 'clear, visible, datum of experience, manifest, in the mind's eye, evident, prominent, palpable, in bodily shape, brilliant, distinct, etc.' (Liddell and Scott, 1994). All these terms revolve around the claim that, whether through perception or directly coming into the mind, objects in the

[49] The impact of the term 'evidence', i.e. what moves people in the street when told there is evidence for x, relates to its metaphorical force — "clear, distinct, plain, visible"; to the fact that it relates to something that appears to the eye; to the suggestion that it points at something objective, tangible, visible, above board, public, pure, innocent and uncontaminated. Even when evidence is not made public, the point is to reassure people that it could. The personal vision of at least one witness (seeing is believing) is crucial here: 'Now Thomas (called Didymus), one of the Twelve, was not with the disciples when Jesus came. So the other disciples told him, "We have seen the Lord!" But he said to them, "Unless I see the nail marks in his hands and put my finger where the nails were, and put my hand into his side, I will not believe it"' (John 20 : 25).

world can be known with certainty, and that epistemological feature is called evidence. This also explains the ambiguous purview of the term, namely that it refers to the *feeling* of certainty itself (subjective evidence); to the *objects* that cause the feeling (objective evidence); and to its *inferential force* (epistemological and social authority) (more on this below). When at the end of the seventeenth century John Locke (1959) did away with all methods of knowledge other than experience via perception (fundamentally) and reflection (secondarily), the force of the mechanism of evidence became seriously undermined, particularly in regards to its direct access to the mind (bypassing perception). This attribute of non-perceptual directness survived for a time in the old notion of intuition and also in the later concept of apperception (Lange, 1900) but it died out with them. The legal usage remains fully extant, but it is no more than a generic name for objects or testimonies that can be used as a source of inferences.

Thus set asunder, the concept of evidence has had no safe port of call. Since the Cartesian challenge, perception has been considered as too fallible to provide the epistemological purchase needed by evidence; considering it as a derivative of certainty causes a tautological loop; basing it on the old concept of dictum by authority is not politically correct. The twentieth century cleverly linked evidence to science, and the tautological consequences of this link have been hidden from view: any narrative generated by science is now considered as sufficient epistemological purchase for evidence, and that is the way it has been defined. When someone asks what legitimates the scientific narratives themselves, the answer is that it is evident or that they are what evidence is about. Evidence can work only if it gets its force from somewhere. Originally it came from magic, and the notion then worked at its best; theologians took it over and the divine was a good replacement for magic. Nowadays, it is science. In all cases, it would seem that the concept of evidence fulfils more than a philosophical need, and that is why societies cling to it at all costs.

Evidence as a Social Practice

The claim that certain things are true can be understood in various ways, e.g. true now and ever (static), inching towards the truth (dynamic progressive). Whichever, it needs to be related to, or anchored in, the mind and behaviour of people. People have to be convinced that a is the case before they fully accept a. Evidence is the mechanism whereby this is achieved. In each historical period, those in control (whether religious, political or scientific leaders) have put together cognitive and emotional packages for the rest of us with the form, 'The world is like w and the reasons to believe it is so are contained in evidence e."

Thus, from the perspective of social order, evidence acts as the long arm of the abstract notion of certainty. In this sense, it can be defined as a social practice or mechanism designed to induce in the majority of the collective social an unconditional acceptance (both cognitive and emotional[50]) of certain world views (*Weltanschauungen*). The latter contain 'truths', whether religious, moral or scientific, that are convenient to social order. Evidence is offered as a proxy for truth since there is little choice for the social collective but to accept and internalize as certain a world view that has been chosen for them.[51] Who decides on what counts as evidence, and which world view is more convenient remains a problem. Earlier solutions revolved around authority and the creation of an elite of seers of evidence. However, the type of society that issued out of John Locke's 'democratic individualism' renders the above solutions incorrect, and new solutions had to be found that ideally were based on impersonal authority of statistics and database systems. These are sold as autonomous epistemological devices, untainted by human hand, and hence impartial and objective. By calling them 'the' evidence, these systems benefit from whatever is left of the force of a once magical term.

The Scaffolding of Evidence

Whether in theology, jurisprudence or epistemology, historical analysis shows that the concept of evidence has been shaped by specific metaphors and dichotomies. One distinction concerns the use of the term to refer to (1) facts or objects (e.g. knife, document, etc.) from which some other fact can be inferred, and (2) the testimony of persons as to the existence of facts. In the former case, there is a real object available to the collective; in the latter, there is a claim by a person who has seen or heard something. In the cultures of the West, the concept of evidence was first (fully) used by organized religion. For example, up to the medieval period, there was a debate in the Christian church as to whether the best grounds for believing in god was rationality (what nowadays would be called a cognitive understanding) or faith. Evidence played a role in the buttressing of rationality and was related to the contents of the Bible. Not always perspicuous, part of the latter often were given more than one interpretation. Who was to decide on the correct one became a problem, and one of the solutions was to create a cast of interpreters.[52]

By challenging the legitimacy of these interpreters, Luther created not only the need to redefine interpretation (hermeneutics) and evidence but also to rethink the relationship between god and men (Lohse, 1987). Another challenge to the old person-based concept of evidence came from John Locke at the end of the seventeenth century, particularly in his conception of the individual as a tabula rasa and the democratizing aspects of epistemology (object will reflect identically in the camera obscure of all minds). As against this, the Platonic influence on both Galileo and Newton generated the great hermetic metaphor of the seventeenth century, namely that evidence is encoded in nature, that nature needs to be interrogated or read, and that the code is mathematics. The probabilistic revolution that started at the end of the eighteenth century led in due course to the mathematization and relativization of the concept of scientific truth.

The history of evidence as a relative concept is a consequence of these changes. Evidence-based medicine is part of this social process, whereby the old authoritarian structures have been hidden behind the statistical techniques (such as meta-analysis), which are presented as impersonal cognitive devices able to extract meaningful information (sufficient for decision making) from large masses of data. They have now become the highest tribunal, and the evidence they issue is presented as the truth. Things, however, are not what they seem, for figures need *interpreting*, and this is still done in the way it always was: in smoke-filled rooms by men

[50] The role of emotions in the acceptance of evidence has not been well studied. This may be because, both in science and the law, evidence is made to appear as the *summum bonum* of objectivity and hence as denuded of emotions. In this regard, it is good to remember Wittgenstein's claim: 'Every explanation is indeed a hypothesis. But someone who is disquieted . . . will not find much help in a hypothetical explanation. The latter will not reassure' (Wittgenstein, 1989).

[51] The force of *videre* issues out of the original metaphor, 'Seeing with your own eyes is believing'. The form of the negotiating gambit is therefore, 'You must believe in this because it is based on evidence' (i.e. on something that you see or you could see). Buttressed by evidence, certainty generates authority. The concept of evidence is therefore central to social order and links stable views of the world with social control.

[52] The highest appeal station for the intermediaries was (and is) Rome. The doctrine of Papal Infallibility was confirmed by the First Vatican Council (1869–70). It was the role of the intermediaries that Luther was to challenge, and by doing so he not only redefined the concept of evidence within the confines of religion but created a new way in which man related to evidence and interpretation.

(and now some women) who decide in the end what is and what is not passed on to the throng.[53]

Interpretation

Central to the cognitive and emotional organization of the world is the concept of interpretation (Grondin, 1994). First a noun naming contradictions and options in everyday life, the Greek term was to become a verb to refer to the action of choosing between options. It then entered Aristotle's epistemology as the name for the link between the sign and the mental image or concept (what the Greek philosopher called 'affections of the mind'). Lastly, since choices and decisions were negotiated in words, interpretation became a term that was used mainly in the realm of language. *Hermeneia* and derivatives were rendered into Latin as 'interpretatione'; soon enough, this voice became incorporated into most European vernaculars.

To start with, the practical acts of interpretation (*exegesis*) concerned sacred and legal books; hermeneutics followed as its theoretical discipline. Within Christian philosophy, the problems posed by biblical interpretation were seen as resulting from the distance that existed between man and God; hence a decision had to be made about whether the Bible needed to be interpreted literally, doctrinally, philologically or historically. Luther challenged the Roman Catholic view that interpretations had to be left to the experts. In encouraging a personal reading of the scriptures, and a direct dialogue with the divinity, he opened up the need for a new subjective relationship between man and God and the concept of inner self, as a private space for such encounter emerged from these momentous changes. During the late eighteenth century, J.G. Herder (Pènisson, 1992) introduced the view that problems with understanding could also be due to different cultural purviews. During this period, interpretation is applied to literary works on the assumption that good hermeneutics (which included a study of the worlds, the history of the ideas) should lead to the one final meaning. Although the reader still defers to the author, the view is also introduced that the former may end up knowing more about the work than the latter. Schleiermacher broadens this definition further by making the interpretative task one coextensive with comprehensive psychology, and Dilthey (at the other end of the nineteenth century) sees hermeneutics as the central task of the human sciences (Grondin, 1994). During the twentieth century, the attention of hermeneutics shifts towards the role of the author and in due course conflates it with that of the reader. The idea that texts have only one meaning is surrendered, and the view that hermeneutics is about texts alone is replaced by the view that it is about the study of human communication.

All these changes are of momentous importance to psychiatry and the derivative biological psychiatry. For a time, the natural sciences presented themselves as immune to hermeneutics on the argument that objectivity was about eliminating subjectivity and rendering knowledge impersonal. These claims are groundless and part of the selling rhetoric of science. Like all other discourses, the scientific discourse is also open to interpretation and hermeneutics, and deferring to statistical significance and the results of laboratory experiments is a cop-out. Whilst that stance can be defended in physics or chemistry, it cannot in relation to psychiatry and biological psychiatry. These are best defined as interstitial disciplines, as doings that inhabit the borderlands between the natural and social sciences. Rhetorical reductionisms and naturalizations of the mind do away with the essence of psychiatry, which remains the specific understanding of human beings with a disorganized psychology. If modelling these processes may be tough, that is not an excuse for giving up and skirting around them (Dupré, 1993, 2001).

Interpretation is essential and pervasive in biological psychiatry, and its practitioners interpret away regardless of whether they have been trained to do so. This makes interpretation haphazard and uninformed. Interpretation occurs at the level of the contact with the patient, the level of the organization of the information, and the level of science making (both context of discovery and justification). The old positivistic view of science jars particularly in the context of biological psychiatry. There is little doubt that even neurobiological research would benefit from a hermeneutic approach to biological psychiatry.

Data Reporting: the Leniency of Fashion

According to the needs of the market place, interpretations of the same biological psychiatry data may suffer a different fate. When in fashion (a sure indicator of large investment by the industry), certain topics or techniques are supported by the major academic funding bodies in the land, and the unsaid message is that they are nearer the truth than older or tired approaches. In addition to an understandable rush by the young, there is an unseemly run by even older researchers who pursue money rather than lifelong research programmes. These fits and instabilities seriously affect long-term team approaches in biological psychiatry, and cherished hypotheses are indecently abandoned as soon as the industry withdraws investment. The current surrender of university research to such oscillations is to be regretted. The publishing industry joins in by showing surprising leniency towards fashionable topics, which are given a much easier passage than 'unfashionable' ones. This practice causes distortions in the public definition of biological psychiatry, and it should be asked seriously whether the privileging of certain topics because they are considered as good investment should not be considered as a form of scientific fraud. Biological psychiatrists should be aware of the way in which the vagaries of the market can destroy any chance of pursuing a balanced and coherent line of research.

CONCLUSIONS

Biological psychiatry is one of the incarnations of psychiatry. It is based on the foundational claims that all mental disorders are disorders of the brain, and that they should be explained by causes and not reasons. In various guises, these claims have individually been made since the seventeenth century, and by the nineteenth century they were defended by organized groups. A particularly keen version of biological psychiatry is predominant at the moment, and its success is due less to anything intrinsic or scientific but to the fact that its definition of mental illness fits in well with the ongoing philosophy of globalism. It is clear that if it was to be decided some time in the future that psychological approaches to mental illness are after all cheaper and a good investment, and that biological therapies are causing too much litigation, etc., universities, the government and the industry without any compunction would pull the epistemological rug from under the feet of biological psychiatry. Let us hope that it does not happen and that (a much improved form of) biological psychiatry goes on for a long time.

Predominance and fashion, however, should not make biological psychiatry immune to criticism. Its *sui generis* and interstitial nature, in fact, creates specific problems that sooner or later biological psychiatry will have to resolve by returning to the drawing board. It seems abundantly clear that the naturalization lark is not working. There are serious problems at the level of data

[53] It is not true to say, for example, that quangos like NICE (National Institute of Clinical Excellence, Great Britain) simply transmit to the throng the results of meta-analysis and literature searches. Whether overtly or covertly, they *interpret* evidence in terms of social, political and financial criteria. (NICE is a special health authority in charge of systematically appraising health interventions.)

capture, processing and interpreting, and all threaten its epistemic validity. The extraordinary complexity of mental disorder demands a humbler attitude on the part of everyone and the incorporation of a hermeneutic dimension to its study. The latter should not just be paid lip service, but should be integrated in whatever organic models are going to be pursued in the future. What biological psychiatry requires is a new model of symptom formation and a new language of description (Berrios, 1999). Thus equipped, biological psychiatry will be harder to research and practise, but it will also be far more likely to generate the kind of narrative that may capture the extraordinary phenomenon of mental disorder, and be more helpful to sufferers.

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II

Measurement Issues

P. Bech

FROM PSYCHOMETRICS TO CLINIMETRICS

It is generally accepted that psychology became a scientific discipline in Wundt's laboratory in Leipzig at the end of the nineteenth century, where measures such as reaction time were used to evaluate the effect of alcohol or opiates on mental states. These psychometric measurements were made under careful and skilled observations, analogous to biological measurements. However, Kraepelin, the founder of biological psychiatry, realized when working in Wundt's laboratory that these tests were too limited for the measurement of higher mental functions (Hippius *et al.*, 1987).

In his monograph on clinimetrics, Feinstein (1987) has argued that clinical data are distinctly human characteristics that differentiate people from animals, tissues or molecules. He continues:

> If we say that cardiac size became smaller, that cardiac rhythm became normal, and that certain enzyme values were lowered, the description could pertain to a rat, a dog, or a person. But if we say that chest pain disappeared, that the patient was able to return to work, and that the family was pleased, we have given a human account of human feelings and observations ...

Kraepelin used clinimetrics when he discriminated between bipolar disorder and schizophrenia in the clinical course of symptoms, or when he found that delusional depression was the extreme severity of depressive states.

The first psychometrist in this clinimetric sense was, however, Binet. He used the basic psychometric principles when he developed the Metrical Scale for Intelligence from 1902 to 1908. This scale has dominated the assessment of intelligence in the twentieth century. It is not a laboratory test but a bedside scale. The psychometric principle, consisting of internal validity, reliability and external validity, will be outlined in this chapter in correspondence with the work by Binet and his colleagues.

The statistical tests related to the psychometric triangle will then be reviewed. The unique scale of global improvement in clinimetrics will be treated separately. Thereafter, the concordance between the procedural algorithms in DSM-III (American Psychiatric Association, 1980) or DSM-IV (American Psychiatric Association, 1994) and the clinical symptom rating scales will be presented.

DSM-III is the first evidence-based diagnostic system for mental disorders. It has been argued that DSM-III is a revised version of Kraepelin's textbooks in clinical psychiatry in order to improve the interobserver reliability of the psychiatric diagnosis. However, the multiaxial approach in DSM-III and DSM-IV is the clinimetric approach, as outlined by Feinstein (1987), through its inclusion of measures of life events or stressors, personality traits and social functioning.

Among the modern philosophical approaches to psychometrics or clinimetrics on the one hand, and biometrics on the other hand, Davidson (1980, 1991) and Chomsky (see Lyons, 1991) should be mentioned. Thus, Davidson (1980) has emphasized the holistic character of the mental field by including the patient's beliefs and motives. This implies (Davidson, 1991) that psychometrics or clinimetrics have to adhere more closely than biometrics to the psychometric principle of internal validity (coherence), reliability (communication) and external validity (correspondence). Davidson (1980) concludes that because of this anomaly between psychometrics and biometrics, only small correlations between psychological and biological phenomena can emerge.

According to Chomsky, however, in the development of modern science, biometrics has been able, step by step, to expand the biological field to cover anything we understand, so that when ultimately beginning to understand the properties of the mind, we simply extend the concept of biometrics to cover these properties as well (Lyons, 1991). In this chapter, the concept of stress has been used as an example of Chomsky's approach. If cortisol is the most valid measure of chronic stress, then this hormone should be called the chronic distress hormone.

In this Chomsky approach to the problems of body and mind, it is held implicitly, however, that proper knowledge can be obtained only in the biological field. This chapter will show that the psychometric measurement of stressors, distress, disability and quality of life collects important clinimetric knowledge. The use of appropriate statistical tests associated with the psychometric principle is expected to increase the correlation between the clinical phenomena and the biological phenomena in biological psychiatry, as has already been the case in clinical neuropsychopharmacology.

THE PSYCHOMETRIC TRIANGLE

When a field of knowledge becomes understood more clearly, the need for quantification or measurement increases (Kuhn, 1961). When Binet (1902) developed his first intelligence tests, he used the psychometric triangle (Bech, 1996; Davidson, 1991) (Figure II.1), which includes (1) the principle of coherence (internal validity), (2) the principle of interpersonal communication (interobserver reliability), and (3) the principle of correspondence (external validity).

At the end of the nineteenth century, experimental psychology had developed using psychophysical methods, reaction time tests or questionnaires to measure intelligence (Galton, 1883; Wundt, 1888; Ribot, 1896). Binet had been working with these methods but was mostly familiar with Ribot's principle of coherence, pointing at the cumulative approach when measuring higher mental states. Thus, Ribot (1896) found that '... in comparing melancholy (clinical depression) with ordinary sadness, we may follow the cumulative method ...' Updating this statement, major depression contains in

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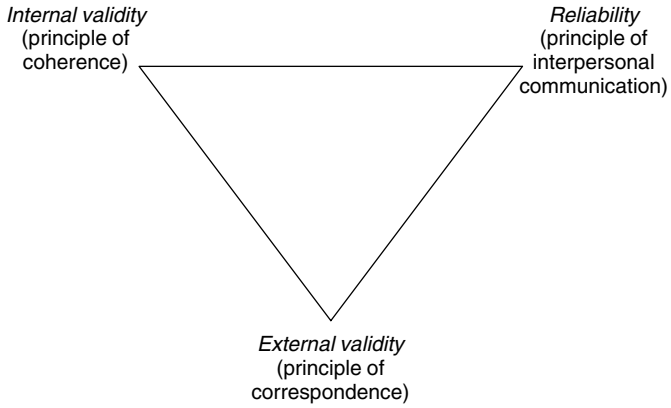

Figure II.1 The psychometric triangle (Bech, 1996)

it minor depression, not unlike the way in which a higher number contains only lower number.

Binet had no exact definition of intelligence, but he considered intelligence to include functions such as reasoning, judgement, comprehension, attention and memory. This was the content validity of intelligence. He then collected tests that reflected these functions. The principle of coherence was applied during his interviews with several children in the pilot studies (Binet and Simon, 1905). If a test did not distinguish between the brighter and the duller children, it was abandoned. In the final version of the Metrical Scale for Intelligence (Binet and Simon, 1905), the tests or subscales were arranged in order of increasing difficulty. The principle of cumulative validity (construct validity) implies that the bright person can solve the easy as well as the difficult tests, while the dull person can solve only the easy tests. Thus, each person passed as many tests or subscales as they were able to until they became too difficult for them to solve. How far the child Binet tested got down the list of subscales could then be compared with how far other children of the same age were able to go.

Each subscale or item in the Metrical Scale for Intelligence was scored dichotomously as a right or wrong response. Thus, the internal validity of Binet's intelligence scale includes both content validity (the components of intelligence) and the cumulative principle of coherence in which the total score is a sufficient statistic (construct validity) (see Figure II.2).

During Binet's pilot studies (Binet, 1902), it became important to take into consideration the bias of the teachers when these classified the children as dull, less dull, rather bright and bright. The same problem was operative for the interviewer when applying the intelligence scale. In psychometric terms, this problem is described as the interobserver reliability of a scale.

The external criteria for validity of the Metrical Scale for Intelligence were the association of the scores with age and school grade as well as with the teacher's assessment on a global scale from dull to bright. Binet assumed that within a single school grade, the eldest children would generally be less bright than the others because they had presumably been held back. Binet and Simon (1908) introduced the concept of mental age, which was determined as being the age at which the tested child would match the performance of the average child. Terman (1916) introduced the intelligence quotient in the Stanford–Binet version of the Metrical Scale for Intelligence.

In 1923, Boring concluded that intelligence is what is measured by intelligence tests such as the Metrical Scale for Intelligence (Boring, 1923). Later, Jensen (1969) concluded that the most important thing we know about intelligence is that it can be measured.

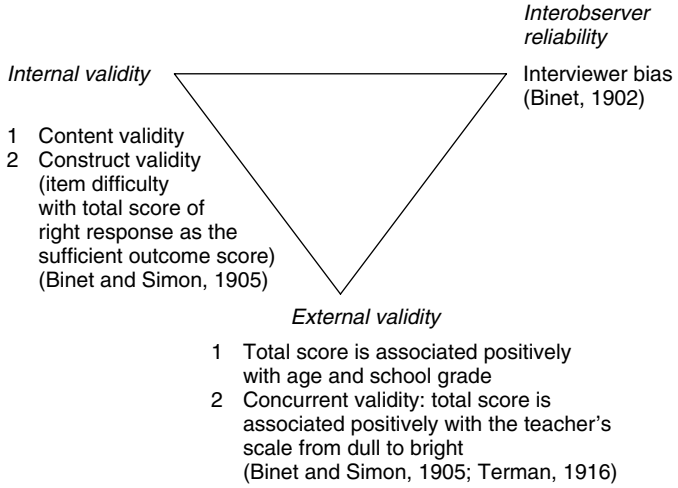

Figure II.2 The psychometric triangle: internal validity, reliability and external validity as used by Binet when constructing the Metrical Scale for Intelligence

STATISTICAL MODELS RELATED TO THE PSYCHOMETRIC TRIANGLE

Internal Validity

In the first major textbook of psychometric methods, Guilford (1936) concluded: '... No single event in the history of mental testing has proved to be of such momentous importance as Spearman's proposal of his famous two-factor theory (Spearman, 1904) ...' Most of the statistical tests in Guilford's first textbook edition of psychometric methods are concerned with correlation coefficients. In his measurement of intelligence, Spearman (1904) used factor analysis. However, it was Binet's psychometric triangle rather than Spearman's correlation analysis that finally emerged as the most valid measurement of intelligence. Thus, a theory of intelligence (content and construct validity) is the first principle of coherence, not a correlation analysis without a theory (explanatory factor analysis). With factor analysis, Spearman (1904, 1927) made valuable contributions to the structure of intelligence but not to its measurement. He identified a general factor that seems common to all types of intellectual activity, and he identified specific factors that seem unique to particular intellectual problems.

In the second edition of his textbook of psychometric methods, Guilford (1954) still considered Spearman's factor analysis (or other types of factor analyses) to be the most important psychometric model for measurements of mental states or activities. However, Mokken (1971) concluded in a comprehensive review of statistical tests in the measuring of mental states that explanatory factor analysis is a method of classification, not of measurement. The use of factor analysis for the classification of target syndromes in clinical psychiatry is described later. In the next section, the confirmatory factor analysis is also mentioned, here as a method of measurement.

Figure II.3 shows the statistical tests found most appropriate by Mokken (1971) for the measurement of the internal validity of a scale. The Guttman scale (Guttman, 1944) can be seen as the Binet approach to measuring the internal validity of intelligence by the cumulative principle. Essentially, as pointed out by Mokken, the Guttman approach is not a statistical approach because Guttman leaves no room for the estimation of errors in his analysis. The Guttman principle is a deterministic approach in which the items should be rank ordered, as in the Binet scale, in accordance with the

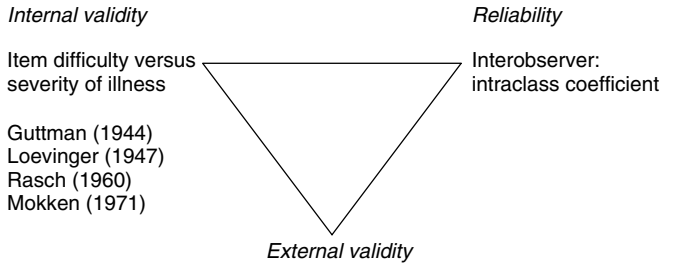

Figure II.3 Statistical models for testing a scale within the psychometric triangle based on the review by Mokken (1971) concerning internal validity

item difficulty (Guttman, 1968). The brightest person will have the right response to both the easiest items and the more difficult items, while the dull person will have a right response to only the least difficult items. Guttman's impact was to apply this scaling principle to other fields of mental states, which was Ribot's original idea.

An example of the Guttman deterministic principle is the scale for post-traumatic anxiety in soldiers who had been under fire (Suchman, 1950). It was found that soldiers with the highest level of post-traumatic anxiety answered yes to 'heart pounding', 'feeling sick at stomach', 'shaking all over', 'losing control of the bowels', and 'feeling of stiffness all over'. Those with mild post-traumatic anxiety experienced only 'heart pounding' and 'feeling sick at stomach'. Thus, the total score of these somatic anxiety items was an appropriate statistic. Suchman concluded that this strong cumulative coherence between the stress symptoms reflects an underlying physiological (hormonal) continuum.

Another example based on the Guttman model is the facet theory, which has been used by Steinmeyer and Möller (1992) for evaluation of the internal validity of the Hamilton Depression Scale. They identified six items as the depressive core items, which were similar to the six items identified by Bech *et al.* (1981) using the Rasch analysis.

Loevinger (1947, 1957) developed an index for errors in the rank order of item difficulty. Mokken (1971) has developed a coefficient of homogeneity for each of the items of a scale. Moreover, the Loevinger coefficient of homogeneity is part of the general Mokken analysis as the Loevinger index can be calculated as the weighted average of the individual Mokken item coefficients.

Figure II.4 shows the basic, cumulative principle in Mokken's coefficient of homogeneity. Mokken uses the term 'monotonous homogeneity' as an expression of the principle of cumulative coherence between items. In Figure II.4, the abscissa indicates the item difficulty (sigma). Four items are shown in Figure II.4: depressed mood, tiredness, guilt feelings and psychomotor retardation. These items are among the core items of major depression. Depressed mood has delta 1, tiredness delta 2, guilt feelings delta 3, and psychomotor retardation delta 4.

The ordinate in Figure II.4 is the probability of a positive score (phi). Hence, phi has a range from 0 (no probability) to 1 (maximal probability). Figure II.4 shows four curves that have the form of item characteristic curves, which is an essential feature of the Rasch analysis (Rasch, 1960) but not necessarily of the Mokken analysis (Mokken and Lewis, 1982). In Figure II.4, it is indicated how the item difficulty (sigma) is measured for phi values of 0.5. The four item curves in Figure II.4 are, thus, rather similar to dose–response curves in pharmacology, in which a phi value of 0.5 equals 50% response. A person who scores positively on psychomotor retardation will also score positively on the other three items. If not, the person has no depression but might have a neurological disorder.

Mokken used the term 'double monotony' to cover the situation in which the curves in Figure II.4 show the probability of a positive item score (phi) as a function that decreases with increasing values of the item difficulty (delta).

The Mokken analysis of a scale is based on the following:

1. The theoretical maximum of phi is 1.
2. The theoretical minimum of phi is 0.
3. It is possible to evaluate a scale as a whole (all items together as the Loevinger coefficient) as well as the scaleability of the individual items (Mokken's coefficient of homogeneity for each item).
4. Confidence intervals of the homogeneity coefficient can be shown.

Mokken has published the following interpretations of the Loevinger coefficients: 0.30 to 0.39 = a weak scale; 0.40 to 0.49 =

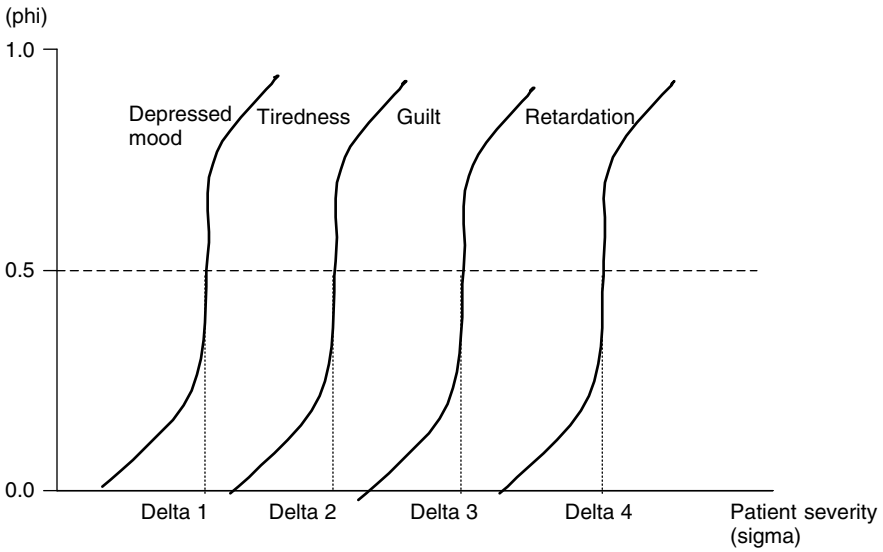

Figure II.4 Probability of positive symptom score on the individual item (phi) of a scale with only four items. The curves are rank ordered in such a way that a patient with scores on retardation also has positive scores on the other items. Sigma signifies the severity of the patient's depression

an acceptable scale; and 0.50 or higher = a strong scale. The most updated programme for the Mokken analysis has been published by Molemaar *et al.* (1994).

The Mokken analysis has been developed with reference to the Rasch analysis. As mentioned already, the Rasch analysis focuses on the item characteristic curves as an essential part of the analysis, in contrast to the Mokken analysis. These curves follow strictly specified S-shaped patterns, as shown in Figure II.4. They are monotonic. According to the Rasch model, the curves for all the items should all have the same shape. The curves are parallel, as are the four curves in Figure II.4, but they are placed as the various points along the abscissa. If the curves shown in Figure II.4 emerge when males are compared with females, younger people with elderly people, etc., then the scale is a measure of the underlying dimension (e.g. major depression). Within the Rasch model, the total score is a sufficient statistic. If a total score is not sufficient, this signifies that two identical scores from two different patients have different clinical meanings in that a very high rating on a few items can yield the same score as a moderate rating on many items.

Thus, compared with the Mokken analysis, the Rasch analysis has the advantage in that the model can be tested empirically by external criteria such as gender and age. The essential importance of the Rasch model compared with the Mokken analysis is that the Rasch analysis is population free, i.e. it is part of the model to evaluate randomly the distribution governing the selection of the population under examination as well as the items of the scale. Mokken also concluded that the superiority of Rasch over his own analysis is that it incorporates an evaluation in respect to the selection of both subjects and items.

Confirmatory factor analysis (Jöreskog, 1990; Jöreskog, 1993) has the advantage over exploratory factor analysis in that it is a direct part of the analysis to test for dimensionality, not just to identify factors. The confirmatory factor analysis, the facet analysis, the Mokken analysis and the Rasch analysis seem to have as a consequence that the higher the homogeneity, the lower the number of items (Bech *et al.*, 1981; European Rating Aggression Group, 1992; Steinmeyer and Möller, 1992; Aish and Wasserman, 2001). Thereby, these analyses converge at the magical number of 7 ± 2, which, as Miller (1956) showed, is the limit of human ratings of psychological dimensions.

In a recent psychometric re-examination of the citalopram dose–effect study by Feighner and Overø (1999), the six-item HAM-D subscale, which in contrast to the full Hamilton Depression Scale (HAM-D; Bech *et al.*, 1981) was shown to fulfil the Rasch analysis, has been found to have a much higher Loevinger coefficient (0.48) than the full HAM-D (Bech *et al.*, 2002). The same pattern was found for the Montgomery Åsberg Depression Scale (MADRS; Montgomery and Åsberg, 1979), in which the six-item subscale had a higher Loevinger coefficient than the full scale. The six MADRS items were 'apparent sadness', 'reported sadness', 'inner tension', 'lassitude', 'inability to feel' and 'pessimistic thoughts'.

Reliability

Another strong criterion in psychometrics is the correlation of a measurement with itself. If we measure body temperature in a healthy person over a few days, the correlation between the results will be close to 1.0. This kind of test–retest reliability is often referred to as the coefficient of stability. For a questionnaire, it is customary to show the coefficient of stability when the scale has been administered over a period of a week. However, in most textbooks on psychometrics, it is the rule to refer to another type of reliability, the split-half reliability coefficient, i.e. the correlation of half of the items with the other half of the items at the same administration (no time lag between two observations). The most frequently used reliability coefficient in this sense is the Cronbach coefficient alpha (Cronbach, 1951), which expresses the mean coefficient of all possible split-half coefficients (Nunnally and Bernstein, 1994). The split-half coefficient correlates the score of half of the items with the score of the other half of the items of a scale, typically odd items versus even items. This procedure is performed on the basis of a single administration of the scale to a sample of subjects.

The Cronbach coefficient alpha is often considered to be a test of internal validity because it seems to refer to the internal consistency of the scale. However, if the coefficient alpha is high, then all the items show the same item difficulty (sigma). In other words, all the items will have one single curve in common, for instance delta 2 in Figure II.4. Moreover, because the number of items is included in the formula resulting in the coefficient alpha, a high coefficient can also be an indicator of the length of a scale. This has been the reason why many scales measuring life events have included many items (Turner and Wheaton, 1995). A high Cronbach coefficient indicates not only that the scale includes many items but also that they can substitute for each other. Thus, the Beck Hopelessness Scale has been found by Beck *et al.* (1974) to have a Cronbach coefficient alpha of 0.93, which has been confirmed by Nimeus *et al.* (1997). However, Aish and Wasserman (2001) have shown by use of confirmatory factor analysis that the 20 items of the Beck Hopelessness Scale can be substituted by one single item: 'My future seems dark to me.' There is no reason to calculate Cronbach's coefficient alpha in order to express the reliability of a questionnaire or to express the internal consistency of a scale. The Rasch analysis and the confirmatory factor analysis are much better statistical analyses of internal validity.

For interview scales (e.g. the HAM-D), the intraclass coefficient is the most correct indicator for interobserver reliability. The intraclass coefficients correspond to degrees of interobserver agreement as: 0.0 to 0.20 = no agreement; 0.21 to 0.40 = very weak agreement; 0.41 to 0.60 = moderate but not acceptable agreement; 0.61 to 0.79 = acceptable agreement; and 0.80 to 1.00 = strong, nearly perfect agreement.

External Validity

There are two major psychometric aspects of external validity: responsiveness and sensitivity. The responsiveness of a scale refers to its ability to measure changes in the individual patient's symptomatology during a treatment period. Clinically, the index of validity in this situation is often the global severity assessment performed by an experienced psychiatrist. When Binet developed his intelligence scale, he used the global intelligence scale assessed by an experienced teacher as the index of validity.

In clinical psychopharmacology, the dose–response effect is often used as an index of responsiveness.

The most frequently used measure for global severity of clinical states is the Clinical Global Impression Scale (CGI) (Guy, 1976). The CGI is scored on a Likert scale from 1 (no clinical symptoms) to 7 (among the most extremely ill patients with the clinical syndrome under examination). In a study on the antimanic effect of a typical antipsychotic in severely manic patients, the Mania Scale (MAS) (Bech *et al.*, 1979) had a higher responsiveness than the CGI (Bech *et al.*, 2001a).

In dose–response studies with tricyclic antidepressants (clomopramine; DUAG, 1999) or serotonin selective reuptake inhibitors (e.g. sertraline; Fabre *et al.*, 1995), it was found that the depression factor of the HAM-D (Bech *et al.*, 1981) had a greater responsiveness than the total HAM-D scale. The HAM-D factor includes six items, which have been found to fulfil the Rasch analysis in contrast to the total HAM-D scale (Bech *et al.*, 1981).

The definition of the term 'sensitivity of a scale' is restricted to the ability of a scale to discriminate between active therapy

and placebo. The different statistical analyses of sensitivity for the meta-analysis of fluoxetine versus placebo have been compared by Bech *et al.* (2000). Among those statistics, the effect size seemed appropriate. This is defined as the difference between the mean of the baseline scores and the mean of the endpoint scores for patients in the active drug group and patients in the placebo group, divided by the pooled standard deviation. It was shown that the effect size for the depression factor of the HAM-D (Bech *et al.*, 1981) was higher than for the total HAM-D (Bech *et al.*, 2000).

In another fluoxetine analysis in which the HAM-D was compared with the MADRS, it was also shown that the HAM-D subscale was superior to the full HAM-D (Faries *et al.*, 2000). When fluoxetine was compared with placebo, the respective effect sizes were 0.32 (subscale) and 0.28 (full scale). A further analysis of Faries' study with the six-item MADRS subscale showed effect sizes of 0.22 (subscale) and 0.20 (full scale).

THE CLINIMETRIC INDEX OF GLOBAL IMPROVEMENT

The clinical global improvement scale is a measure of the overall therapeutic effects of an intervention during the treatment period. In principle, it is a measure of change during treatment in which the therapist makes an assessment of the reversibility of the mental state of the patient when retrospectively recalling the state before treatment and comparing it with the state after treatment. The change in before versus after treatment can be either an improvement or a worsening. However, often only the clinical improvement is measured by a scale in which 0 = unimproved or worse; 1 = improved slightly; 2 = improved much; and 3 = improved very much.

Feinstein (1987) has investigated this global clinical improvement scale in more detail and concludes that this kind of measurement is unique for psychometrics or clinimetrics, as it has no analogue in biometric measurements of change in the laboratory. It is a human rating of human changes of feelings or observations over time.

Cronholm *et al.* (1974) considered the global clinical improvement scale to be an idiographic measurement. Thus, the clinician can weight the individual clinical symptoms differently from patient to patient: '... in a way a new scale is constructed for each patient ...' (Cronholm *et al.*, 1974).

The interobserver reliability of the global clinical improvement scale has been investigated only rarely. It is, however, part of the Cronholm–Ottosson Depression Scale (Ottosson, 1960; Bech, 1991a). In a study by Isaksson *et al.* (1968), the interobserver reliability of the global improvement scale was only 0.56, while the Cronholm–Ottosson Depression Scale obtained a coefficient of 0.72.

However, in the original study by Ottosson, the global improvement scale was administered by experienced psychiatrists. When assessing the ability to discriminate between various dosages of antidepressive therapy, the global improvement scale was found to be superior to the Cronholm–Ottosson Depression Scale (in which the mean scores before and after treatment were used) (Ottosson, 1960). However, a re-examination of Ottosson's data showed that the Cronholm–Ottosson Depression Scale was superior to the global improvement scale when the 50% reduction of before-treatment scores was used (Bech, 1991a).

The use of clinical improvement scales has confirmed that the most commonly accepted analogue to 'much' or 'very much' improvement is a 50% reduction of baseline scores on symptom rating scales such as the HAM-D after around six weeks of antidepressive treatment (Bech, 1989).

When investigating the sensitivity of a modified version of the Cronholm–Ottosson Depression Scale, the MADRS, the global clinical improvement scale was used as the index of validity. The results showed that the MADRS was superior to the HAM-D. However, as pointed out by Faries *et al.* (2000), the treatments used in the Montgomery–Åsberg study were tricyclic antidepressants. In their meta-analysis of tricyclic antidepressants versus placebo trials, Faries *et al.* showed that the effect size was 0.23 for MADRS but only 0.15 for the HAM-D. However, a further analysis of the Faries study showed that the respective effect sizes were 0.25 for the MADRS subscale and 0.23 for the HAM-D subscale.

The most frequently used global improvement scale is the CGI. However, this scale also includes a severity scale, from 1 = not at all ill to 7 = among the most extremely ill patients within the diagnosis under consideration, e.g. depression or mania.

In a recent study of manic patients (Bech *et al.*, 2001b), the MAS (Bech *et al.*, 1979) was compared with Guy's (1976) version of severity in the CGI. In this study on severely manic patients treated with different antipsychotics, it was shown that the MAS was superior to the global severity scale in terms of responsiveness. Thus, after one week of therapy, 53% of the patients had a 50% reduction of baseline scores on the MAS, while only 30% had a 50% reduction of the baseline scores on the CGI. This difference was statistically significant.

PROCEDURAL ALGORITHMS VERSUS RATING SCALES

The first evidence-based diagnostic system for mental disorders was the *Diagnostic and Statistical Manual for Mental Disorders*, Third Edition (DSM-III) (American Psychiatric Association, 1980). The basic principle of the DSM-III, compared with the previous two editions (DSM-I; American Psychiatric Association, 1952; DSM-II; American Psychiatric Association, 1968) was the degree of interobserver reliability. It was demonstrated that in relation to the clinical target syndromes in psychiatry (schizophrenia, mania, depression, anxiety), DSM-III in contrast to DSM-II obtained an acceptable intraclass coefficient (Spitzer and Fleiss, 1974).

The innovations in DSM-III are the use of procedural algorithms when defining the various target syndromes as well as a multiaxial system in which concepts such as stress, stressors, distress and disability can be stipulated compartmentally.

This use of procedural algorithms in the definition of the symptomatic (distress) syndromes of the clinical target syndromes in psychiatry has been responsible for the rather high interobserver reliability. The internal validity of the clinical target syndromes in DSM-III is, however, not based on the cumulative principle. Thus, for the diagnosis of major depression in accordance to DSM-III, any five out of a total of nine symptoms must have been present. In DSM-IV (American Psychiatric Association, 1994), the algorithm has been modified so that at least one of depressed mood and lack of interest should be present. These two symptoms are the ones with the lowest delta number in the HAM-D (Bech *et al.*, 1981). This lack of the cumulative principle implies that the algorithm is resistant to qualification or measurement (Bech, 1991b; Quine, 1963). The algorithm is for the purpose of diagnosis. However, in the ICD-10 (World Health Organization, 1993), different contents of algorithms can categorize depression as mild, moderate or severe.

The items in the DSM-III system were selected according to two principles. On the one hand, they were to reflect the core symptoms based on shared phenomenology over time; on the other hand, they were to have discriminating power for the diagnosis they reflect. In this context, Frances *et al.* (1990), when editing DSM-IV, emphasized that '... unfortunately, items at the core of the definition are sometimes poor at discriminating the disorder, and items that are more discriminating may not be close to the core ...' However, factor analyses of comprehensive rating scales have identified most of the clinical target syndromes. Thus, on the

basis of factor analysis, using rating scales such as the Wittenborn Scale (Wittenborn, 1955), the Lorr Multidimensional Scale (Lorr, 1953), the Lorr Syndromes Scale (Lorr *et al.*, 1963), the Symptom Checklist (SCL; Parloff *et al.*, 1954), Frank (1975) concluded that the target syndromes of schizophrenia, mania, depression and anxiety (including phobia or obsessive–compulsive states) were valid at the symptom level. The same pattern was found by Pietzcker *et al.* (1983) when using the Arbeitsgemeinschaft für Methodik und Dokumentation in der Psychiatric (AMDP) Scale for factor analysis.

	How much of the time ...	All the time	Most of the time	Slightly more than half the time	Slightly less than half the time	Some of the time	At no time
1	**Have you felt low in spirits or sad?**	☐	☐	☐	☐	☐	☐
2	**Have you lost interest in your daily activities?**	☐	☐	☐	☐	☐	☐
3	**Have you felt lacking in energy and strength?**	☐	☐	☐	☐	☐	☐
4	**Have you felt less self-confident?**	☐	☐	☐	☐	☐	☐
5	**Have you had a bad conscience or feelings of guilt?**	☐	☐	☐	☐	☐	☐
6	**Have you felt that life wasn't worth living?**	☐	☐	☐	☐	☐	☐
7	**Have you had difficulty in concentrating, e.g. when reading the newspaper or watching television?**	☐	☐	☐	☐	☐	☐
8a	**Have you felt very restless?**	☐	☐	☐	☐	☐	☐
8b	**Have you felt subdued?**	☐	☐	☐	☐	☐	☐
9	**Have you had trouble sleeping at night?**	☐	☐	☐	☐	☐	☐
10a	**Have you suffered from reduced appetite?**	☐	☐	☐	☐	☐	☐
10b	**Have you suffered from increased appetite?**	☐	☐	☐	☐	☐	☐

Figure II.5 Major (ICD-10) Depression Inventory

These studies by Frank and Pietzcker *et al.* have demonstrated that factor analysis is a method of classification, as shown by Mokken (1971). The number of items needed to define the various target syndromes has not been established by factor analysis from a measurement point of view. The comprehensive rating scales used by Frank and Pietzcker *et al.* are checklists of symptoms rather than ratings. The number of items in the various target syndromes in DSM-IV is rather limited, following the concept of the magical number of 7 ± 2, as first introduced by Miller (1956).

Rating scales developed to measure the individual target syndromes and found valid according to a Mokken (1971) or Rasch (1960) analysis also have as their number of items 7 ± 2. The basic principle in this analysis of internal validity of measurement is that no single item of the scale in itself is sufficient, but that a limited number of items are sufficient if these items can be rank ordered according to the cumulative principle. It is not unimportant which of the items are used for a rating scale when the total score is a sufficient statistic in contrast to algorithms (Bech, 1991b).

Figure II.5 shows the Major Depression Inventory (Bech *et al.*, 2001c). This questionnaire is based on the DSM-IV symptoms for major depression. In order to include the ICD-10 system, the DSM-IV symptom of guilt feelings has been subdivided into two items covering low self-confidence (an individual item in ICD-10) as well as guilt feelings. Each item is scored in terms of frequency using the DSM-IV and ICD-10 timeframe of the last two weeks.

The Major Depression Inventory can be scored both as a rating scale in which the total score is a sufficient statistic and as a diagnostic scale using, for instance, the procedural algorithm for major depression according to DSM-IV. In both situations, items 8 and 10 are scored so that the highest values of 8a or 8b and 10a or 10b are used.

As a screening tool for DSM-IV major depression, the sensitivity and specificity of the Major Depression Inventory, when using the Schedule for Clinical Assessment in Neuropsychiatry (SCAN; World Health Organization, 1998) as the index of diagnostic validity, were 0.90 and 0.82, respectively (Bech *et al.*, 2001b). As a rating scale to measure the severity of depressive states, the Major Depression Inventory has been shown to fulfil the Rasch analysis, i.e. the total score is a sufficient statistic (Olsen *et al.*, 2002).

The ten items in the Major Depression Inventory seem to have a discriminating power for the diagnosis of major depression and to be close to the core symptoms of depression. Although Kessler and Mroczek (1995) have argued that measuring severity requires a scale that represents more aspects of the domain being measured than a diagnostic scale, empirical studies with such scales as the Major Depression Inventory seem to converge the number of items in severity scales and diagnostic scales (Bech, 1993).

THE MEASUREMENT OF CHRONIC STRESS

The main outcome of DSM-IV has been the procedural algorithms in axis 1, which classify the clinical target syndromes in psychiatry based on symptoms. The psychometric importance of these algorithms is their acceptable interobserver reliability rather than their validity. In DSM-IV, it is assumed that when assessing these target syndromes based on current symptoms, no bias from the other axes should be operating. In this sense, the assessment of the target syndromes of schizophrenia, mania, depression or anxiety should be atheoretical. One of the few syndromes in which a theory of aetiology is operating is the diagnosis of post-traumatic stress. A measurement of the severity of stressors is essential for this syndrome, because the concept of stress is response-based, as emphasized by Selye (1973) when defining chronic stress, which he was the first to do in medical literature.

Post-traumatic stress disorder is an acute stress reaction to a discrete, sudden trauma. Only a small percentage of people with post-traumatic stress disorder will develop a chronic stress reaction as defined by Selye (1973). In chronic stress, the more continuous stressors (e.g. daily hassles) are the ones that should be measured. Axis 4 in DSM-IV provides a measure of stressors in which both acute stressors and chronic stressors are considered.

The relationship between chronic stressors and personality traits has been shown most penetratingly by Eysenck and Eysenck (1976) in their questionnaire measuring neuroticism (or emotional instability) and extraversion (in stress reaction often referred to as Type A behaviour). Thus, the monotony of daily work is perceived as a stressful understimulation by people with high extraversion, while people with high neuroticism have a tendency to catastrophize demands, to get worked up over nothing, and to worry unnecessarily over things that might happen.

From a psychometric point of view, too many life event scales have been constructed with too many items based on the Cronbach coefficient alpha when showing the 'reliability' of the scale (Turner and Wheaton, 1995).

Selye's original model of stress placed stress as the biological response to stressors (Selye, 1936; Selye, 1973). Among the biological reactions, it was the hypothalamic-pituitary-adrenocortical axis (HPA) that Selye found most important, especially the measurement of cortisol. The subjective experience of the high production of cortisol was referred to by Selye as distress. The 'peripherical' experience of distress therefore included sweating, dry mouth, muscle weakness, heart pounding and tension. The 'central' experience was worrying, anxiety and depression.

The first distress scale was constructed by Parloff *et al.* (1954). This scale included 41 distress symptoms (Bech, 1993). The SCL has since been expanded to include 90 symptoms (SCL-90; Lipman, 1986). The SCL-90 questionnaire includes most of the DSM-IV symptoms of psychopathology.

The International Classification of Impairments, Disabilities and Handicaps (World Health Organization, 1980) is an important attempt to expand the Selye concept of chronic stress (Bech, 1998). 'Impairments' refer to the biological disease process in which cortisol should be considered as an important nonspecific response of the body to the demands of a chronic illness. 'Disabilities' refer to the clinical symptoms when measured not as psychopathological (axis 1) syndromes but as their impact on social performance in terms of behavioural restriction. The consequences of the social or behavioural reactions are then measured subjectively in health-related quality of life (Bech, 1998). Figure II.6 summarizes the five axes of DSM-IV when applied to the concept of chronic stress. Axis 3 (somatic or biological conditions) is shown to classify the biological state of stress according to Selye (1973). Axis 1 covers the clinical distress syndromes. Axis 4 covers both the acute and the chronic stressors. Axis 2 covers the most important personality traits, among these the Eysenck dimensions of neuroticism and extraversion. Finally, axis 5 covers the measurement problems of disability and the subjective health-related dimensions, which will be discussed in the next sections.

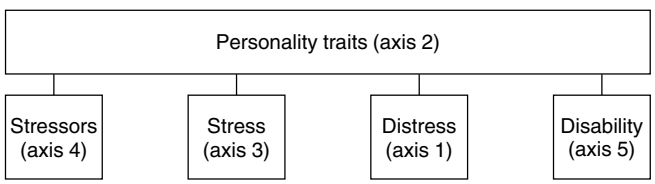

Figure II.6 The measurement of stress with reference to the five axes in DSM-IV

ILLNESS MEASURED IN UNITS OF DISABILITY

The most frequently used questionnaire for the measurement of disability is the Medical Outcomes Study SF-36 (short form, 36 items) questionnaire (Ware, 1996). This is a mixture of disability and subjective quality-of-life items.

Figure II.7 shows the content validity of SF-36. Apart from one item (measuring health transition), the items can be listed to cover eight subscales. The first measures the performance in physical functioning. The second subscale measures the limits in the kind of work or other activities that have been operating as a result of decreased physical health (role — physical). The third subscale measures bodily pain. The fourth measures self-reported health on a five-point scale from excellent to poor. The fifth subscale measures the limits in the kind of work or other activities that have been operating as a result of distress (role — emotional).

From a measurement point of view, the SF-36 has been an experiment of psychometric research when measuring health-related disability or functioning. The specifications of SF-36 in relationship to the psychometric triangle are shown in Figure II.8. Another advantage of SF-36 is the use of a 0-to-100 scale for all eight subscales, as shown in Figure II.9. The Danish norm values of SF-36 for people aged 25 years and 75 years are compared with a group of patients with major depression. As can be seen, only the subscale of emotional health has no age effect. The impact of major depression is pervasive in that this illness has an impact on all eight subscales in SF-36 (Wells *et al.*, 1992).

Finally, the SF-36 has had a major impact on the translation procedures involved when a scale is used outside the language in which it has been developed. Gandek and Ware (1998) have presented the scientific translation procedures for SF-36 when translated from American English into the various European languages.

	Components	
1	Physical functioning	(10 items)
2	Role – physical	(4 items)
3	Bodily pain	(2 items)
4	General health	(5 items)
5	Vitality	(4 items)
6	Social functioning	(2 items)
7	Emotional health	(5 items)
8	Role – emotional	(3 items)
	(Self-evaluated health transition)	(1 item)
	Total	36 items

Figure II.7 Content validity of the Medical Outcomes Study SF-36

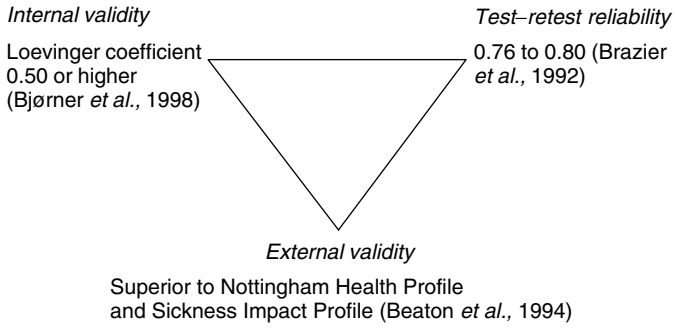

Figure II.8 The psychometric triangle for SF-36

Finally, the last item in SF-36, self-evaluated health transition, measures five levels on which the patients compare their current health with their health a year ago (from 'much better' to 'much worse'). This item is thus a clinimetric index of change. The validity of this item when compared with the General Health Rating Index is very high (Davies and Ware, 1981).

THE SUBJECTIVE QUALITY OF LIFE DIMENSIONS

Figure II.10 shows the eight components of SF-36 in relation to the Psychological General Well-Being (PGWB) questionnaire, which was developed by Dupuy (1984), although the most comprehensive study was made by Ware *et al.* (1979). The purely disability-oriented components of SF-36 are not included in the PGWB. Three of the more quality-of-life-related components (mental health, vitality, general health) are included in the PGWB. The two new components, positive well-being and self-control, are pure quality-of-life components.

In contrast to SF-36, the PGWB is commonly used as a rating scale in which the total score of the 22 items included in the questionnaire is a sufficient statistic. Higher scores mean a better quality of life.

Figure II.11 shows the properties of the PGWB within the psychometric triangle when used as a total score of the 22 items.

Many scales analogous to the PGWB have been constructed, e.g. the Hospital Anxiety Depression Scale (HADS) (Zigmond and Snaith, 1983) or the World Health Organization (WHO) 28 Well-Being Scale (Meadows and Bradley, 1990). Both the HADS and the WHO Well-Being Scale have subscales for anxiety and depression covering the mental health subscale of SF-36. However, these subscales are too crude to afford a valid measure of clinical depression.

Positive well-being is the most important subjective quality-of-life aspect. In the PGWB, a subscale of five items measuring positive well-being analogously to the WHO (five) Well-Being Scale has been extracted from the WHO 28 Well-Being Scale (Bech, 1999; Heun *et al.*, 1999). Like the SF-36 subscales, this scale is scored from 0 (worst thinkable well-being) to 100 (best thinkable well-being). Three of the items are found in the SF-36 mental health subscale. The remaining two items have recently been added to SF-36 in order to incorporate a pure positive well-being subscale. This SF-38 has been used in a Danish health status project in the normal population.

The first evaluation of the WHO (five) Well-Being Scale in patients with mental disorders has shown that the scale discriminates significantly between the five levels of the self-evaluated health item in SF-36 (Folker and Jensen, 2001). The level of 'much better' discriminated an average of 15 points, which is

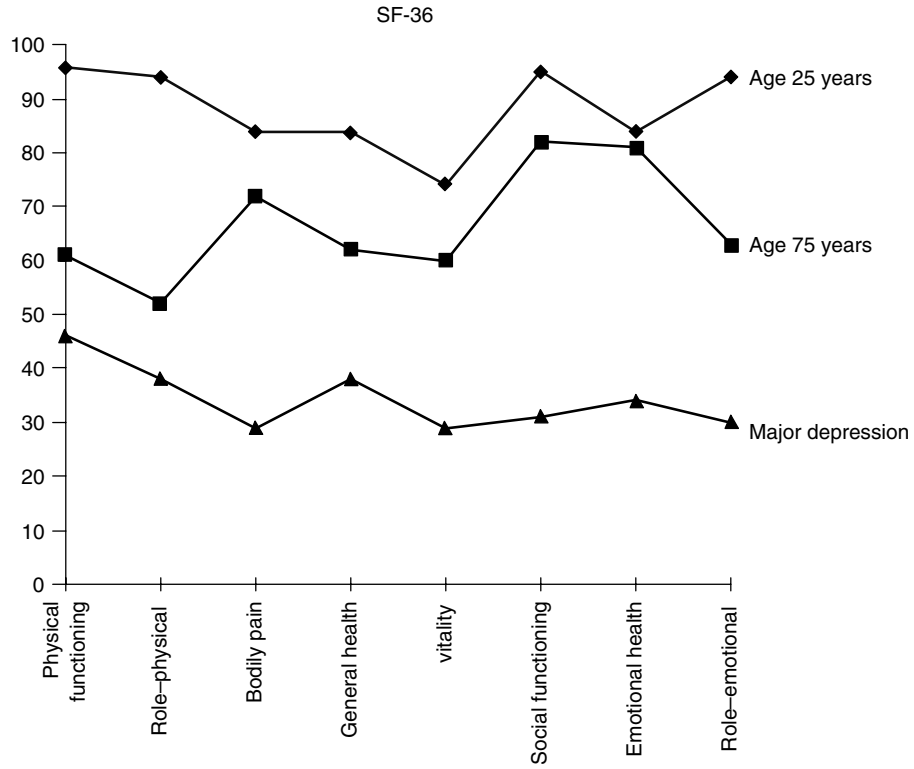

Figure II.9 Standardization of SF-36 in a Danish normal population including only people aged 25 years and 75 years. The profile of a patient with major depression is also included to show the importance of age when using the SF-36 as the goal of treatment

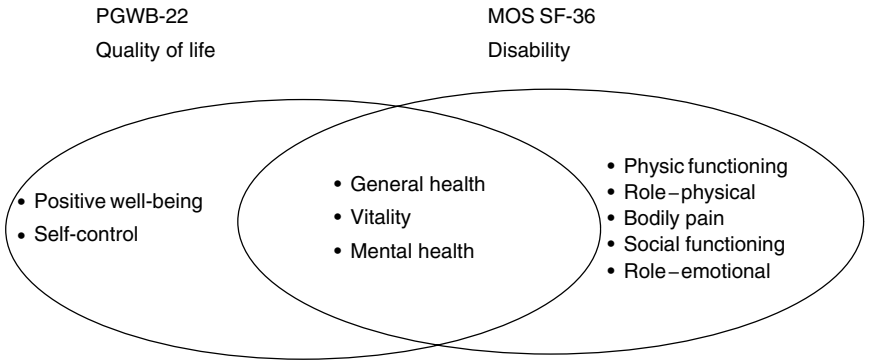

Figure II.10 Comparative content of disability and health-related quality of life scales

comparable to the findings reported by Ware (1996). In terms of WHO (five) Well-Being Scale scores, 'much better' corresponded to 78 and 'much worse' to 30. The level of 'no change' corresponded to a WHO (five) Well-Being Scale score of 45, which equals the average score of patients with major depression before treatment.

CONCLUSION

This chapter has dealt with the basic psychometric triangle of internal validity, reliability and external validity. The clinimetric field has included scales or questionnaires measuring clinical symptoms, life events, distress, disability and quality of life.

The statistical models or tests associated with the psychometric triangle have been described. The test for internal validity of a scale controls for the cumulative coherence among the individual items in the scale. These tests (e.g. Rasch analysis, Mokken analysis, confirmatory factor analysis) have shown that a relatively small number of items (7 ± 2) is often sufficient. Historically, however, the Cronbach coefficient alpha has been misused to imply that many more items are needed.

For questionnaires, the test for reliability is an analysis of test–retest stability as measured with a coefficient of stability. For interviewer-based scales, the intraclass coefficient is the most relevant.

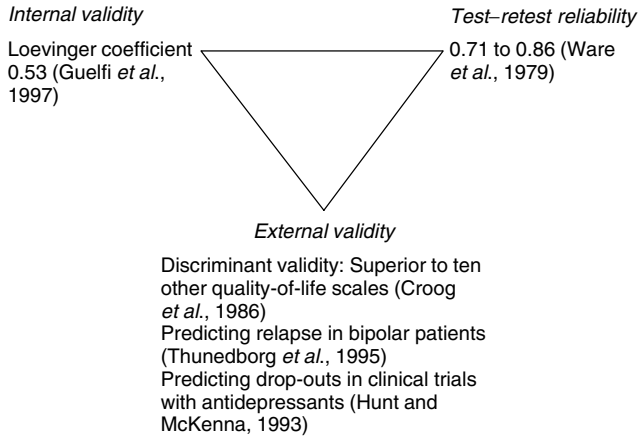

Figure II.11 The psychometric triangle for PGWB

The external validity of a scale is evaluated by responsiveness and sensitivity. While responsiveness refers to the ability of a scale to measure changes in the individual patient's symptomatology over time (analogous to the global clinical improvement scale), sensitivity is restricted to the ability of a scale to discriminate between active therapy and placebo.

The correlation between clinimetrics and biometrics has been discussed with reference to the measurement of stress. The psychometric triangle has also been found to apply when mental disorder or illness is measured in units of disability as well as in subjective quality-of-life units.

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III

Animal Models in Biological Psychiatry

Martin Sarter and John P. Bruno

INTRODUCTION

Historically, animal research has served as a main empirical approach to the development and testing of neuropsychopathological theories (e.g. Suomi *et al.*, 1975). Furthermore, animal models have played a major role in efforts to determine the behavioural and neuronal mechanisms underlying drug effects (e.g. Dews, 1958). Generating the perfect animal model does not represent a separate goal of research in biological psychiatry; rather, the model and its constant evolution represents an integral part of biological psychiatry (McKinney, 2001). Animal models of neuropsychiatric disorders range from attempts to produce complex models that incorporate both the neurobiological and behavioural/cognitive abnormalities underlying a disorder, to animal assay models that typically utilize the effects of relatively simple pharmacological or neuronal manipulations for the detection or screening of potentially effective drugs (see Figure 1 in Chapter XVI-1).

The discussion about the proper evaluation of animal models has been continuing (e.g. Willner, 1991; Geyer and Markou, 1995; Geyer and Markou, 2000; McKinney, 2001). This discussion centres around the relative significance of the different aspects of validity (face, predictive, construct validity). The literature generally, but not universally, agrees concerning the extremely limited significance of phenomenological similarities between the model and the human conditioned model (face validity). Furthermore, the importance of the demonstration of predictive validity, which typically involves the demonstration of effects of drugs on measures generated by the model that mirror and predict their efficacy in patients, is obvious and indisputable (Geyer and Markou, 2000). Approaches to demonstrate construct validity, and the actual meaning of this type of validity, have remained less clear, and often have been confused with other aspects of validity. In most general terms, construct validity refers to a theory-driven, experimental substantiation of the behavioural and/or neuronal components of the model. Similar to the constant development and revision of all theoretical aspects of neuropsychiatric disorders, the demonstration of construct validity represents a continuous task (Geyer and Markou, 2000). This chapter will stress that the demonstration of construct validity, despite its inherent complexities, represents the most important and a necessary component of the development and validation of an animal model in biological psychiatry (see also Willner *et al.*, 1991).

ANIMAL MODELS CONSTITUTE THEORIES ABOUT DISORDERS

The effects of chronic mild stress (CMS) on consummatory behaviour of rodents are postulated to model anhedonia, a core symptom of depression (e.g. Willner, 1997). The behavioural and cognitive effects of systemic muscarinic and nicotinic receptor blockade in humans and animals are claimed to model aspects of senile dementia (e.g. Parrott, 1992; Little *et al.*, 1998). The behavioural and neurochemical effects of repeated psychostimulant exposure are thought to model the positive symptoms of schizophrenia (e.g. Robinson and Becker, 1986). These few examples indicate what animal models really are — they are theories, and in fact complex ones, about the aetiology and neuronal mediation of psychiatric disorders (Willner, 1991). For example, the CMS model reflects a multitude of theories, including the relationship between stress and consummatory behaviour, the role of stress in depression, the reduction in saccharin intake as a symptom of anhedonia, and anhedonia as a core symptom of depression (Willner, 1997). Each aspect of this model involves complex and contested biopsychological questions (see also Hatcher *et al.*, 1997; Hagan and Hatcher, 1997). Likewise, the repeated psychostimulant exposure model theorizes that the neurochemical consequences of repeated psychostimulant exposure, particularly the sensitization of mesolimbic dopaminergic systems, and the associated behavioural effects, such as stereotypy, are related causally. Furthermore, this model is based on the theory that increases in mesolimbic dopaminergic transmission mediate the positive symptoms of schizophrenia, and so forth. Thus, data produced by animal models expand not only the theory of the disorder but also the theory underlying the model.

The recognition of animal models as theories is much more than just a philosophical exercise, as it has substantial implications for the use of animal models in biological psychiatry research and for the interpretation of data generated by animal models. In reality, an animal model often gains acceptance by virtue of its technical ease and its resulting popularity. Such models rapidly acquire the character of laboratory assays. Examples for such assays are the effects of muscarinic receptor antagonists on the performance of rodents in simple one-trial memory tasks as a model of amnesia or even senile dementia (e.g. Iversen, 1997), and the use of a spontaneously hypertensive rat strain as a model of attention deficit hyperactivity disorder (ADHD; e.g. Papa *et al.*, 1998). Obviously, the degree to which data generated by these assays can be interpreted in terms of amnesia or ADHD often involves sobering complexities (e.g. Sarter *et al.*, 1992a; Sarter *et al.*, 1992b; Sagvolden and Sergeant, 1998). Therefore, the typical question 'How good is this animal model?' can be answered only by addressing the soundness of the theory about the disorder and the related theories underlying the model in addition to other, more obvious questions. Likewise, data generated by a model need to be interpreted within the theoretical framework that justifies the model. As will be stressed below, these are the main reasons why construct validity represents the most important criterion among the several criteria available to determine the validity of an animal model.

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DETERMINING THE VALIDITY OF ANIMAL MODELS

The rationale underlying the demonstration of construct, predictive and face validity of animal models in psychiatry has been discussed extensively in the literature and does not need to be repeated here (see Willner, 1991 for a particularly noteworthy chapter; see also Weiss and Kilts, 1998 and Robbins, 1998). Instead, the present discussion will focus on paradigmatic issues and the clarification of the complexities in applying these validation criteria, and on typical pitfalls. Below, the limited significance of face validity, as opposed to construct validity, will be discussed. This will be followed by a paradigmatic analysis of animal models of schizophrenia that attempts to exemplify our main hypothesis that, in developing animal models, the demonstration of construct validity represents the most vital step.

Face Validity Rarely Matters

Face validity represents the most straightforward validation criterion, as it refers to the degree of phenomenological similarity between the model and the disorder to be modelled. It is also the most seductive aspect of validity, as an animal that, for example, does not move much or consumes little palatable food may be readily characterized as being depressed. Likewise, an animal traversing a pool using relatively irregular swimming patterns appears to lack memory for the position of the invisible platform. However, there are two main reasons why face validity represents the weakest of all validation criteria. First, and most obviously, the species-specific behavioural repertoire of laboratory animals, especially rodents, confounds such interpretations. For example, a stressed animal may consume less saccharin because it exhibits a general reduction in fluid intake while in fact maintaining its preference for saccharin (Hatcher *et al.*, 1997). Increased latencies of rats to find the platform in a water maze may reflect not so much loss of memory about its location but the confounding effects of 'amnesic' treatments on sensorimotor skills and exploratory habits (e.g. Whishaw, 1989).

Second, and more importantly, many cardinal symptoms of a psychiatric disorder, such as hallucinations or impairments in verbal recall, cannot be modelled directly in animals. However, core components of the (cognitive) theory describing the mechanisms mediating the development of such symptoms may be modelled. The prime example for this issue is the positive symptoms associated with schizophrenia. While there have been attempts to interpret stimulant-induced behaviour, such as stereotypy and locomotor hyperactivity, in terms of positive symptoms (Segal *et al.*, 1981), it is difficult to substantiate such speculations. Alternatively, contemporary theories concerning the attentional dysfunctions that give rise to positive symptoms provide the basis for animal models that focus on such attentional dysfunctions (e.g. Braff, 1993; Swerdlow and Geyer, 1998; Sarter and Bruno, 1999). The modelling of such attentional dysfunctions in animals may be unrelated phenomenologically to positive symptoms or the attentional impairments in schizophrenic patients. However, such models may represent core cognitive dysfunctions and, depending on the methods used to induce such dysfunctions in animals, may also represent the core neurobiological aspects of the disease that may indeed be sensitive to antipsychotic treatments (Sarter *et al.*, 2001; Sarter and Bruno, 1999). Thus, the importance of face validity in the animal modelling of neuropsychiatric disorders has been overestimated considerably.

The widespread overestimation of the importance of face validity is due in part to the dominating role of nosology in biological psychiatry. As clinicians focus on the classification and categorization of disorders in accordance with symptom clusters, such schemes emerge as important criteria for animal models. For example, current efforts to establish animal models that represent both the positive and negative symptoms of schizophrenia (e.g. Lahti *et al.*, 1999), with attentional impairments often being classified as a negative symptom, are nosology driven but may be less important in light of data and conceptualizations that postulate relationships between different symptom clusters, particularly between the cognitive impairments and psychosis (e.g. Strauss, 1993). The determination of the usefulness of animal models that follow clinical nosology almost by definition is restricted to considerations of face validity.

Lastly, the intermittently expressed demand to focus strictly on species-specific behaviours in animal models is based on the general idea that only naturalistic studies can inform properly about the behavioural or cognitive processes affected by neuronal manipulations (e.g. Gerlai and Clayton, 1999). Obviously, data from studies employing presumably more 'naturalistic' settings that exploit species-specific capabilities (e.g. the use of olfactory stimuli in rodents) do not generalize readily to other species, thus the demonstration of face validity would be very difficult. Although 'natural tasks' may be useful in identifying phenomena of interest, they often preclude the rigorous control and systematic manipulation of variables that are essential for meaningful investigations of behaviour. Consequently, naturalistic studies have only limited potential in identifying specific changes in cognitive or behavioural functions. In fact, animal models that use highly artificial, yet theoretically valid, tasks for the probing of, for example, cognitive functions in laboratory animals may be most effective in generating valid conclusions about the mechanisms mediating performance abnormalities (Sarter and Bernston, 1999). The demonstration of such mechanisms may be more important in terms of the validity of the model than the degree to which the actual behavioural measure shows face validity. Thus, animal models focusing on naturalistic settings and species-specific behaviour *per se* may be the least valid, or at least they are most difficult to validate.

Construct Validity is Necessary

Construct validity refers to a complex, multifaceted approach that in essence aims to map a theory about the biopsychological mechanisms of a human disorder on to a biopsychological theory of a particular animal behaviour. For example, the positive symptoms of schizophrenia are widely accepted to result from a hyperactive mesolimbic dopaminergic input system (see below). If an animal can be created that shows persistent dopaminergic hyperactivity, and if this hyperactivity is demonstrated to produce a particular behaviour, the underlying (cognitive) mechanisms of which can be related to the (cognitive) mechanisms mediating the development of positive symptoms in patients, then construct validity would be established (see below for additional necessary components of such a model).

Another important means to establish construct validity focuses on the effects of variations of theoretically or clinically important factors. If the model is valid, then the main dependent measure(s) of the model would be expected to vary as predicted. For example, the theory of sustained attention predicts that increases in the event rate will result in a steeper decrement in performance over time; if a task designed for the measurement of sustained attention in rodents is valid, then increases in the event rate would be expected to increase the slope of the decline in performance over blocks of trials (e.g. McGaughy and Sarter, 1995). In general, in order to establish construct validity of an animal model of neuropsychiatric disorders, the main neuroscientific, behavioural and cognitive points that constitute a theory of the condition modelled need to be connected with the equivalent points constituting the model.

An animal model may exhibit good face validity and solid predictive validity (e.g. by predicting efficacy of therapeutic agents), and researchers may be inclined to suggest that, in such cases, the demonstration of construct validity may be unnecessary (Geyer and

Markou, 2000). Moreover, it may be assumed that such models do not require an extensive experimental and theoretical analysis of, for example, the role of associational mechanisms mediating shock-induced suppression of spontaneous behavioural responses in animal models of anxiety. Efforts to model complex theoretical relationships between neurobiological and behavioural variables that are a core focus of construct validation have been suggested to not necessarily benefit the scientific usefulness of such a model. After all, one may argue that such a model predicts clinical drug efficacy and thus it does not matter whether it agrees with the relevant neurobiological and behavioural theories of the disorder or the behaviour modelled.

There are several animal models that match the scenario outlined above, and they seem to be favoured in pharmaceutical research settings. Among these models, those used to assess the anxiolytic effects of antianxiety drugs, mostly benzodiazepine receptor (BZR) agonists, represent prominent cases. As discussed by Sanger (1991), the models based on punishment-induced suppression of responses generally have good face validity, and their predictive validity is impressive, specifically concerning BZR agonists. While the degree to which such models assess aspects of pathological anxiety remains unsettled, this absence of construct validity apparently has not been of much concern, as the models 'worked'. With the discovery of non-BZR, clinically effective anxiolytic drugs (particularly buspirone) that have little effect in these traditional animal models, the limitations of using models without construct validity has been impressively revealed (Sanger, 1991; Rodgers, 1997). Thus, this example illustrates that models with face and predictive validity may 'work' but for the 'wrong' or at least largely unknown reasons. Moreover, such models are prone to produce false negatives, as their predictive validity may be limited to the class of compounds that originally supported the usefulness of the model. In any event, such a model *per se* does not provide the basis for the safe prediction that new groups of therapeutic compounds acting via different neuropharmacological and behavioural/cognitive mechanisms will be detected as such. Thus, the demonstration of construct validity does not represent a luxurious experimental activity but, differing with Geyer and Markou's (p. 16, 2000) conclusion that '... predictive validity is the only necessary and scientifically meaningful criterion ...', the focus on construct validity is, in fact, a necessary step for successfully validating an animal model. To this end, more recent efforts to develop new models for research in the field of anxiety have focused on construct validity by attempting to address the cognitive mechanisms mediating the biased processing of fear- and anxiety-related stimuli and contexts (e.g. Bernston *et al.*, 1998; Stowell *et al.*, 2000).

The perspectives discussed above differ quite radically from the conclusions drawn by Weiss and Kilts (1998). They argue that the modelling of symptoms, as opposed to theory-driven analysis, must remain the primary goal of animal modelling. Moreover, they suggest that theoretical validity represents a secondary, and possibly even unnecessary, component. The example discussed above, as well as the various animal assay models used traditionally in the field of schizophrenia (such as the catalepsy test or the conditioned avoidance response; see Weiss and Kilts, 1998), illustrate not only the practical limitations of such a perspective but also that the focus on face and predictive validity in certain fields may, in fact, have contributed to the paucity of theoretical development in those fields. This can be illustrated particularly well in the area of depression. While animal assay models, such as the behavioural despair test or the tail suspension test, have dominated animal research in this area, models that could potentially assist in developing contemporary hypotheses about the neuronal mechanisms mediating depression-associated cognitive processes have remained sparse (Healy, 1998).

A PARADIGMATIC ANALYSIS OF ANIMAL MODELS OF SCHIZOPHRENIA

The present knowledge concerning the aetiology, neurobiology and cognitive dysfunction of schizophrenia suggests that an optimally valid animal model of schizophrenia may incorporate the following perspectives and criteria (discussed below), all but the last of which relate to construct validity. This discussion is designed not to review comprehensively the evidence in support for the various components of the model, but to describe paradigmatically the construction of an animal model of schizophrenia and to exemplify further the relative unimportance of face validity compared with construct validity.

Developmental Dysmorphogenesis

Neuroanatomical and neuropsychological evidence has strongly supported the general hypothesis that abnormal brain development mediates the development of schizophrenia. Although the exact nature of such developmental dysmorphogenesis remains unclear, evidence suggests defective migration of neurons and resulting cytoarchitectural irregularities (e.g. Jones, 1997; Rajkowska *et al.*, 1998), abnormal interneuronal circuitry (Woo *et al.*, 1998), and cortical proapoptotic processes (Jarskog *et al.*, 2000). These abnormalities are generally thought to mediate the childhood neuropsychological precursor symptoms of schizophrenia (e.g. Cannon and Mednick, 1993; Dworkin *et al.*, 1993), and later to yield the mesolimbic dopaminergic hyperactivity hypothesized to be related directly to positive symptomatology (Laruelle, 2000; Lieberman *et al.*, 1997; Grace, 2000).

Although the importance of modelling the developmental component of schizophrenia has been widely appreciated (Weinberger and Lipska, 1995; Lipska and Weinberger, 2000), the range of manipulations and the relatively undeveloped underlying rationales reflect the absence of clear and prevailing hypotheses about the nature of the developmental dysmorphogenesis. As discussed extensively in Lipska and Weinberger (2000), such models investigated the effects of gestational malnutrition, prenatal exposure to influenza virus, Borna disease virus or lymphocytic choriomeningitis virus, prenatal exposure to X-ray irradiation or several toxins, including the antimitotic agent methylazoxymethanol (MAM), post-natal exposure to stress, and the effects of neonatal lesions. Although these manipulations generally disrupt the integrity of neuronal systems to a more global and profound extent than indicated by current neuropathological evidence from the brains of schizophrenic patients, and thus do not show good face validity in this respect, several of these manipulations are capable of producing secondary effects that are key to the disorder, including increased behavioural and neurochemical responsivity to the effects of psychostimulants (see below). Furthermore, some of the behavioural and neurochemical effects of these manipulations are attenuated by antipsychotic treatment. Thus, although the immediate neuronal effects of X-ray exposure or neonatal lesions may not show good face validity in terms of reflecting the dysmorphogenesis observed in the brains of schizophrenia, these manipulations may result in behavioural and neurochemical consequences that model important aspects of the disorder that are sensitive to antipsychotic treatment. Thus, the construct validity of such manipulations may be of sufficient quality to warrant further research using such models, despite their limited face validity.

The Role of Stress

The acute manifestation of the behavioural and cognitive correlates of the developmental dysmorphogenesis in schizophrenia often interacts with, or is even triggered by, stress (e.g. Norman and Malla, 1993). Based on the activation of dopaminergic systems

by stressors (e.g. King *et al.*, 1997; George *et al.*, 2000), stress has been speculated to tax developing prefrontal circuits, thereby revealing deficient prefrontal organization in patients, and thus triggering first episodes of schizophrenic symptoms and, subsequently, relapse (e.g. Weinberger, 1987).

Changes in the reactivity of the behavioural and neurochemical effects of stressors were demonstrated in various models, including neonatal hippocampal and prefrontal lesions. However, it is less clear whether the demonstration of stress-induced increases in nucleus accumbens (NAC) dopamine release in adult animals with neonatal hippocampal lesions depends on the time of the lesion, because the effects of stress in neonatally lesioned animals were rarely compared with those in animals with lesions produced in adulthood (Lillrank *et al.*, 1999; Brake *et al.*, 2000; but see Wood *et al.*, 1997). Furthermore, neonatal hippocampal lesions attenuate the reactivity of NAC dopamine to stress (Brake *et al.*, 1999; Lillrank *et al.*, 1999), while prefrontal lesions increase the effects of stress on NAC dopamine (Brake *et al.*, 2000). Based on the substantiated hypothesis about hyperdopaminergic functions in schizophrenia (see below), the effects of neonatal hippocampal lesions therefore may violate a main component of the construct validity of this model. Alternatively, a more precise determination of the developmental window during which such lesions need to be produced in order to result in increased behavioural and dopaminergic reactivity in adulthood (Wood *et al.*, 1997) may resolve this problem. Such a model, together with evidence from studies on the effects of prefrontal lesions, would support the suggestion that early loss of telencephalic input to the mesolimbic dopaminergic system in general yields increased behavioural and dopaminergic responses to stress and psychostimulants (Saunders *et al.*, 1998; Coutureau *et al.*, 2000).

The Role of Puberty

It is not clear whether puberty merely serves as a marker of the degree of general brain development required to permit the development of schizophrenic symptoms in interaction with the effects of stressors, or whether neuroendocrinological events associated with puberty interact directly and necessarily with the neuronal maturation of defective circuits in schizophrenia (e.g. Clark and Goldman-Rakic, 1989; Frazier *et al.*, 1997). In animals with neonatal hippocampal lesions or prefrontal lesions, sensorimotor gating and other behavioural deficits, and increased behavioural and dopaminergic reactivity to the effects of amphetamine, were demonstrated specifically at postpubescent time points (e.g. Lipska *et al.*, 1995; Wood *et al.*, 1997; LePen *et al.*, 2000; Flores *et al.*, 1996; but see Chambers *et al.*, 1996). At the very least, these findings relate to the typical time of onset of symptoms in patients and thus add face validity to these models. Puberty-associated cognitive maturation enables the perception and experience of stress, and thus puberty may minimally mark a developmental stage necessary for triggering first episodes. Alternatively, the puberty-defining increases in gonadal hormones and the associated increases in tropic hormones and their regulating hypothalamic factors may interact necessarily with the maturation of defective circuits to form the basis for the emergence of schizophrenic symptoms. Substantiation of these hypotheses would add important construct validity to animal models of schizophrenia and, at the same time, would significantly extend current theories of schizophrenia.

Increases in Mesolimbic Dopaminergic Activity

After decades of conflicting scenarios concerning the status of striatal dopaminergic transmission in schizophrenia, the demonstration of enhanced amphetamine-induced decreases in striatal dopamine D2 receptor binding in schizophrenic patients (Laruelle *et al.*, 1999; Breier *et al.*, 1997) has strongly supported the hypothesis that a hyperdopaminergic dysregulation represents a neuronal marker of schizophrenia (see also Grace, 1991; Moore *et al.*, 1999b). Administration of amphetamine to schizophrenics presumably resulted in a greater release of striatal dopamine than in normal subjects, therefore displacing the labelled dopamine receptor ligand from striatal binding sites to a greater degree in schizophrenics than in control subjects. Moreover, the psychostimulant-induced displacement in labelled D2 receptor binding was correlated specifically with the presence of positive symptoms and, importantly, its demonstration did not depend on prior treatment with antipsychotic drugs. These data also support the hypothesis that the dopaminergic system in schizophrenic patients is sensitized (Laruelle and Abi-Dargham, 1999; Lieberman *et al.*, 1997; Laruelle, 2000; Strakowski *et al.*, 1997).

Although the neuropharmacological interpretation of the increased releasability of dopamine in schizophrenic patients is complex (e.g. Seeman and Kapur, 2000), this finding represents a neuronal hallmark of the disease that, if present in an animal model, contributes significantly to its construct validity. (Given the strong evidence in support of hyperdopaminergic activity in patients, increased releasability of dopamine in animal models may increasingly also be considered as supporting face validity.) In fact in the absence of precise hypotheses about the developmental dysmorphogenesis in schizophrenia (see above), any developmental manipulation yielding increased releasability of dopamine may therefore be of interest to explore further the mechanisms mediating the abnormal regulation of mesolimbic dopaminergic transmission.

The evidence in support of an increased releasability of dopamine in schizophrenia has enhanced further the construct validity of animal models based on psychostimulant-induced sensitization of mesolimbic dopaminergic transmission (Robinson and Becker, 1986; Segal and Kuczenski, 1997; Castner *et al.*, 1999). Likewise, the effects of the *N*-methyl-D-aspartate (NMDA) receptor antagonists phencyclidine (PCP) and ketamine have been attributed in part to dysregulation of the mesolimbic dopaminergic system (Jentsch and Roth, 1999; Kegeles *et al.*, 2000). Recent studies have focused on the consequences of repeated psychostimulant administration in forebrain circuits efferent to the mesolimbic regions. For example, repeated amphetamine administration was demonstrated to sensitize robustly cortical acetylcholine release (Nelson *et al.*, 2000). Abnormally high increases in cortical acetylcholine release have been hypothesized to represent an integral component of the abnormal regulation of limbic-telencephalic circuits, and to mediate the impairments in information processing that contribute to, or even determine, the development of positive symptoms (Sarter and Bruno, 1999; see below).

Impairments in Information Processing

Since the original descriptions by Kraepelin and Bleuler, impairments in cognitive functions, specifically in the ability to discriminate between relevant stimuli and associations and those that are irrelevant for the task or cognitive process at hand, have been hypothesized to contribute to, or at least be associated with, the core symptoms of schizophrenia. In the 1960s, the mostly descriptive analyses (Venables, 1964; McGhie and Chapman, 1961; Shakow, 1962) stressed the patient's inability to filter irrelevant sensory stimuli and associations from processing, and the resulting exhaustion of attentional resources for the processing of relevant inputs (e.g. patient 15 in McGhie and Chapman: '. . . if something else is going on somewhere, even just a noise, it interrupts my thoughts and they get lost . . .'; p. 51). More contemporary, cognitive psychology-inspired theories focus on the patient's inability to employ top-down processes to select significant cognitive cues and stimuli as well as rejecting distracting inputs (Braff, 1993; Andreasen *et al.*,

1998; Javitt *et al.*, 2000). Gray's (1998) theory hypothesizes explicitly that the long-term, escalating consequences of such attentional impairments impede the subject's ability to use and update past experiences to interpret and respond properly to current information processing and thus contribute to the development of positive symptoms, particularly delusions.

The precise cognitive features of the attentional impairments in schizophrenia, and their relationships to the main symptom clusters, are complex and poorly understood. However, the results from numerous studies support the notion that attentional capacities are reduced in schizophrenics and exhausted by the processing of task-irrelevant information (e.g. Grillon *et al.*, 1990; Bentall and Slade, 1985; Salo *et al.*, 1996; see also Light and Braff, 2000 and Mar *et al.*, 1996). Moreover, several studies suggested that such attentional impairments are related intrinsically to the neurobiological bases and the positive symptoms of this disorder (e.g. Sperber *et al.*, 1994).

Clinical research that focused on the descriptive classification of symptoms using standard scales (e.g. scales for the assessment of negative/positive symptoms) and the assessment of the patient's attentional impairments using regular psychometric tests (e.g. continuous performance task, CPT) typically has not been designed to test hypotheses about the role of attentional impairments in the development of positive symptoms (Elliott and Sahakian, 1995; Green *et al.*, 2000). In fact, the clinical literature occasionally classified such impairments as negative symptoms, or suggested inconsistent relationships between attentional impairments and positive symptoms (but see Addington *et al.*, 1991 and Brockington, 1992).

The modelling of the cognitive components of schizophrenia clearly has not progressed well (e.g. Moore, 1999) for several reasons. First, the focus on nosology and face validity (see above) has impeded efforts to identify those elementary cognitive dysfunctions that mediate the development of the disorder's cardinal symptoms. The perspective that cognitive impairments are unrelated to positive symptoms, and the associated attempts to model and treat such impairments separately from positive symptoms, represents a logical extension of the nosological perspective and the associated classification of cognitive impairments as negative symptoms (e.g. Tandon and Greden, 1989; see also Ellenbroek and Cools, 2000a).

Clearly, among all attempts to model the core cognitive impairments of schizophrenia, the disruption of pre-pulse inhibition has been developed and validated most extensively (for a comprehensive review, see Swerdlow and Geyer, 1998 and Geyer *et al.*, 2001). Other attempts to model such impairments have focused on the hyperattentional performance of amphetamine-sensitized rats in tasks designed to assess specific attentional functions (Crider *et al.*, 1982; Deller and Sarter, 1998). Furthermore, these impairments were hypothesized to be due to increases in the reactivity of cortical cholinergic inputs (Nelson *et al.*, 2000; Sarter and Bruno, 2000) and associated increases in mesolimbic dopaminergic activity (Moore *et al.*, 1999a). As mentioned previously, despite the obvious limitations in achieving face validity when modelling positive symptoms in animals, models are capable of reproducing the fundamental impairments in information processing that mediate, at least in part, the development of schizophrenic symptoms. Relevant attentional functions can be measured in laboratory animals, including rodents (e.g. Muir, 1996; Bushnell, 1998; McGaughy and Sarter, 1995; Turchi and Sarter, 1997), and thus the attentional effects of psychotogenic manipulations can be assessed. Although historically animal models of schizophrenia did not address cognitive variables, the construct validity of any model remains incomplete without reflecting the cognitive variables of schizophrenia (see also Ellenbroek and Cools, 1990b and Ellenbroek *et al.*, 1996).

Effects of Antipsychotic Drugs

Generally, atypical antipsychotic drugs have been assumed to attenuate more effectively the cognitive symptoms in schizophrenia than conventional antipsychotic D2 receptor antagonists (e.g. Breier, 1999; Kinon and Lieberman, 1996; Remington and Kapur, 2000; Arnt and Skarsfeldt, 1998). Although the clinical evidence in support of this assumption has remained limited (e.g. Goldberg and Weinberger, 1994; but see Green *et al.*, 1997 and Kern *et al.*, 1999), data supporting the differential efficacy of typical and atypical antipsychotic drugs in different animal models (e.g. Morrow *et al.*, 1999) have substantiated the contrasting effects of the two classes of therapeutic drugs. For example, typical antipsychotic drugs were reported to attenuate the effects of psychostimulant sensitization, while atypical antipsychotic treatments in fact augment some of the effects of repeated psychostimulant administration. Conversely, atypical antipsychotic drugs appear to be particularly potent in reversing phencyclidine-induced behavioural and cognitive impairments, while haloperidol exacerbates such impairments (Jentsch and Roth, 1999).

Animal models may also reveal therapeutic mechanisms that go beyond the traditional focus on typical and atypical antipsychotic drugs. For example, treatment with dopamine D2 receptor antagonists *per se* has been hypothesized to result in cognitive impairments via downregulation of D1 receptors. Thus, D1 receptor stimulation has been considered a complementary therapeutic approach for the cognitive symptoms in schizophrenia (Castner *et al.*, 2000; Goldman-Rakic, 1999). Predictive validity represents an obvious component of any animal model of schizophrenia, specifically in light of the necessarily overwhelming focus on models with construct validity (Geyer and Markou, 2000). For such models, the demonstration of beneficial effects of clinically effective drugs represents the main empirical test of their underlying theory.

CONCLUSION

This brief discussion of the main components of an animal model of schizophrenia illustrates the spectrum of developmental, neuroscientific, behavioural, cognitive and pharmacological components that, in combination, need to be addressed to create a model of schizophrenia. As discussed by Tallman (2000), schizophrenia does not represent a more complex disorder than other psychiatric disorders, and thus the requirements for developing an animal model for schizophrenia are not fundamentally different from those for models of, for example, depression or senile dementia (see also Sarter *et al.*, 1992b). The alternative to engaging in the construction of animal models that combine all levels of analysis, and to demonstrating construct validity, is to revert back to animal assay models. Animal assay models may not only generate data with very limited validity but also deprive the researcher from achieving the main goal of research using animal models, i.e. to test and extend a theory about the disorder of interest.

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IV

Monoaminergic Transmitter Systems

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NORADRENALINE

Introduction

Noradrenaline (NA) together with dopamine (DA) and adrenaline (A) are the most important mammal catecholamines. This group of compounds is characterized by a benzene ring with two attached hydroxyl groups (a catechol nucleus) and an opposing side chain of ethylamine or its derivatives. Stimulation of sympathetic nerves leads to release of a substance originally called 'sympathin' (Cannon and Uridil, 1921), subsequently identified as NA by von Euler (von Euler, 1946a, b). A few years later, NA was also identified in the brain (Holtz, 1950) and as its distribution was not following vasculature, it was proposed that NA in fact is a neurotransmitter in the central nervous system (CNS) (Vogt, 1954). Its wide distribution and potential significance in normal CNS function and pathophysiology were established in the early 1950s (Carlsson, 1959; Montagu, 1956; Vogt, 1954). In similarity to DA and A, and also 5-hydroxytryptamine (5-HT), there are considerable species differences in NA distribution as well as receptor types and their localization (Smeets and Gonzáles, 2000).

Biochemistry

L-Tyrosine, an aromatic amino acid derived from food proteins, is the precursor for the catecholamines. Interestingly, catabolism of phenylalanine in the liver by phenylalanine hydroxylase also leads to synthesis of L-tyrosine. A genetic deficiency of that enzyme results in accumulation of phenylalanine and the disorder phenylketonuria with subsequent retardation as the major symptom. Hydroxylation of L-tyrosine by tyrosine hydroxylase (TH) results in L-3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA is decarboxylated by aromatic L-amino acid decarboxylase to DA, the final product in DAergic neurons. In cells that contain DA-β-hydroxylase (DBH), DA is hydroxylated to NA. Lastly, cells that possess phenylethanolamine *N*-methyltransferase (PNMT) methylate NA to A. Only a limited neuronal cell population in hind brain contains PNMT and consequently, in contrast to DA and NA, only very small amounts of A are synthesized in the CNS.

The rate-limiting step in the synthesis of catecholamines is TH since its maximal velocity is less than those of other enzymes in the synthetic pathway. TH activity is acutely regulated in an inhibitory fashion by a pool of catecholamines readily accessible to the enzyme, i.e. end-product inhibition (Masserano *et al.*, 1989), and enhanced following electrical stimulation of neurons, as shown in the early 1970s by Roth and collaborators (Roth *et al.*, 1975), or by phosphorylation mediated by cAMP-dependent protein kinase A, Ca^{2+} calmodulin-dependent protein kinase and protein kinase C (Edelman *et al.*, 1978; Masserano *et al.*, 1989). TH is long-term regulated by increased TH gene expression, which is induced by a variety of stressors increasing the neuronal activity. This trans-synaptic induction results in enhanced synthesis of TH enzyme protein.

Catabolism and turnover of catecholamines were extensively studied and mapped in the 1970s and 1980s and only a few, hopefully representative, papers are cited here (Berry *et al.*, 1994; Butcher *et al.*, 1990; Carlsson, 1975a, b; Costa *et al.*, 1972; Elchisak *et al.*, 1977; Elsworth *et al.*, 1983; Kopin, 1985; Mårdh *et al.*, 1981, 1983; Sharman, 1981; Widerlöv and Lewander, 1978).

Catecholamines are metabolized by two enzymes: monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT). MAO, both the A and B types, is found throughout the body, and in CNS MAO has been demonstrated in both neurons and the glial cells. In the CNS, particularly in locus coeruleus (LC), the MAO-A form predominates. Deamination by MAO leads to 3,4-dihydroxyphenylglycolaldehyde (DHPGA) and subsequently to 3,4-dihydroxyphenylglycol (DHPG) and 3-metoxy-4-hydroxyglycol (MHPG). Another pathway is conversion of DHPGA to 3,4-dihydromandelic acid (DHMA) and, finally, to vanilylmandelic acid (VMA). COMT catabolizes NA to normetanephrine (NMN), which is converted to 3-metoxy-4-hydroxyphenylglycoaldehyde (MHPGA). MHPGA in turn is catabolized to either VMA or 3-metoxy-4-hydroxyphenylglycol (MHPG). Significant quantitative differences exist between different species; in humans MHPG is the principal metabolite found in the CSF, whereas in plasma and urine VMA predominates. While MHPG concentrations in CSF represent a reasonable measure of central NAergic activity, measurement of only urinary VMA does not adequately reflect the peripheral NAergic state since MHPG is also synthesized peripherally and, moreover, MHPG can be converted to VMA.

NA Nuclei, Pathways and Receptors

The anatomy of NA (nuclei and pathways) in the CNS was originally mapped by Dahlström and Fuxe (1964) and subsequently modified by Lindvall and Björklund (1983). Three main cell groups located in the pons and medulla are recognized: (1) the LC, (2) the lateral tegmental cell system, and (3) the dorsal medullary group. The LC is the most important NA structure, projecting both rostrally and caudally, and innervating essentially all parts of the telencephalon and diencephalon, e.g. all layers of the neocortex, hippocampus, amygdala, thalamus and hypothalamus. Although the number of cells in the LC is small (clearly less than 10 000), due to the extremely high number of axon terminal ramifications (virtually >10 000 axon; Moore and Bloom, 1979). LC exerts a major influence on brain and behavioural responses. In contrast, the lateral tegmental system and the dorsal medullary group provide integration between the central and peripheral NAergic systems, as well as control/modification of the cardiovascular, visceral and autonomic functions. NA is colocalized with neuropeptide Y in

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some brain regions (Evenitt *et al.*, 1984; Hökfelt *et al.*, 1983; for review see Aoki and Pickel, 1990).

Neurons in the LC with their wide projections to the brain are central to NA release and actions in the CNS. Interestingly, in parallel to the feedback controls between the frontal cortex–nucleus accumbens–A9/A10 areas, LC is subjected to a reciprocal regulation from the prefrontal cortex by glutamatergic input (Jodo *et al.*, 1998). However, there are also two brainstem nuclei that provide major synaptic inputs to the LC: the nucleus paragigantocellular (PGi) and the nucleus prepositus hypoglossi (P_RH; Aston-Jones *et al.*, 1986). The PGi mediates, via glutamatergic and corticotrophin releasing factor (CRF) inputs, the excitatory effects of environmental stimuli and is thus crucial for vigilance, attention and behavioural responses to external stimuli. The P_RH nucleus exerts a primary inhibitory action via GABAergic and encephalinergic inputs. Other structures, such as ventral tegmental area (VTA), raphe nuclei, hypothalamus and paraventricular nucleus (PVN), also provide LC input and thereby modulation of behaviour via effects on NA release in terminal areas. In addition to external stimuli, the LC also responds to noxious stimuli (Elam *et al.*, 1986a) and, moreover, monitors the internal autonomic and vegetative state. Thus, LC responds even to entirely peripheral events, such as a small alteration in blood volume (Svensson and Thorén, 1979), as well as to changes in blood pressure, a regulatory influence that seems largely mediated via vagal afferents from the cardiopulmonary region (Elam *et al.*, 1984, 1985). In addition, the LC responds to hypercapnia and hypoxia (Elam *et al.*, 1981) analogously to the peripheral sympathetic nerves, as well as to entirely visceral stimuli, such as distension of the urinary bladder, the distal colon or even the stomach (Elam *et al.*, 1986b; for extensive review see Svensson, 1987). Generally, the activity of the LC neurons is strongly positively correlated to the waking–sleep cycle, being essentially quiescent during REM sleep. LC cell firing is stimulated by sensory stimuli and is decreased during maintenance/automatic behaviours (Aston-Jones and Bloom, 1981; Foote and Aston-Jones, 1995; Valentino and Aston-Jones, 1995). LC lesion experiments, recordings of neuronal firing, and stimulation and inhibition, respectively, of α_1 and α_2 receptors have demonstrated the significant role LC plays in the function of, for example, cortex, thalamus, hypothalamus, PVN, hippocampus and amygdala, and the corresponding behavioural manifestations such as attention, vigilance, arousal, fear stimulus discrimination, eating behaviour and learning (Aston-Jones *et al.*, 1994; Ressler and Nemeroff, 2000; Wellman, 2000).

The NA and A receptors (adrenergic receptors) all belong to the G-protein-coupled receptor superfamily. The original classification of Ahlquist (Ahlquist, 1948) — based on the response of sympathetically innervated peripheral organs to sympathomimetics and their antagonists — into α and β receptors is still valid. Subsequently, pharmacological studies and the development of molecular biology have enabled receptor cloning and subdivision into α_{1A}, α_{1B} and α_{1D}; α_{2A}, α_{2B}, α_{2C} and α_{2D}; and β_1, β_2 and β_3 (Bylund *et al.*, 1994; Hieble *et al.*, 1995). In this context it is important to keep in mind that adrenergic receptors are found not only pre- and postsynaptically on neurons but also on glial cells, which respond to NA and other agonists and are coupled to the same second messenger systems (Salm and McCarthy, 1992; Stone and Ariano, 1989).

α_1 adrenoceptors are characterized by a high sensitivity to the agonists phenylephrine and metoxamine and to the antagonists phenoxybenzamine, prazosin, and 5-methylurapidil and WB-4101. Both α_{1A} and α_{1B} are postsynaptic and are found throughout the rat CNS although their relative density is different in different brain regions (Morrow and Creese, 1986; Ruffolo *et al.*, 1991, 1993, 1994). α_1 receptors are excitatory. By inhibiting the non-voltage-dependent K^+ current they cause a slow depolarization and thereby increase cell excitability (McCormick, 1991; Nicoll *et al.*, 1990). As regards intracellular effects of α_1 receptor stimulation, G-protein-mediated stimulation of phospholipase C (PLC) leads to phosphoinositolphosphate (PIP_2) breakdown to inositol triphosphate (IP_3) and diacylgycerol (DAG). IP_3 stimulates release of Ca^{2+} from endoplasmic reticulum, while DAG both increases phosphokinase C (PKC) activity and when phosphorylated to phosphatidic acid (PA) acts as Ca^{2+} ionophore. In this context, it should be pointed out that the relationship between the described electrophysiological effects and the effects on Ca^{2+} and PKC have not been fully elucidated.

α_2 adrenoceptors are characterized by activation by α-methyl-NA and clonidine and blockage by yohimbine, idazoxan and rauwolfscine (Bylund *et al.*, 1994; Ruffolo *et al.*, 1993, 1994). According to the original suggestion of Langer, this receptor is presynaptic (Langer, 1974). The α_{2A} and α_{2C} receptors are widely but unevenly, with regard to brain regions and to each other, distributed in the brain. High α_{2A} and α_{2C} receptor densities are found in the LC, amygdala and hippocampus, and all LC neurons contain α_{2A} receptor subtype. On the other hand, α_{2B} is localized mostly in the thalamus (Nicholas *et al.*, 1993b, 1996; Scheinin *et al.*, 1994; Talley *et al.*, 1996; Wamsley *et al.*, 1992).

In contrast to the electrophysiological properties of α_1 receptors, α_2 receptors hyperpolarize the cell membrane as a consequence of activating an inward-rectifying K^+ current and thereby have inhibitory effects. Thus α_2 receptors on NAergic nerve terminals act as autoreceptors inhibiting NA release and the somatodendritic α_2 receptors in LC inhibit the firing of LC neurons (Aghajanian and VanderMaelen, 1982a; Andén *et al.*, 1970; Cedarbaum and Aghajanian, 1977; Langer and Arbilla, 1990; Langer *et al.*, 1971; Starke *et al.*, 1989; Svensson *et al.*, 1975). Interestingly, this K^+ response is the same as the one associated with DA-D_2 and 5-HT_{1A} receptors (McCormick, 1991). With regard to the second messenger system, α_2 receptors are negatively coupled to adenylyl cyclase, through action on Gi, with a subsequent decrease in cAMP synthesis.

The β adrenoceptor subdivision into β_1 and β_2 was originally proposed by Lands *et al.* (1967) based on physiological responses of several tissues to sympathomimetic compounds. Both β_1 and β_2 have been extensively investigated as prototypes for the G-protein-coupled superfamily of receptors and cloned early on (Frielle *et al.*, 1987, 1988; Kobilka *et al.*, 1987; Ostrowski *et al.*, 1992; Shorr *et al.*, 1981, 1982). β_3 receptor, found in the periphery, has been cloned some years later (Emorine *et al.*, 1989). Isoproterenol and salbutamol are classical β receptor agonists, while propranolol and pindolol are non-selective antagonists. Selective antagonists for β_1 and β_2 are atenolol and ICI-118, 551, respectively (Hieble and Ruffolo, 1991).

Radioligand binding studies as well as mRNA studies demonstrate that both β receptors are widely distributed in the brain. Although in many areas the distribution is about even, β_1 predominates in the cerebral cortex, the dentate gyrus, and CA1 and medial dorsal hypothalamic nuclei, while β_2 is more abundant in the cerebellum and reticular, paraventricular and central thalamic nuclei (Nicholas *et al.*, 1993a, 1996; Palacios and Kuhar, 1983; Rainbow *et al.*, 1984). Electrophysiologically, β receptors stimulate Ca^{2+} currents, augment the hyperpolarization–activated cation current and enhance the rate of cell firing under conditions of high excitation (McCormick, 1991; Nicoll *et al.*, 1990). The extensively studied effect of β receptors on the second messenger system is stimulation of adenylyl cyclase via Gs followed by an increase in cAMP (Kobilka, 1992).

Interestingly, the relationship between the electrophysiological and second messenger effects is better understood than that for α_1 and α_2 receptors, since stimulating adenylyl cyclase (for example with forskolin) or using α-bromo-cAMP (a cAMP analogue, not susceptible to breakdown as cAMP) elicits the same electrophysiological responses.

β receptors have been used as a paradigm to study two phenomena: receptor desensitization and receptor downregulation/upregulation. Following exposure to β agonists, β receptors

show a decrease in responsivity within minutes or even seconds. This is caused by the uncoupling of the receptor from Gs and adenylyl cyclase (Kobilka, 1992). The uncoupling is a consequence of phosphorylation of the receptor by PKA and β adrenergic receptor kinase (βARK). Longer (hours) agonist exposure results in loss of receptor binding sites; that is, receptor downregulation. This phenomenon is due to diminished receptor mRNA and increased degradation of receptor protein (Collins, 1993; Collins *et al.*, 1991). Receptor upregulation is opposite to downregulation. In parallel to many other systems, receptor upregulation is a sequelae of decreased agonist availability. Thus 6-OHDA lesion of the catecholaminergic nerve terminals results in increased β_1 and β_2 receptor binding.

Transmitter Release and Physiology of NA Neurons

In the CNS, NA is concentrated and stored in synaptic vesicles by a vesicular transporter. The same transporter protein is found in DAergic, NAergic and 5-HTergic neurons, indicating that these three monoamines are concentrated by the same mechanism. Of historical interest was the finding in the early 1950s that reserpine (derived from the plant *Rauwolfia serpentina*, and used for centuries in India, and in the 1950s in the USA as an antipsychotic) inhibits catecholamine and 5-HT neuronal vesicle transporters. This leads to inhibition of monoamine accumulation with subsequent depletion and profound behavioural depression. A classical early study in the field was conducted by Carlsson and co-workers (Carlsson *et al.*, 1957).

In similarity to other neurotransmitters synaptically released, NA is released by depolarization of the nerve terminals. Depolarization of nerve terminal membrane opens the Ca^{2+} channels with Ca^{2+} entry and fusion and discharge of the vesicle, a process called vesicular exocytosis. This phenomenon has been extensively studied for several decades (Thureson-Klein, 1983; Trifaro *et al.*, 1992). Release of NA is regulated by presynaptic α_2 autoreceptors mostly of the α_{2A} subtype. This was first demonstrated in the periphery by showing that nerve stimulation-induced NA release is enhanced by α_2 receptor antagonists and inhibited by α_2 receptor agonists (Langer and Arbilla, 1990; Langer *et al.*, 1971; Starke, 1971; Starke *et al.*, 1989). The inhibition is probably achieved by a decrease in depolarization-evoked entry of Ca^{2+} into the nerve terminal and also hyperpolarization of nerve terminal by increasing K^+ conductance (Aghajanian *et al.*, 1977; Starke *et al.*, 1989). Release can also be modulated by somatodendritic α_2 autoreceptors found in the LC neurons (Svensson *et al.*, 1975). By increasing K^+ conductance, the neuronal cells are hyperpolarized and the firing rate decreased (Aghajanian *et al.*, 1977; Cedarbaum and Aghajanian, 1977). Although the validity of findings of release autoregulation by presynaptic α_2 receptors is generally accepted, a different hypothesis, namely that agonist inhibitory effects and antagonist enhancing effects occur at two different sites, has also been proposed (Kalsner, 2001). Most of the NA release in the CNS is secondary to neuronal activation of LC.

NA Reuptake

In similarity to 5-HT, NA released into the synaptic cleft is either metabolized or transported back into the neurons by a high-affinity transporter. The transporter is a presynaptically located glycoprotein, and this intraneuronal high-affinity uptake is called uptake 1. NA can also be taken up extraneuronally, by a different mechanism which has lower affinity for and is less selective for NA. This uptake has been termed uptake 2 (Amara and Kuhar, 1993; Brownstein and Hofmann, 1994). Both uptake 1 and uptake 2 accumulate NA against a substantial concentration gradient and require a source of metabolic energy. This energy is provided by the transmembrane Na^+ electrochemical gradient achieved by the Na^+-K^+ ATPase. Many therapeutic drugs and substances of abuse inhibit the uptake of NA. Tricyclic antidepressants have been developed expressly for therapeutic use and inhibit both the NA and 5-HT transporters but in the therapeutic concentrations used have no effect on DA. On the other hand, drugs of abuse such as cocaine, amphetamine and methylphenidate are effective inhibitors of both NA and DA uptake (and to a certain extent also of 5-HT, e.g. cocaine). Reuptake of NA and 5-HT, as one of the mechanisms of action of antidepressants, is dealt with in other chapters of this book.

Functional Role and Clinical Significance of Brain NA Neurons

Although an extensive review of this topic is far beyond the scope of this chapter, the function of the largest central NA system, which originates in the LC and has been extensively studied for several decades, will be briefly discussed. Early studies in awake animals, in particular the monkey, revealed LC activity to be correlated with vigilance, showing phasic activation responses to environmental sensory stimuli, particularly if associated with novelty or fear, but showing low activity in association with behaviours such as grooming, sweet water consumption or sleep (Foote *et al.*, 1983). Thus a role in attentional functioning was confirmed and, in principle, the system may serve as a significance enhancer with respect to salient environmental stimuli. As mentioned above, this function also appears to apply to the internal milieu, and a general role in the so-called defence reaction appears very likely (Svensson, 1987). Subsequent studies over the past two decades have largely confirmed and extended such a biological role of the largest brain NA system (Aston-Jones *et al.*, 1999) and accordingly it is not surprising that brain NA neurons have been found to be involved in the mode of action of both many antidepressant drugs as well as a number of antipsychotics, and drugs affecting wakefulness and attention, such as nicotine (Mitchell, 1993; Svensson and Engberg, 1980). Blockage of central α_2 receptors may be involved in both antidepressant (Linnér *et al.*, 1999; Svensson, 2000a; Svensson and Usdin 1978) and antipsychotic drug effects (Hertel *et al.*, 1999), although the specific roles of the different receptor subtypes in this regard remain to be clarified. It could be added that blockage of brain α_1 adrenoreceptors seems to contribute to the therapeutic effect of a number of antipsychotics (Andersson *et al.*, 1994; Mathé *et al.*, 1996; Svensson *et al.*, 1999; Wadenberg *et al.*, 2000). Thus brain NA neurons represent a highly important target for major groups of psychoactive drugs.

DOPAMINE

Introduction

DA was discovered as an independent neurotransmitter in the brain during the late 1950s (Carlsson, 1959; Carlsson *et al.*, 1957, 1958). Since that time DA has emerged as one of the most important neurotransmitters in brain from a behavioural as well as psychopharmacological perspective, with an established role not only in the pathophysiology and treatment of Parkinson's disease, but also in schizophrenia and the mode of action of antipsychotic drugs, as well as in drug dependence. Indeed, during the past five years alone, more than 16 000 papers on DA have been published. The present review will therefore only briefly outline its biochemistry, anatomy and physiology, with some emphasis on the more recent developments in the field.

Biochemistry

The major processes of synthesis, storage and metabolism of this neurotransmitter have been known for decades. DA is synthesized from the amino acid tyrosine in a two-step enzymatic process. First, tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase, which is the rate-limiting enzyme in DA synthesis. Subsequently, L-DOPA is rapidly converted to DA by aromatic L-amino acid decarboxylase. In noradrenergic neurons, DA is further converted by DA β-hydroxylase to NA. DA is stored in vesicles and physiologically released by a calcium-dependent process initiated by nerve impulse activity. Released DA is approximately 80% effectively and rapidly transported back into the nerve terminal by a DA-specific transporter. Extravesicular DA is intracellularly metabolized by the enzyme monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC). Released, extracellular DA is sequentially degraded by the actions of catechol-*O*-methyltransferase (COMT) and MAO to 3-methoxytyramine (3-MT) and homovanillic acid (HVA). In the rat brain DOPAC is the major DA metabolite. Nevertheless, both DOPAC and HVA, in both sulphate-conjugated and free forms, are found in high concentrations along with small amounts of 3-MT in rat brain. In the human brain, free HVA appears instead as the main metabolite, with only small amounts of DOPAC (Cooper *et al.*, 1996).

DA Nuclei, Pathways and Receptors

The large mesotelencephalic DA systems originate in the midbrain tegmentum, and the distribution of DA cell bodies in this region is restricted largely to two nuclei in the rat, the substantia nigra zona compacta (SN-ZC or A9) and the ventral tegmental area of Tsai (VTA or A10), as revealed by Dahlström and Fuxe (1964). The neurons in the SN-ZC project primarily to the caudate nucleus and putamen, i.e. striatum, and the system was thus called the nigrostriatal DA system (Andén *et al.*, 1964). The VTA exhibits somewhat more diverse projections than the SN-ZC. Thus, the DA neurons in the VTA project to, for example, ventral striatum (VSTR), including the nucleus accumbens (NAC), amygdala, hippocampus and olfactory tubercle as well as several limbic cortical sites, such as medial prefrontal, cingulate and entorhinal cortices (Andén *et al.*, 1966; Björklund and Lindvall, 1984; Ungerstedt, 1971).

In humans, the cortical DA projection is much more widespread than in the rat, corresponding to the relatively larger size of the frontal cortex in humans. These DA pathways are often collectively referred to as the mesolimbocortical DA system. However, cortically projecting DA neurons appear not to innervate subcortical sites, and vice versa (Fallon, 1981; Swanson, 1982). These cortical and subcortical DA projections seem, at least partially, to arise from different anatomical subdivisions of the VTA. The ventrally located paranigral nucleus of the VTA primarily contributes the subcortical, mesolimbic DA projection, e.g. to the NAC and other striatal sites. In contrast, the parabrachial pigmented nucleus of the VTA also contains the somata of DA neurons from which the cortical DA innervation originates (Deniau *et al.*, 1980; Fallon, 1981; Oades and Halliday, 1987; Phillipson, 1989; Simon *et al.*, 1979; Swanson, 1982). In addition, these mesocortical DA neurons exhibit several functional differences from the mesolimbic DA cells; mesocortical DA neurons display a more variable firing pattern, differential coexistence with neuropeptides, as well as altered autoreceptor and heteroreceptor regulation in comparison with the subcortically projecting DA neurons (Grenhoff *et al.*, 1988a; Roth and Ellsworth, 1995). Coexistence and interaction of DA with some neuropeptides, e.g. cholecystokinin (Hökfelt *et al.*, 1980; Schalling *et al.*, 1990) and neurotensin (NT), has been extensively investigated (Bean *et al.*, 1990; Hertel *et al.*, 1996; Lambert *et al.*, 1995; Quirion *et al.*, 1985).

Dopamine Receptors

There are currently five known DA receptor subtypes categorized according to structural, functional and pharmacological characteristics, and divided into two main families (D_1-like and D_2-like) based upon their sequence homology. The D_1-like receptors (D_1 and D_5) activate adenylyl cyclase, whereas the D_2-like receptors (D_2, D_3 and D_4) inhibit adenylyl cyclase. The D_2 receptor was cloned from rat brain based on its homology with the previously cloned β adrenoceptor (Bunzow *et al.*, 1988). The other D_2-like receptors, the D_3 and D_4 receptors, were cloned by low-stringency hybridization using probes derived from the D_2 receptor (Sokoloff *et al.*, 1990; van Tol *et al.*, 1991). These receptors also have been extensively studied *in vivo* and *in vitro* using a variety of methodologies, including behavioural, physiological, neurochemical, pharmacological and, more recently, molecular approaches.

Both the D_1 and D_2 families are found postsynaptically, whereas the presynaptic receptors are regarded to belong to the D_2 family. There appears to be some differential distribution of DA receptor types in various DA terminal regions in the rat. Dense D_1 binding has been found in the dorsolateral striatum and moderate binding has been detected in the neocortex (Boyson *et al.*, 1986). Dense D_5 binding is mainly restricted to thalamic, hypothalamic and hippocampal neurons. High D_2 binding is observed in, for example, dorsolateral striatum, NAC, VTA and SN-ZC (Gehlert and Wamsley, 1985).

Specifically, the D_2 receptor has been found mainly in the striatum, in the olfactory tubercle, and in the core of the NAC (Bouthenet *et al.*, 1991; Jackson *et al.*, 1994). The D_2 receptor is expressed in the core of the NAC by GABAergic neurons coexpressing enkephalins (Le Moine and Bloch, 1995; Le Moine *et al.*, 1990), as well as in the septal pole of the shell of the NAC, where it is expressed by NT-containing neurons (Diaz *et al.*, 1994). D_2 receptor mRNA is also present in the prefrontal, cingulate, temporal and entorhinal cortices, in the septal region, in the amygdala, and in the granule cells of the hippocampal formation (Bouthenet *et al.*, 1991; Jackson *et al.*, 1994). It is furthermore found in the hypothalamus, the substantia nigra pars compacta and the VTA, where it is expressed by dopaminergic neurons (Bouthenet *et al.*, 1991; Meador-Woodruff *et al.*, 1989; Weiner *et al.*, 1991). Immunohistochemical analysis with specific antibodies revealed that D_2 receptors are present in medium spiny neurons of the striatum, where they are more concentrated in spiny dendrites and spine heads than in the somata. D_2 immunoreactive terminals are frequently detectable, forming symmetrical, rather than asymmetrical, synapses (Hersch *et al.*, 1991; Levey *et al.*, 1993). The D_2 receptors are also present in perikarya and dendrites within the substantia nigra pars compacta and are much more concentrated in the external segment of the globus pallidus than in other striatal projections (Levey *et al.*, 1993). D_2 receptor immunoreactivity has also been detected in the glomerular and internal plexiform layers of the olfactory nerve and in the central nucleus of the amygdala (Levey *et al.*, 1993).

The D_3 receptor is largely expressed in forebrain and limbic areas (Sokoloff *et al.*, 1990). In rat brain, the D_3 receptors are mainly expressed in the islands of Calleja, where D_3 receptor mRNA is expressed in granule cells (Bouthenet *et al.*, 1991; Levesque *et al.*, 1992), and in the medium-sized spiny neurons of the rostral and ventromedial shell of the NAC. In contrast to the D_2 receptor, low levels of the D_3 receptor are found in the dorsal striatum. Moreover, D_3 mRNA is also found in the substantia nigra pars compacta, and in the ventral tegmental area, where, compared to the D_2 receptors, it is expressed only in a minority of dopaminergic neurons. D_3 receptors are also found in Purkinje cells and, at low expression levels, in hippocampus, in the septal area, and

in various cortical layers of the medial–temporal lobe (Bouthenet *et al.*, 1991).

The D_4 receptor is predominantly expressed in the frontal cortex (O'Malley *et al.*, 1992), a region that receives dense input from midbrain DA neurons and is associated with cognitive and emotional processes. Low levels of D_4 receptor mRNA have been found also in the basal ganglia. This receptor also appears to be highly expressed in the amygdala, hippocampus, hypothalamus, mesencephalon and retina. Experiments using immunohistochemistry and electron microscopy have revealed that, in both the cerebral cortex and hippocampus, D_4 receptors are present in both pyramidal and non-pyramidal neurons that have been identified as GABAergic (Mrzljak *et al.*, 1996).

In short, the patterns of D_3 and D_4 receptor expression are predominantly restricted to the mesocortical and the mesolimbic systems. Overall, it appears that D_2 receptors are more prominently expressed in areas associated with motor control, while D_3 and D_4 receptors are more exclusively located in areas where the DA system is thought to serve a role in modulating emotion and cognition. Interestingly, the D_3 and D_4 receptors are linked to the heteromodal association neocortex via their expression in the NAC and prefrontal cortex (Ross and Pearlson, 1996; Schwartz *et al.*, 2000). The heteromodal neocortex network includes the dorsolateral prefrontal cortex, Broca's area, planum temporale and the inferior parietal lobule (Ross and Pearlson, 1996). These interconnected association areas are involved in higher integrative functions, such as executive tasks (for example, working memory, motivation and planning), speech and focused attention. Dysfunctions of these brain regions have been implicated in schizophrenia. Indeed, neuropathological abnormalities have also been described in these brain areas of schizophrenic patients (Roberts, 1991).

Before the discovery of the various subtypes of DA receptors, it was generally accepted that the therapeutic effects of antipsychotic drugs could be mainly attributed to blockade of D_2 receptors. The notion of a DA receptor blocking component *per se* in the mode of action of antipsychotics was originally hypothesized by Carlsson and Lindquist (1963), and among the many subsequent studies an apparent tight correlation between the clinical potency of various antipsychotics and their affinity for DA-D_2 receptors was observed (Creese *et al.*, 1976; Seeman *et al.*, 1976). Although this correlation subsequently has been seriously questioned, the fact remains that no drug has yet been identified as an effective antipsychotic agent without any significant affinity for DA-D_2 receptors. For classic antipsychotic drugs (e.g. haloperidol) it has been demonstrated that antipsychotic treatment leads to striatal D_2 occupancies between 60% and 80%, and that occupancies above 80% in most cases are associated with extrapyramidal side effects. On the contrary, the atypical antipsychotic drug clozapine has showed considerably lower D_2 occupancy, i.e. in the range of 20–60% (Farde *et al.*, 1992; Goyer *et al.*, 1996). However, clozapine displays a unique clinical profile and possesses relatively high affinity for many different neurotransmitter receptors, including other DA receptors, several subtypes of 5-HT receptors, α adrenergic receptors, etc., and it has accordingly been proposed that also blockade of non-D_2 receptors must contribute to its therapeutic effect in schizophrenia (Svensson, 2000b). Since it had been suggested that D_3 and D_4 receptors may be of particular significance in schizophrenia and in the mode of actions of antipsychotic drugs (Reynolds, 1996; Seeman *et al.*, 1997), several selective D_3 and D_4 antagonists have subsequently been developed. Compared to typical and atypical antipsychotics, selective D_3 or D_4 receptor antagonists have, however, generally been found rather inactive in animal behavioural tests predictive of antipsychotic or extrapyramidal activity, although at present it is too early to draw any firm conclusions in this regard.

Physiology of DA Neurons and Transmitter Release

DA neurons in the SN-ZC and VTA show spontaneous spike firing which is driven by an endogenous pacemaker conductance (Grace and Bunney, 1983, 1984; Grace and Onn, 1989) and is modulated by afferent inputs. Generally, the discharge of the cells is an important determinant of the DA release process (Keefe *et al.*, 1992; Mathé *et al.*, 1999). The firing pattern of the DA neurons is characterized by two differential modes of firing; single-spike firing and burst firing (Grace and Bunney, 1983, 1984; Wang, 1981). Single-spike firing is a relatively regular, low-frequency firing pattern, i.e. 1–10 Hz. In contrast, burst firing is typically recognized as the transient high-frequency discharge of multiple action potentials. The burst firing mode has been shown to elicit much more efficient release of DA in terminal areas than a regular firing pattern of the same average rate, and burst firing also causes significant activation of postsynaptic neurons as indicated by induction of c-*fos* and NG1-A (Bean and Roth, 1991; Chergui *et al.*, 1996, 1967; Gonon, 1988; Suaud-Chagny *et al.*, 1992). Moreover, this postsynaptic response shows both a spatial and a temporal specificity with respect to brain region, genes activated and cell phenotype (Chergui *et al.*, 1997). Physiologically, the pulse of DA produced by a burst of action potentials is rapidly attenuated by efficient uptake of transmitter from the synaptic cleft (Grace and Bunney, 1984). In addition, bursts seem to specifically facilitate release of colocalized neurotransmitters, such as NT and cholecystokinin (Bean and Roth, 1991). Such transient changes in impulse activity normally occur in relation to basic attentional and motivational processes in response to reward-predicting stimuli, and apparently serve to initiate goal-oriented behaviours (Nishino *et al.*, 1987; Schultz, 1986, 1998; Schultz *et al.*, 1993). In both awake and anaesthetized mammals *in vivo*, DA cells typically display a firing pattern which includes both single-spike and burst firing, with frequent switches between these two modes of firing. In contrast, in DA cells in a midbrain slice preparation, i.e. in cells that have been largely deprived of active neuronal inputs, burst firing is absent and little variability in the firing pattern is observed (Grace and Onn, 1989). Generally, the structure of the firing patterns of midbrain DA neurons *in vivo* has been proposed to reflect the neuronal response to coordinated synaptic inputs emerging from several neuronal circuit interactions (Hoffman *et al.*, 1995).

Regulation by Afferent Inputs

Generally, the midbrain DA neurons receive glutamatergic, GABAergic, cholinergic, serotonergic and noradrenergic inputs.

Glutamate

Spontaneous burst activity of DA neurons *in vivo* appears to be directly dependent upon activation of somatodendritic *N*-methyl-D-aspartate (NMDA) receptors via afferent excitatory amino acid (EAA) inputs, which mainly stem from the prefrontal cortex (PFC), the subthalamic, laterodorsal (LDTg) and pedunculopontine (PPTg) tegmental nuclei. The PFC seems to regulate a subpopulation of DA neurons in the VTA that in turn innervate the PFC, whereas other VTA neurons projecting to the NAC, which are innervated from the PFC, are GABAergic (Carr and Sesack, 2000; Carr *et al.*, 1999; Christie *et al.*, 1985; Sesack *et al.*, 1989). At any rate, the different glutamatergic afferents to the VTA seem to be importantly involved in the overall control of DA release in terminal fields (Charléty *et al.*, 1991, Chergui *et al.*, 1993, Gariano and Groves, 1988; Grenhoff *et al.*, 1988b; Murase *et al.*, 1993; Svensson and Tung, 1989). In addition, the NMDA receptors are involved in the control of the regularity of firing, another functionally important determinant of DA neuronal discharge, which seems critical for the capacity of a neuronal system to adapt to environmental demands

(Grenhoff *et al.*, 1988b; Servan-Schreiber *et al.*, 1990). NMDA-receptor activation mediates a slow excitatory postsynaptic potential (EPSP) in these cells (Mercuri *et al.*, 1996), whereas metabotropic glutamate agonists have been found to depress both excitatory and inhibitory input to the DA neurons (Bonci *et al.*, 1997). In fact, the latter effect seems to be shared by muscarinic receptors, which also depress both excitatory and inhibitory afferents via a presynaptic action (Grillner *et al.*, 1999, 2000). Some biochemical support for differential regulation of VTA DA neuronal subpopulations by EAAs was reported in the late 1980s. Thus, although DA release in the PFC was found to be preferentially modulated by NMDA receptors in the VTA, the DA release in the NAC seemed largely controlled by AMPA and kainate receptors in the VTA (Kalivas *et al.*, 1989; Mathé *et al.*, 1998). Needless to say, by electrical stimulation of afferents in a brain slice preparation, synaptic potentials can be elicited in DA neurons through activation of both AMPA/kainate and NMDA receptors (Johnson and North, 1992b; Mereu *et al.*, 1991). Metabotropic glutamate receptors (mGluRs) have also been shown to mediate both a slow excitatory as well as an inhibitory synaptic potential (Fiorillo and Williams, 1998; Shen and Johnson, 1997). More recently, even the presence of glutamate in the DA neurons themselves has been reported, forming functional glutamatergic synapses, so-called autapses (Sulzer *et al.*, 1998).

The glutamatergic input to the VTA has considerable interest in relation to the putative pathophysiology of schizophrenia, which has been considered as a hypoglutamatergic state (Kim *et al.*, 1980) and, in particular, a reduced activation of the NMDA receptor (Bunney *et al.*, 1995). Several early studies showed that negative symptoms are correlated with decreased capacity to activate the dorsolateral prefrontal cortex, so-called hypofrontality (Ingvar, 1987; Ingvar and Franzén, 1974; Weinberger *et al.*, 1986), a brain region that contributes an excitatory amino acid input to the VTA. Interestingly, experimentally induced 'hypofrontality' — suppressed functional activity of the corresponding brain region in the rat, i.e. the medial prefrontal cortex — as produced by local cooling or direct intracerebral application of lidocaine, was found to cause a selective abolition of the burst firing mode in VTA DA neurons, as well as regularization of their firing pattern (see above), although basal firing rate did not change (Murase *et al.*, 1993; Svensson and Tung, 1989). In other words, a pacemaker-like firing was seen, similar to the firing pattern seen in the deafferented slice preparation or following intracerebral application of kynurenate, a non-selective excitatory amino acid receptor antagonist (Grenhoff *et al.*, 1988b). If also present in humans, such a dysfunction of the VTA neurons, especially those that in turn project to the PFC (see above), might well contribute to explain the decreased capacity to process positive or negative reinforcement signals generally associated with impaired frontal lobe function as well as with schizophrenia (Svensson and Tung, 1989). Significantly, pretreatment with 5-HT_{2A} or 5-$HT_{2A/C}$ receptor antagonists, which clinically have been found to ameliorate dysthymia and negative symptoms in schizophrenia, as well as to improve motivation and drive, was subsequently shown to antagonize the selective reduction in burst firing of VTA neurons in 'hypofrontal' animals (Svensson *et al.*, 1989, 1995).

GABA

The firing of VTA DA cells is also influenced by γ-amino butyric acid (GABA). The GABAergic input originates in the striatum and in the pallidum (Bolam and Smith, 1990; Fonnum *et al.*, 1978; Smith and Bolam, 1990) as well as from intrinsic GABAergic interneurons in the midbrain (Di Chiara *et al.*, 1979; Grofova *et al.*, 1982; Stanford and Lacey, 1996), and mediates inhibition of DA neuronal activity by both GABA-A and GABA-B receptors (Johnson and North, 1992b). GABA may induce a hyperpolarization through both GABA-A and GABA-B receptor activation and thus reduce the average firing rate and attenuate burst firing (Erhardt *et al.*, 2002; Grace and Bunney, 1980; Johnson and North, 1992b).

Cholinergic, Serotonergic and Noradrenergic Inputs

The midbrain DA neurons receive a cholinergic input from the PPTg and the adjacent LDTg (Beninato and Spencer, 1987; Bolam *et al.*, 1991; Clarke *et al.*, 1987). Both muscarinic and nicotinic receptors are present on the dopaminergic cells (Cortès and Palacios, 1986; Schwartz, 1986; Wada *et al.*, 1989) and pharmacological activation of these receptors leads to excitation of the neurons (Calabresi *et al.*, 1989; Lacey *et al.*, 1990). Also the serotonergic raphe nuclei provide a serotonergic afferent input to the DA neurons (Dray *et al.*, 1976; Fibiger and Miller, 1977; Hervé *et al.*, 1987; Phillipson, 1979; Ugedo *et al.*, 1989; van Bockstaele *et al.*, 1994) and a noradrenergic input has been demonstrated as well (Grenhoff and Svensson, 1989; Grenhoff *et al.*, 1993; Phillipson, 1979). Thus, burst activity of VTA DA neurons seems to be modulated by a noradrenergic input from the LC to the VTA and, for example, administration of the α_1 adrenoceptor antagonist prazosin selectively depresses burst firing (Grenhoff and Svensson, 1993). In addition, electrical stimulation of the LC elicits monoamine-mediated short-latency bursts in VTA DA cells, an effect that in turn was specifically antagonized by prazosin administration (Grenhoff *et al.*, 1993). Stimulation of α_1 adrenoceptors seems to increase the excitability of DA cells via inhibition of K^+ efflux (Grenhoff *et al.*, 1995).

Dopamine-Receptor-Mediated Regulation

The general physiological and biochemical activity of DA neurons originating in the midbrain is profoundly regulated by DA receptors. The original experiments by Carlsson and Lindqvist (1963), which studied antipsychotic drug action utilizing biochemical methodology, proposed a feedback regulation of DA neuronal activity by postsynaptic DA receptors. This notion was subsequently confirmed by several electrophysiological studies (Aghajanian and Bunney, 1973, 1977; Bunney and Aghajanian, 1978; Bunney *et al.*, 1973; Einhorn *et al.*, 1988). Moreover, DA activity is also powerfully controlled by so-called autoreceptors, i.e. various DA receptors located along the DA neuron responding to its own neurotransmitter by an inhibition of transmitter synthesis and release, as well as of the firing rate. Conversely, a stimulation of DA activity is observed following administration of DA-D_2 receptor antagonists (Andén *et al.*, 1973; Bunney *et al.*, 1973; Kehr *et al.*, 1972; Roth, 1973; for review see Carlson, 1977; Roth and Elsworth, 1995) and, consequently, the autoreceptors are thought to maintain a certain degree of tonic suppression of DA neuronal activity. At the cellular level, activation of somatodendritic autoreceptors by DA or DA agonists, such as apomorphine, hyperpolarize DA neurons through opening of K^+ channels via pertussis-toxin-sensitive G-protein (Aghajanian and Bunney, 1973; Innis and Aghajanian, 1987; Lacey *et al.*, 1987). Under physiological conditions such an endogenous autoreceptor activation is thought to be maintained by DA released from dendrites of neighbouring DA neurons, rather than by autoinhibition from the releasing neuron itself. Release of DA is also modulated by presynaptic DA autoreceptors located on the nerve terminals, since local or systemic administration of DA agonists has been reported to decrease DA release in the striatum (Kehr *et al.*, 1972; Westerink and de Vries, 1989; Zetterström and Ungerstedt, 1984). Generally such homeostatic regulatory mechanisms may serve a compensatory purpose, serving to antagonize dramatic changes in the functioning of the DA system and thus maintaining its activity within a stable range. However, mesocortical DA neurons appear largely to lack nerve impulse and synthesis-modulating autoreceptors (Chiodo *et al.*, 1984; Roth and Elsworth, 1995) and,

moreover, DA terminals in the deep cortical layers do not seem to be equipped with DA transporters (Sesack *et al.*, 1998). Thus the released transmitter may, especially following burst activity in the neurons, diffuse extrasynaptically, and previous studies indicate that a substantial portion of such DA may be taken up and metabolized in noradrenergic nerve terminals in the region (Gresch *et al.*, 1995). Importantly, the extrasynaptic DA may allow for activation of numerous extrasynaptic D1 receptors in the prefrontal cortex, specifically located on the pyramidal neurons (Smiley *et al.*, 1994), which in turn are thought to regulate the communication between such neuronal ensembles. As a corollary, brain NA uptake inhibitors would be expected to increase the availability of DA in the PFC, by preventing its uptake into noradrenergic terminals, a notion that recently has been confirmed by experimental results (Linnér *et al.*, 2001).

Postsynaptic Effects of DA

The postsynaptic effects of DA in its target areas have been the subject of numerous studies for decades. These actions are manifested both at the level of the individual postsynaptic neuron, with a cascade of intracellular transduction mechanisms being affected (for recent review see Greengard, 2001), and also include presynaptic effects and modulatory effects at the network level, which in addition may vary between different brain regions. This review will focus on some of these postsynaptic actions that may be of particular interest in relation to psychiatric disorders and their pharmacological treatment.

Striatum

Within this much-studied brain region DA has mostly been found to decrease the excitability of neurons in both the dorsal striatum and the NAC by means of D_1 receptor stimulation (Calabresi *et al.*, 1987; O'Donnell and Grace, 1996; White and Wang, 1986), although this notion has been challenged (Gonon, 1997). Here the D_1-mediated inhibition may act synergistically with D_2-receptor-mediated inhibition if agonists are applied at the same time. However, the D_1-induced reduction in excitability can be reversed to facilitation if a D_2 agonist is applied subsequently (Onn *et al.*, 2000). Such a temporal dissociation may have physiological significance. Thus, during burst activation of the DA neurons, e.g. in association with a rewarding stimulus, the ensuing large DA release (see above) would not only activate intrasynaptic D_1 and D_2 receptors, but also probably spill over, out of the synaptic cleft, to stimulate additional extrasynaptic D_1 receptors (Hersch *et al.*, 1995; Jansson *et al.*, 1999). However, with maintained high activity of the DA neurons the continued D_1 receptor activation may serve to attenuate a subsequent D_2 receptor activation, which preferentially is an intrasynaptic event (Onn *et al.*, 2000). Such a mechanism may help to explain the rather rapid attenuation of the response to rewarding stimuli, which is a well-known psychological phenomenon. The cellular mechanisms are different for the D_1- and the D_2-receptor-mediated responses. Thus, whereas D_1 stimulation modulates sodium conductances as well as high-voltage-activated calcium conductances involving PKA-mediated processes (Surmeier *et al.*, 1995), D_2 receptor stimulation modulates voltage-dependent potassium conductances. The D_1 response subsequently involves the phosphorylation of a DA- and cAMP-regulated phosphoprotein, DARPP-32, and this protein is, in turn, a critical component in the whole intracellular cascade of events that mediates D_1-receptor-regulated functions, which include, for example, modulation of glutamatergic transmission (Fienberg *et al.*, 1998; Greengard, 2001). Needless to say, DARPP-32 is by no means exclusively located in D_1 expressing neurons, but is also found in other striatal projection areas and, for example, in enkephalin containing striatal neurons (Langley *et al.*, 1997). D_2 receptor activation may here cause dephosphorylation of DARPP-32 (Nishi *et al.*, 1997). In short, DARPP-32 may generally serve to modulate DA-mediated functions at the striatal outflow level.

Apart from its effects at the cellular level, DA may also influence neuronal functioning in its target areas at the network level, e.g. by modulation of gap junction conductance and the associated interactions between neighbouring neurons, as elegantly demonstrated by Grace and colleagues. Here it appears that whereas D_2 receptor stimulation may cause an increased intercellular coupling, D_1 stimulation seems ineffective. Moreover, the level of coupling *per se* seems to be tonically suppressed by DARPP-32 (Onn *et al.*, 2000).

Finally, DA may modulate the postsynaptic response of striatal neurons to other neurotransmitters as well as the presynaptic regulation of transmitter release. Thus, for example, D_1 receptor activation has been shown to augment NMDA-receptor-mediated currents (Cepeda *et al.*, 1998), i.e. to facilitate glutamatergic transmission, and the D_1-mediated responses have been shown to be preferentially mediated by burst firing of the DA neurons (Chergui *et al.*, 1996, 1997; Gonon, 1997). Other studies show that D_2 receptor stimulation may, at the presynaptic level, decrease release of GABA from intrinsic neurons as well as glutamate release from corticostriatal nerve terminals. Generally, there exists a complex interaction and balance between dopaminergic and glutamatergic neurotransmission in the striatum, where DA may suppress the release of glutamate, whereas glutamate seems to enhance DA release. Overall this arrangement may contribute to maintain a restricted range of activity in striatal neuronal circuits, especially over time, albeit allowing for rapid changes in DA signalling in association with environmental cues. Recent work shows that DA also contributes to synaptic plasticity within the striatum. Thus, following high-frequency stimulation of corticostriatal, glutamatergic afferents, long-term potentiation (LTP) may be obtained, whereas low-frequency stimulation of the same striatal afferents may elicit long-term depression (LTD), and such processes require the presence of the DA afferent input (Centonze *et al.*, 1999). Such phenomena are mostly thought to be related to learning and memory and may thus, in this brain structure, have a bearing on, for example, the learning of various motor behaviours.

Prefrontal Cortex

The role of DA in the PFC has attracted successively increased attention over the years and still remains somewhat controversial, although studies *in vivo* clearly demonstrate an inhibitory effect of DA on PFC neuronal firing. Results *in vitro* have, however, revealed that DA and D_1 agonists may affect the excitability of PFC neurons via several mechanisms, involving both sodium and potassium conductances as well as L-type calcium conductances in the proximal dendrites of the pyramidal neurons (Yang *et al.*, 1999). Generally, the DA input is thought to regulate the glutamatergic input to these cells. The inhibitory effect of DA on pyramidal neurons is, instead, probably related to DA-induced excitation of GABAergic interneurons (Zhou and Hablitz, 1999). To add to the complexity and physiological sophistication of this machinery, studies have shown that the effects of DA on PFC neurons depend on their membrane potential, which normally may vary. Thus D_1 agonists may increase excitability only under certain conditions, i.e. in the depolarized state (Lavin and Grace, 2001).

Mediodorsal Thalamus

Here the dopaminergic input, which originates in the midbrain, may generally contribute to the control of thalamocortical information

processing. Specifically, experiments *in vitro* indicate that DA, acting through D_2 receptors, may facilitate oscillatory activity within the mediodorsal nucleus (Lavin and Grace, 1998).

Basolateral Amygdala

This brain region also receives an innervation from the midbrain DA neurons and here the effects of DA seem to vary between different cell types in a complex way. In addition, DA may serve to control afferent input to these cells from various sources in a differential fashion.

Ventral Pallidum

This DA projection area also receives a dopaminergic innervation from the midbrain DA neurons and, generally, this brain region represents an interface between the limbic and extrapyramidal systems. Therefore, DA in this region may contribute to influence motivated behaviour.

Summary

The effects of DA in its target areas are thus very complex and executed all along the cortico-striato-pallido-thalamocortical loop, which is of prime interest in relation to schizophrenia and its pharmacological treatment. In fact, several loops are interconnected and neurons of the shell region of the NAC not only receive a dopaminergic input from the VTA but also receive inputs from the cerebral cortex (infralimbic, agranular, piriform and insular areas), hippocampus and amygdala. In turn, these neurons project back to the VTA, but also to entorhinal cortex and the PFC, after relaying in the ventral pallidum and mediodorsal thalamus. Thus, the shell of the NAC is involved in a series of feedback and feedforward loops, involving the PFC as well as the VTA. Interestingly, the PFC is included in the heteromodal association neocortex, which in turn comprises other interconnected association areas, such as Broca's area and the inferior parietal cortex. These brain regions are generally involved in highly integrative mental functions, such as speech, learning and memory, and focused attention. The dopaminergic input may in a physiological sense serve to control efficiency in these heteromodal, cortico-ventrostriatal circuits, in analogy with its purported role in motor, cortico-dorsostriatal circuits. DA has been suggested to help maintain stability in such circuits at baseline conditions, but may at the same time serve to enhance or suppress signals, for example related to learning in association with rewarding or non-rewarding stimuli. DA activity may also contribute to direct attention to salient environmental stimuli and cues, and facilitate effective mental and motor strategies. In short, the complex effects of DA in its target areas suggest that this transmitter allows a gating mechanism for selection, acquisition and maintenance of appropriate behavioural programmes.

Behavioural and Clinical Significance of DA Neurons

The DA neurons of the midbrain were in the 1950s found to be implicated in motor tasks related to normal movements, but also, as subsequent studies showed, in motivational and reward-related behaviour as well as cognitive functions (for review see Koob *et al.*, 1998; Le Moal and Simon, 1991; Schultz, 1997, 1998).

The nigrostriatal DA neurons have long been known to be critically involved in the pathophysiology of Parkinson's disease (Carlsson, 1959; Ehringer and Hornykiewicz, 1960), and degeneration of the DA cells in the ventrolateral part of the substantia nigra zona compacta and to a lesser degree also in the VTA is responsible for the typical symptoms of Parkinson's disease, namely bradykinesia, rigidity and tremor. The degeneration of the DA neurons leads to a reduced DA output to neostratium (e.g. caudate-putamen), increasing the activity in the indirect loop to globus pallidus interna and substantia nigra pars reticulata of the basal ganglia, leading to an increased inhibition of motor output from the thalamus (for review see DeLong, 1990). As proposed by Carlsson (1959), the DA precursor L-DOPA was found to provide the first effective treatment for the disease by Birkmayer and Hornykiewicz in 1961, and is still the most effective treatment of Parkinson's disease despite severe side effects affecting up to 75% of the patients after five years of treatment.

The mesolimbocortical DA neurons are profoundly implicated in reward-related behaviour (Robbins and Everitt, 1999), and many drugs of abuse, including cocaine, amphetamine, opiates, ethanol and nicotine, are known to directly or indirectly cause an increased release of DA in terminal areas (Di Chiara, 1995). Moreover, specific lesions of NAC-projecting DA neurons reduce self-administration of nicotine (Corrigall *et al.*, 1992), cocaine and amphetamine (Roberts *et al.*, 1980; Wise and Bozarth, 1987). Rats can also learn to electrically self-stimulate the dopaminergic neurons of the VTA (Corbett and Wise, 1980; Garris *et al.*, 1999; Wise and Rompre, 1989; Wolske *et al.*, 1993). The cholinergic input to the DA neurons has been shown to be implicated in mediating rewarding brain stimulation of the lateral hypothalamus (Yeomans and Baptista, 1997; Yeomans *et al.*, 1985, 1993). In several *in vivo* and *in vitro* studies nicotine has been shown to excite DA neurons (Calabresi *et al.*, 1989; Grenhoff *et al.*, 1986; Picciotto *et al.*, 1998; Pidiplichko *et al.*, 1997), whereas opiates have been shown to inhibit the GABAergic input to these cells, leading to a disinhibition of the DA neurons (Johnson and North, 1992a).

Studies in the monkey (Schultz *et al.*, 1993, 1998) have further analysed the role of the midbrain DA neurons in reward-related tasks and demonstrated that these neurons are, indeed, activated by a primary reward and especially if it is unexpected. If the reward is coupled to a conditioning signal (visual or auditory), the increased firing of the DA neurons will switch from the moment of appearance of the reward to the instant when the conditioning stimulus appears, and not when the actual reward is delivered. Moreover, if the conditioning stimulus is not followed by the expected reward the DA cells decrease their firing. DA neurons can thus detect deviations from the expected reward, so that a positive signal is delivered to the brain if the reward is better than expected, no change if the reward is just as expected, and negative if less than expected. Thus, DA neurons seem to serve two functions: (1) to provide a tonic, generally enabling input on postsynaptic neurons in terminal fields, and (2) to send a phasic, global reinforcement or teaching signal to the brain, which helps to adapt behaviour and motor acts according to the motivational value of environmental stimuli (for review see Redgrave *et al.*, 1999; Schultz, 1997, 1998; Schultz *et al.*, 1997, 1998).

The prefrontal projection of the mesocortical DA neurons in the VTA is considered to modulate the activity of neurons in the PFC related to cognitive functions such as working memory, planning and execution of behaviour, inhibitory response control and maintenance of focused attention (Le Moal and Simon, 1991). Also reward-related stimuli increase dopaminergic activity in this region (Taber and Fibiger, 1997). In particular, D_1 receptor activation has been demonstrated to be required for the adequate performance of working memory tasks (Sawaguchi and Goldman-Rakic, 1994). Indeed, experimental results in both rats and monkeys indicate that not only too little, but also too much D_1 receptor activation may impair cognitive functioning, e.g. working memory (Murphy *et al.*, 1996).

In addition to their implication in reward-related behaviour and cognitive functioning, a putative overactivity of the mesolimbocortical DA system has been considered to be involved in

the symptomatology of schizophrenia (Carlsson, 1988). The 'DA hypothesis of schizophrenia' was based on the fact that: (1) all effective antipsychotic drugs used for treatment of schizophrenia share antidopaminergic activity, in particular through blockade of D_2 receptors, and (2) drugs which directly or indirectly facilitate dopaminergic neurotransmission in brain, e.g. amphetamine or L-DOPA, can precipitate or aggravate psychosis. However, this classical hypothesis was successively challenged by the relative difficulties of finding unequivocal evidence for dopaminergic hyperactivity in the brains of schizophrenic patients, by the relative lack of effect of the typical neuroleptics on negative symptoms, and by the fact that the atypical antipsychotic drug clozapine, despite its lower occupancy of D_2 receptors, has a significantly higher efficacy in refractory patients, as well as an advantageous effect on negative and some cognitive symptoms (Farde *et al.*, 1988, 1992; Kane *et al.*, 1988, Nordström *et al.*, 1995). Clozapine is not only a D_2 receptor antagonist but also has affinity for D_1, D_4 and α_2 adrenergic, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7, muscarinic and histaminergic receptors (for review see Roth *et al.*, 1998), and in contrast to classical neuroleptic drugs it increases DA efflux in the prefrontal cortex (Moghaddam and Bunney, 1990; Nomikos *et al.*, 1994). This is of particular interest in view of the role of prefrontal DA in cognitive functioning and may have a bearing on its effectiveness against cognitive and negative symptoms (Hertel *et al.*, 1999). Consequently, the original DA hypothesis of schizophrenia, proposing a global hyperactivity of brain dopaminergic systems, needed to be revised. A differential dysfunctioning of the dopaminergic systems with hyperactivity, or hyperactivity in some areas and hypofunction in others, seemed more likely (Weinberger *et al.*, 1986). Such a dysfunction of brain DA systems might, in turn, rather than involving any particular pathology of the DA neurons themselves, be related to alterations in the input to these neurons (Nauta, 1976, Svensson and Tung, 1989). Indeed, subsequent preclinical results obtained with the so-called phencyclidine model of schizophrenia proposed the notion of a hyperactive subcortical, striatal DA projection concomitant with an impaired prefrontal DA projection in schizophrenia (Jentsch *et al.*, 1997; Svensson *et al.*, 1993, 1995). Significantly, this experimentally derived notion has subsequently been directly borne out by clinical studies in schizophrenic patients (Breier *et al.*, 1997; Egan *et al.*, 2001; Laruelle *et al.*, 1996). Thus, a novel strategy in the development of improved antipsychotic drug treatment emerged, in the sense that rather than simply to attempt to antagonize a presumed generally overactive DA transmission in brain, one should aim for normalization or stabilization of the above hypo- and concomitantly hyperfunctioning different DA projections at the same time (Svensson *et al.*, 1995). Indeed, clozapine and some other atypicals appear to do so (see above), and it appears very likely that their activity at several receptors other than D_2 receptors may contribute to such an effect (for review see Svensson *et al.*, 1999).

5-HYDROXYTRYPTAMINE

Introduction

A serum vasoconstrictor discovered over a century ago (Brodie, 1900), was isolated, characterized, and named serotonin by Rapport and collaborators in 1948 (Rapport *et al.*, 1948). Soon thereafter, Rapport identified serotonin as 5-hydroxytryptamine (5-HT). Enteramine, a smooth muscle contracting substance that had been isolated from the enterochromaffin cells in the gastrointestinal tract (and also contained in a variety of tissues) by Ersparmer and Vialli in the 1930s, was found to be identical with 5-HT by Ersparmer in the early 1950s (Ersparmer, 1966; Ersparmer and Asero, 1952). A few years later, Twarog and Page demonstrated the existence of 5-HT in mammalian brain (rats, rabbits and dogs) (Twarog and Page, 1953).

The widespread and uneven distribution of 5-HT in the brain led to the proposal that it is a neurotransmitter in the CNS (Bogdansky *et al.*, 1956; Brodie and Shore, 1957). Subsequently, Dahlström and Fuxe, using the then newly developed Falck-Hillarp's histochemical fluorescence methods, mapped the serotonergic cell bodies and showed their extensive projections to all brain regions (Dahlström and Fuxe, 1964; Fuxe, 1965).

Phylogenetically, 5-HT belongs to the oldest known neurotransmitters and is ubiquitously found not only in vertebrates and invertebrates, including the most primitive organisms endowed with a nervous system, but also in plants, e.g. vegetables and fruits (Tyce, 1985; Umbriaco *et al.*, 1990).

Biochemistry

5-HT is synthesized from the essential amino acid tryptophan (TRP). TRP is a major building block for protein synthesis and only a small amount of ingested TRP is used to synthesize 5-HT in a variety of cells, but not in platelets. Tryptophan hydroxylase hydroxylates TRP to 5-hydroxytryptophan (5-HTP), which is decarboxylated to 5-HT by aromatic-L-amino acid decarboxylase (AADC) and pyridoxal-5-phosphate (vitamin B6) as coenzyme. The rate-limiting step in 5-HT synthesis is considered to be the availability of 5-HTP, since AADC activity is at least an order of potency higher than that of tryptophan hydroxylase (Kema *et al.*, 2000). Of the total body 5-HT, only 1–2% is found in the brain (Cooper *et al.*, 1995). 5-HT does not cross the blood–brain barrier (BBB) and thus the brain is dependent on its own synthesis of the transmitter. This process, in turn, is determined by concentrations of free plasma TRP and transport across the BBB. TRP competes with other amino acids — isoleucine, leucine, phenylalanine, tyrosine and valine — to be moved across the BBB by the same large neutral amino acid (LNAA) transporter. The fact that TRP transport is dependent on both its concentration and those of five other amino acids constitutes the physiological basis for the rapid tryptophan-depletion (RTD) test. The test has in the past decade been used to assess the serotonergic function in brain, in particular in depression, and the mechanisms of action of antidepressant and prophylactic treatments (Bell *et al.*, 2001; Delgado *et al.*, 1999; Moore *et al.*, 2000; Reilly *et al.*, 1997). The goal of the RTD test is to decrease brain 5-HT synthesis and release by temporarily reducing/eliminating availability of TRP. This is achieved by administering a load of LNAA not containing TRP to the experimental subjects. The procedure stimulates liver protein synthesis, thereby using TRP and secondarily decreasing TRP transport into the brain.

Using positron emission tomography (PET), Nishizawa and coworkers (1997) have shown that in healthy subjects, men had approximately 50% higher rates of 5-HT synthesis than women and that following tryptophan depletion these differences were increased severalfold. These results are of interest in view of the greater prevalence of and vulnerability to depression in women. Other human studies have shown significantly reduced CSF 5-HIAA levels following the RTD test (Carpenter *et al.*, 1998; Williams *et al.*, 1999), while animal experiments have revealed decreased brain 5-HT and 5-HIAA as well as reduced release of 5-HT from serotonergic raphe nucleus following dietary interventions similar to those in humans (Bel and Artigas, 1996; Gartside *et al.*, 1992a, b; Moja *et al.*, 1989). In this context, for the interpretation of the RTD depletion test it might be relevant that TRP is also a constituent of other CNS regulatory proteins, e.g. a variety of enzymes, receptors, ion channels, neuropeptides, etc., and that possible alterations in their synthesis and function have thus far not been elucidated.

The RTD test has contributed to our knowledge of the role of 5-HT in the mechanisms of action of antidepressant treatments and,

to a limited extent, the pathophysiology of depressive disorders. Perhaps the most interesting, albeit also speculative, conclusions are that while a 5-HT dysfunction may play a role in eliciting depression in patients vulnerable to alterations in 5-HT function and that synaptic 5-HT availability is of therapeutic importance in maintaining euthymic mood in patients treated with selective serotonin reuptake inhibitors (SSRI) (but not with predominantly NAergic drugs), the primary determinants of depression are likely to involve compounds and circuits other than 5-HT, but modulated by it.

The major 5-HT metabolic pathway is deamination by monoamine oxidase (MAO), in particular MAO-A. The intermediary MAO product, 5-hydroxyindoleacetaldehyde, is a substrate for two enzymes: aldehydedehydrogenase, resulting in 5-hydroxyindoleacetic acid (5-HIAA), and alcohol dehydrogenase, leading to formation of 5-hydroxytryptophol (5-HTOL). 5-HIAA is by far the major metabolic product of 5-HT and its measurements in human CSF and brain tissue and in awake freely moving animals also in microdialysates from specific brain regions, have been a standard and fruitful tool to assess 5-HT release and metabolism. Determination of 5-HTOL is a useful index to study effects of ethanol on 5-HT. Minor metabolic routes for 5-HT are *N*-acetylation by serotonin-*N*-acetyltransferase and 5-*O*-methylation by hydroxyindole-*O*-methyltransferase leading to synthesis of melatonin (5-*O*-methyl-*N*-acetylserotonin). Lastly, in parallel to other biologically active compounds, elimination also takes place via conjugation with sulphuric and glucuronic acids.

5-HT Nuclei, Pathways and Receptors

5-HT cells are localized in brainstem nuclei called the raphe nuclei. The original classification of Dahlström and Fuxe (1964) into nine cell clusters, B_9-B_1, based on rostral to caudal topography, has generally been superseded by categorizing the cells into the caudal linear nucleus, the dorsal raphe nucleus (DRN), the median raphe nucleus (MRN) and the caudal nuclei. For a more detailed description see, for example, Jacobs and Azmitia (1992). The DRN is the largest nucleus, containing 50% or more of the 5-HT neurons.

Afferent input into the raphe nuclei comes from several brain regions, most notably hypothalamic areas, substantia nigra, LC, ventral tegmental area (VTA), prefrontal cortex, amygdala, NAC, etc., and is mediated by, for example, GABA, excitatory amino acids, NA, substance P, NT and vasointestinal polypeptide (VIP). Notably, the serotonergic neurons in the dorsal raphe neurons receive an excitatory noradrenergic input mediated by postsynaptic α_1 adrenoceptors, and thus inhibition of this input causes inhibition of serotonergic cell firing (Svensson, 2000a; Svensson *et al.*, 1975).

Efferents from the rostral nuclei, mostly the DRN and MRN, project ascendingly to telencephalon and diencephalon. The caudally located nuclei, on the other hand, project downward to the spinal cord and the cerebellum. In contrast to the more circumscribed DA pathways, virtually all brain regions receive 5-HT input (for review see Piñeyro and Blier, 1999) and these 'all encompassing' projections constitute the anatomical/functional basis for the wide array of 5-HT roles in CNS (Heinz *et al.*, 2001; Jacobs and Fornal, 1995; Lanctôt *et al.*, 2001; Shiah and Yatham, 2000; Rajkowska, 2000).

Actions of 5-HT are mediated by a multitude of 5-HT receptors. Although each receptor is activated by 5-HT itself, an array of agonists and antagonists has been synthesized to act more selectively on each subtype. A detailed description of 5-HT receptors is beyond the scope of this chapter and only the most salient points are taken up here. In parallel to classification of other receptors, the amino acid sequence of the receptor protein, the intracellular transduction mechanisms (that is, G-protein-linked or ligand-gated ion channel) and drug-related characteristics (selective agonists and antagonists) have been employed to identify/classify the 5-HT receptor subtypes. The pioneering work of Peroutka and Snyder (1979) using labelled 5-HT, LSD and spiroperidol established the existence of multiple 5-HT receptors. Several reviews on this subject are available (Barnes and Sharp, 1992; Hoyer and Martin, 1996, 1997; Hoyer *et al.*, 1994). The currently recognized receptor families are: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7. 5-HT_1 receptors are further subdivided into A, B, D, E and F; 5-HT_2 into A, B and C; and 5-HT_5 into A and B. Anatomical distribution is wide and most brain regions possess multiple receptor subtypes. Thus, in the limbic structures such as hippocampus, septum and amygdala there are 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B} and 5-HT_6 receptors; in striatum 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4 and 5-HT_6 receptors; in raphe nuclei 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5B}, and 5-HT_7 receptors; in cortex 5-HT_{1B}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_3 receptors; in hypothalamus 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{5A} receptor; in choroid plexus 5-HT_{2C} receptor. The abundant and widespread localization of 5-HT receptors in the limbic system, raphe nuclei, mesolimbic pathways and choroid plexus gives an indication of the role 5-HT plays in regulation of affect, its own release, modulation of DA function and cerebrospinal fluid characteristics, respectively (Blier, 2001; Kilpatrick *et al.*, 1996; Piñeyro and Blier, 1999).

Transduction mechanisms involve the G-protein-coupled superfamily. Thus, all five 5-HT_1 receptors induce membrane hyperpolarization and are negatively linked to adenylyl cyclase; 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 are positively coupled to adenylyl cyclase with generation of cAMP; activation of the three 5-HT_2 subtypes induces depolarization and these receptors transduce via activation of phospholipase C and effects on phosphoinositides with generation of two second messengers, inositol triphosphate and diacylglycerol. In contrast, the 5-HT_3 receptor belongs to the ligand-gated ion channel family (analogous to, for example, glycine receptors, $GABA_A$). This is unique not only for the 5-HT receptors but also among other monoamine and diamine neurotransmitter receptors (Hoyer and Martin, 1996; Hoyer *et al.*, 1994). Last but not least, effects of a given transmitter are determined not only by its receptor subtypes and their regional distribution, but also by pre- and/or postsynaptic localization and whether they exert inhibitory or facilitatory actions. The 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}, and also 5-HT_3 are both pre- and postsynaptic, while 5-HT_{1E} and 5-HT_{1F} are postsynaptic. The three types of 5-HT_2 receptor are postsynaptic (although 5-HT_{2C} may also be presynaptic). The 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 are postsynaptic. The role of 5-HT pre- and postsynaptic receptors in 5-HT synthesis and release is reviewed in a subsequent section.

Transmitter Release and Activity of 5-HT Neurons

In neurons, 5-HT is stored in vesicles and released by exocytosis, a process by which synaptic vesicle fuses with the cell membrane. Exocytosis, the main mechanism for release of transmitters, is triggered by depolarization (classically induced by K^+) and involves membrane proteins, for example synapsin I. A second release mechanism is carrier-mediated (Levi and Raiteri, 1993); 5-HT from the cytoplasmic pool is released by that kind of mechanism. The carrier-mediated release is not modulated by presynaptic receptors and is blocked by serotonin transporter (SERT) inhibitors (Levi and Raiteri, 1993). SERT mediates the 5-HT release evoked by drugs of abuse such as PCP, MDA and 'ecstasy' (MDMA) (Berger *et al.*, 1992; Bonanno *et al.*, 1994).

5-HT release from the neuronal cell body is regulated by somatodendritic 5-HT_{1A} autoreceptors which exert feedback control on neuronal firing. In addition, release is controlled by terminal 5-$HT_{1B/1D}$ autoreceptors, in e.g. cortex and hippocampus.

In contrast to release via 5-HT_{1A} receptors, this release can be modulated without changing the firing activity of 5-HT neuron (Crespi *et al.*, 1990). Experiments in rodents indicate that 5-HT_{1B} and 5-HT_{1D} receptors negatively regulate 5-HT release from MRN (Piñeyro and Blier, 1999; Piñeyro *et al.*, 1995, 1996). The release in cortex and hippocampus is negatively regulated by activation of 5-HT_{1B} terminal autoreceptors (Middlemiss and Hutson, 1990).

Heteroregulation of release in DRN and MRN occurs via GABA and GABA agonists that exhibit inhibitory effects (Becquet *et al.*, 1990) while substance P and direct and indirect DA agonists have facilitatory effects (Ferré *et al.*, 1994; Reisine *et al.*, 1982). As regards heteroregulation in the terminal projection areas, α_2 adrenoceptors appear to play an important regulatory role. Available evidence indicates that hetero- and auto-α_2 adrenoceptors regulating release of 5-HT and NA, respectively, have different properties (Blier *et al.*, 1993; Martire *et al.*, 1989; Mongeau *et al.*, 1993; Raiteri *et al.*, 1990). Acetylcholine increases 5-HT release in forebrain by nicotinic receptor activation, while histamine inhibits it, most likely acting via the H_3 heteroreceptor (Fink *et al.*, 1990; Summers and Giacobini, 1995).

SSRIs are highly efficient in increasing extracellular concentrations of 5-HT. Thus, acute administration of a high dose (e.g. $10\,mg\,kg^{-1}$) of any of the typical SSRIs, such as citalopram, sertraline or fluoxetine, will cause increased 5-HT levels in microdialysates from serotonergic terminal areas, such as striatum, cortex and diencephalon (Dailey *et al.*, 1992; Fuller, 1994; Invernizzi *et al.*, 1994; Rutter and Auerbach, 1993). Administration of lower doses of SSRIs (e.g. $1\,mg\,kg^{-1}$) does not elevate 5-HT in microdialysates from the projection areas but has a significant effect on 5-HT in the raphe nuclei. In turn, this increase in somatodendritic extracellular 5-HT leads to activation of inhibitory 5-HT_{1A} autoreceptor located on the soma and dendrites of 5-HT neurons (Bel and Artigas, 1992, 1993; Invernizzi *et al.*, 1994).

The immediate consequence is decreased serotonergic cell firing and, consequently, inhibition of 5-HT release in the terminal projection areas. The long-term sequelae are 5-HT_{1A} autoreceptor desensitization (Arborelius *et al.*, 1995; Svensson 1978) and a rebound of release (Arborelius *et al.*, 1996). Elucidation of these mechanisms has led to animal studies and clinical augmentation trials with 5-HT_{1A} antagonists (Bordet *et al.*, 1998; Maes *et al.*, 1996; Zanardi *et al.*, 1998). This topic, including the usage of pindolol, an agonist/weak antagonist of 5-HT_{1A} somatodendritic receptors (Arborelius *et al.*, 2000; Cremers *et al.*, 2001), is further reviewed in subsequent sections. Interestingly, in animal experiments, prolonged administration of SSRIs, such as paroxetine ($10\,mg\,kg^{-1}$) or fluoxetine ($5\,mg\,kg^{-1}$), was found also to diminish the effects of 5-$HT_{1B/1D}$ receptor inhibition of electrically induced 5-HT release (El Mansari and Blier, 1996; Piñeyro and Blier, 1999).

In similarity to SSRIs, acute administration of MAO inhibitors (MAOI) decreases 5-HT neuronal firing. Long-term treatment with MAOI also produces a recovery of 5-HT cell firing and increases the release of 5-HT. However, the mechanisms involved appear to be different from those following SSRIs since the terminal 5-HT_{1B} autoreceptor sensitivity appears not to be altered. A likely explanation is desensitization of α_2 adrenergic heteroceptors as proposed by Blier and co-workers (Blier and Bouchard, 1994; Blier *et al.*, 1986a, b). Interestingly, mianserin and mirtazapine (two antidepressants that antagonize α_2 adrenoceptors) also desensitize α_2 adrenergic heteroceptors on 5-HT neurons (Haddjeri *et al.*, 1997). Lastly, clinically used 5-HT_{1A} agonists, e.g. buspirone, also require a few weeks for their antidepressive/anxiolytic effects to develop. This delay in onset is apparently due to the recovery of the initial inhibitory 5-HT_{1A} autoreceptor effects with concomitant stimulation of the postsynaptic receptors (Haddjeri *et al.*, 1998).

The activity is both auto- and hetero-regulated. 5-HT decreases the firing rate of 5-HT neurons (Aghajanian *et al.*, 1972). This effect is mediated via somatodendritic 5-HT_{1A} receptors, as shown by the effects of 5-HT_{1A} agonists; 8-OH-DPAT is perhaps the one most extensively studied; other compounds explored *in vivo* are LSD, buspirone, (S)-UH-301 and (+)-WAY100135 (Aghajanian *et al.*, 1972, 1987; Arborelius *et al.*, 1994, 1995, 1996; Blier and Montigny 1994; Fornal *et al.*, 1994). α_1-Adrenoceptor antagonists also suppress the firing activity of 5-HT neurons (Baraban and Aghajanian, 1980; Haddjeri *et al.*, 1996). Importantly, administration of SSRIs and MAOIs results in a rapid 5-HT increase in extracellular concentrations at the somatodendritic level (Artigas, 1993; Sharp and Hjorth, 1990) and, as a consequence, activation of the 5-HT_{1A} autoreceptors and decreased 5-HT release. However, chronic administration of tricyclic antidepressants as well as SSRIs, 5-HT_{1A} agonists and MAOIs results in desensitization of somatodendritic 5-HT_{1A} autoreceptors. In turn, this leads to a greater 5-HT activity in terminal areas compared to the initial effects of antidepressants on 5-HT (Arborelius *et al.*, 1995, 1996; Blier and de Montigny, 1983, 1985; Blier *et al.*, 1986a, b; Svensson, 1978). Indeed, such a mechanism may well, at least partly, account for the typical delay in onset of the drugs' effects, as originally proposed by Svensson (1978).

Manipulation of 5-HT neuronal firing has accordingly been tried to shorten the time for onset of the therapeutic effects of antidepressant drugs. Artigas and co-workers gave pindolol to depressed patients treated with SSRIs and obtained a rapid improvement in their clinical condition (Artigas *et al.*, 1994). The results were initially attributed to pindolol's blockade of somatodendritic 5-HT_{1A} autoreceptors and thereby antagonism of the feedback inhibition of 5-HT neuronal firing caused by SSRIs. In a recent study, acute (−)pindolol reduced the firing rate of the 5-HT neurons. However, it did not abolish the inhibition of the 5-HT neuronal activity caused by citalopram but, in fact, further decreased the firing rate. The results were interpreted as showing that pindolol is both an agonist and a weak antagonist of the 5-HT_{1A} somatodendritic receptors (Arborelius *et al.*, 2000).

Pindolol is also a partial β adrenoceptor agonist, with significantly higher affinity for the β adrenoceptor than the 5-HT_{1A} receptor (Hoffman and Lefkowitz, 1990; Sánchez *et al.*, 1996). In view of this fact, Cremers and co-workers (Cremers *et al.*, 2001) hypothesized that its beneficial effects might be due to the action on β adrenoceptor and not the 5-HT_{1A} autoreceptor. This hypothesis was tested in guinea-pigs. In microdialysis studies of the ventral hippocampus, systemic administration of paroxetine augmented, as expected, the extracellular levels of 5-HT. Pretreatment with pindolol in doses resulting in brain levels $>600\,nmol\,l^{-1}$ were required to augment the 5-HT increases elicited by systemic paroxetine, while concentrations of only $3\,nmol\,l^{-1}$ were needed to antagonize the β adrenoceptor-agonist-induced increases in brain cAMP. Considering that approximately 100-fold higher plasma pindolol concentrations compared to the clinical plasma levels are necessary to enhance SSRI effects, it was hypothesized that SSRI augmentation is not secondary to blockade of somatodendritic 5-HT_{1A} autoreceptors.

In summary, the experiments reviewed above demonstrate the plasticity of the 5-HT receptor system and that the net result of the autoreceptor transmission changes is an increased release of 5-HT.

5-HT Reuptake

In parallel to DA and NA, following release into the synaptic cleft 5-HT is either metabolized or actively transported back into the neuron by a high-affinity transporter, SERT. SERT is a membrane polypeptide located presynaptically. Intracellular 5-HT is subsequently carried by a vesicular transporter into secretory vesicles, where it is stored. SERT has been solubilized, purified and reconstituted after purification from a variety of sources, e.g. rat

brain and platelets, human CNS and platelets (Biessen *et al.*, 1990; Ramamoorthy *et al.*, 1993a). Moreover, it was found to be encoded by a single gene on chromosome 17 and has also been cloned (Blakely *et al.*, 1991; Lesch *et al.*, 1993; Ramamoorthy *et al.*, 1993b). SERT is expressed both in neuronal and non-neuronal cells. Early binding studies were carried out with 3H-imipramine (Langer *et al.*, 1980), and subsequently with SSRIs, e.g. ^{3}H-paroxetine and ^{3}H-citalopram (D'Amato *et al.*, 1987; Descarries *et al.*, 1990; Habert *et al.*, 1985). Imipramine binding shows a significantly higher density than binding of SSRIs, a phenomenon explained by the fact that it binds to a high- and a low-affinity site. SSRIs, on the other hand, bind only to the high-affinity site, the site that appears to be related to uptake of 5-HT. SERT has been successfully studied in human subjects *in vivo* using PET. SERT was clearly identified in several brain regions, such as the frontal cortex, occipital cortex, hypothalamus and brainstem (Kuikka *et al.*, 1995). As for cellular localization of SERT, its presence in neurons has been demonstrated by immunocytochemistry. In contrast, in glial cells, depending on the antibody used SERT has been found by some (Anderson *et al.*, 1992; Lawrence *et al.*, 1995) but not by other research groups (Piñeyro and Blier, 1999; Qian *et al.*, 1995). SERT mRNA is found in neurons of the raphe nuclei coinciding with the localization of 5-HT cell bodies (Austin *et al.*, 1994; Fujita *et al.*, 1993). SERT mRNA has also been identified in the hippocampus, neostriatum and the frontal cortex, although the levels are substantially lower (Lesch *et al.*, 1993).

The mechanisms of uptake have been investigated for almost three decades, originally in platelets and following SERT cloning in different expression systems (Blakely *et al.*, 1991; Corey *et al.*, 1994; Demchyshyn *et al.*, 1994; Ramamoorthy *et al.*, 1993a, c; Rudnick, 1977). 5-HT is the specific substrate for SERT. However, 5-HT derivatives as well as PCP, amphetamine and cocaine are also substrates, although substances of abuse, e.g. cocaine, exert most of their effects by antagonizing DA uptake. In contrast, TRP, 5-HTP, 5-HIAA, and also NA and DA (in concentrations up to 10 μM) do not bind significantly (Corey *et al.*, 1994; Barker and Blakeley, 1995; Ramamoorthy *et al.*, 1993a, b; Wölfel and Graefe, 1992). 5-HT is taken up as cation and the process is dependent on the extracellular Na^+ and Cl^- and intracellular K^+ fluxes. The Na^+ gradient is the driving force for the 5-HT uptake, and Na^+ and 5-HT fluxes are directly coupled by SERT. Obviously, tricyclic antidepressants and SSRIs have SERT as their primary target. The question of the number of binding sites has been a subject of extensive exploration. Binding studies of labelled 5-HT, imipramine, paroxetine; chemical modification of SERT; and using antibodies directed against different parts of SERT (Langer and Schoemaker 1988; Langer *et al.*, 1980, 1987; Marcusson *et al.*, 1992; Tarrant and Williams, 1995) indicate that there are two separate but overlapping recognition sites for 5-HT, tricyclic antidepressants and non-tricyclic antidepressants. Important in this context is the fact that in addition to SERT, tricyclic antidepressants also bind at the NA reuptake transporter. Consequently, the relative potency of binding at those two sites constitutes, in part, the biological basis for the different clinical profiles of some classical tricyclics; for examples, chlomipramine and imipramine have more potent effects on 5-HT, while desipramine and nortriptyline primarily affect NA.

Behavioural and Clinical Significance

In the late 1960s Aghajanian and collaborators described the electrophysiological characteristics of presumed 5-HT neurons within the midbrain raphe nuclei in the anaesthetized rat. The cells were found to display a rather regular and slow firing pattern (Aghajanian *et al.*, 1968). Later they definitely established that the neurons were indeed serotonergic by combining single-cell recording with an intracellular double-labelling method (Aghajanian and VanderMaelen 1982b), and subsequent studies in awake animals, e.g. by Jacobs and collaborators, further documented the physiological characteristics of these neurons (see below).

Interestingly, the serotonergic neurons in the DRN seem to be subjected to a tonic excitatory input from noradrenergic neurons, at least as judged by experiments in anaesthetized animals. Thus, when the noradrenergic input is inhibited after administration of either clonidine, which suppresses the activity of NA cells, or by blocking α_1 adrenoceptors, which are located on the serotonergic neurons, the activity of midbrain 5-HT neurons is inhibited (Baraban and Aghajanian, 1980; Svensson *et al.*, 1975). *In vitro*, the majority of serotonergic cells in the DRN are silent, probably due to interruption of the noradrenergic input, but they can be activated by adding NA or the α_1 adrenoceptor agonist phenylephrine into the medium (Aghajanian *et al.*, 1987).

The activity of 5-HT cells in the DRN has been found to change in relation to the sleep–wake–arousal cycle (Jakobs and Azmitia, 1992). Thus, the activity is at its highest during active waking, but becomes slower and more regular during quiet waking. When an animal is entering slow-wave sleep (SWS) the 5-HT cell activity becomes even slower and also irregular, whereas during REM sleep the 5-HT neurons in the DRN are completely silent. These changes in activity of 5-HT cells in the DRN have been found to be associated with corresponding changes in the extracellular concentration of 5-HT in the DRN (Portas and McCarley, 1994). Thus, the concentrations of 5-HT appear to be highest during waking, to decrease during SWS and to become even further reduced during REM sleep. However, the light–dark cycle does not appear to influence the activity of these neurons, at least not in the cat. In several studies Jacobs and co-workers have studied the activity of 5-HT cells in the DRN in behaving cats. They found that the activity of the 5-HT neurons was not altered by a wide range of physiological challenges, e.g. changes in body temperature, blood pressure, environmental stimuli, such as confronting the animal with a natural enemy, or by exposure to painful stimuli. However, studies in cats have also shown that during some types of repetitive motor activities, i.e. running or chewing and biting, the activity of a subgroup of DRN 5-HT neurons is increased, whereas during orienting behaviours the activity of some 5-HT neurons is suppressed. It was hypothesized that a high 5-HT neuronal activity, associated with repetitive motor behaviours, might function to suppress sensory inputs. However, the reduced 5-HT activity, found in association with orienting behaviour, might instead serve to facilitate sensory functions (for review see Jacobs and Azmitia, 1992).

Pharmacological studies using electrophysiological techniques demonstrate that serotonin in brain may indeed serve to specifically modulate sensory responsiveness. Thus, the serotonergic input to the noradrenergic LC has been shown to selectively suppress glutamate-evoked responses of LC neurons, while leaving basal activity largely unaffected (Aston-Jones *et al.*, 1991; Chouvet *et al.*, 1988). In addition, SSRIs such as fluoxetine, sertraline and citalopram were subsequently found to significantly suppress LC hyperactivity evoked by excitatory amino acid inputs, which mediate activation responses to various sensory stimuli (Akaoka and Aston-Jones, 1993; Engberg 1992). This mechanism might have clinical significance. Thus, the intense arousal associated with panic attacks in all probability involves profound activation of brain NA neurons in the LC and, moreover, such LC activations are most likely driven by some of its major, excitatory afferent inputs, which utilize excitatory amino acids as neuronal messengers. Accordingly, a continued treatment of panic disorder with SSRIs may help to suppress part of the most intense arousal reactions through the NA–5-HT interaction in the LC, and such an effect may gradually

contribute to extinguish the overall neurobiological hyperreactivity (Svensson 1987, 2000a).

In short, a critical behavioural function of serotonin in the brain may be to dampen the responsiveness of the individual to sensory stimuli of salient nature. Although it is beyond the scope of the present review to cover the full pharmacological significance of central serotonergic systems in psychiatry, the multitude of indications for the SSRIs, for example, demonstrate the profound importance of serotonin in modulating behaviour, including aggression, sexual behaviour and anxiety as well as depression, a modulatory influence that may even include the personality in itself. Thus, brain 5-HT remains one of the most important transmitters in biological psychiatry.

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V

The Excitatory Amino Acid System

Daniel T. Monaghan, Patrick A. Howson, David E. Jane and Richard J. Bridges

As one of the last major neurotransmitter systems to be identified, our appreciation of the roles of L-glutamate in synaptic signalling has progressed from the point where it was considered a nonspecific excitant to our present recognition of it as the primary excitatory transmitter in the mammalian central nervous system (CNS). Beyond its participation in standard fast synaptic communication, this excitatory amino acid (EAA) contributes to higher-order processes ranging from synaptic plasticity to neuropathology. The identification and characterization of the receptors through which L-glutamate mediates these processes has led to an understanding of the fundamental signalling machinery of most excitatory synapses in the brain. Instead of being passive transducers of chemical to electrical signals in the dendrite, these receptors display a diversity of physiological and molecular properties that enable them to play dynamic roles in the integration and modulation of synaptic signals. In this chapter, we provide a overview of three components critical to the operation of an EAA synapse: the ionotropic receptors, the metabotropic receptors, and the glutamate transporters.

IONOTROPIC GLUTAMATE RECEPTORS

L-Glutamate excites, or depolarizes, almost all neurons in the vertebrate CNS by activating ligand-gated ion channel receptors. These ionotropic glutamate receptors (Table V.1) span the plasma membrane, and the binding of L-glutamate causes a conformational change that opens a pore formed by the receptor. The opened ion channel allows the flux of Na^+, K^+ and sometimes Ca^{++} ions. It is the influx of these positively charged ions that causes the cell to depolarize. Early studies by Watkins and colleagues identified three classes of L-glutamate ionotropic receptors responsible for the depolarizing actions of L-glutamate. These were named for glutamate analogues that selectively activate each receptor class, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), and kainate (for review, see Watkins, 1981). With the development of receptor-selective antagonists, it then became possible to prove that these receptors are responsible for synaptic transmission throughout the brain. These discoveries have established glutamate as the major excitatory neurotransmitter in the vertebrate CNS and, in the process, have revealed several important and unexpected aspects of synaptic transmission.

NMDA Receptors

NMDA receptors have a distinctive role in synaptic transmission due to their unique physiological properties. When NMDA receptor antagonists first became available, initial studies could not identify a specific synaptic response mediated by these receptors, even though NMDA receptors are distributed throughout the brain. Soon, it was discovered that NMDA receptors have novel physiological properties that enable them to play an important role in synaptic transmission, but not in mediating the primary fast synaptic response in a glutamate-using synapse. Under normal physiological conditions, a single stimulation of a synaptic pathway results in a glutamate receptor-mediated synaptic response but with little detectable NMDA-receptor-mediated component (Collingridge *et al.*, 1988). Instead, most of the synaptic current is mediated by AMPA receptors. The apparent absence of an NMDA receptor synaptic component is due to the voltage-dependent properties of the receptor. In contrast to most other ion channel receptors, NMDA receptor currents are both ligand gated and voltage gated (Flatman *et al.*, 1983; MacDonald *et al.*, 1982). To observe more robust NMDA receptor responses, the cell must be moderately depolarized from the resting potential. Thus, partial depolarization results in larger NMDA-evoked currents, even though the voltage-gradient driving force responsible for these currents is reduced. The voltage dependency of NMDA receptors is due to the preferential blockade of NMDA receptor channels by Mg^{++} ions at negative membrane potentials (Mayer *et al.*, 1984; Nowak *et al.*, 1984). Hence, at the normal negative resting membrane potential, physiological concentrations of Mg^{++} ions potently block NMDA receptor channels. However, if the cell has been depolarized previously, such as during high-frequency stimulation, Mg^{++} ions can no longer block activated NMDA receptors, and a larger current results. Thus, NMDA receptor responses are dependent upon the immediately preceding history of the cell, with larger NMDA receptor responses occurring if the cell is currently depolarized from a previous synaptic signal.

Another important property of the NMDA receptor is calcium permeability. Unlike most ligand-gated ion channels, the NMDA receptor has a high calcium permeability in addition to sodium and potassium permeability. In many cases, it is this influx of calcium that is thought to be responsible for the subsequent biological actions induced by NMDA receptor activation. With the combination of these two distinctive physiological properties (voltage dependency and calcium permeability), NMDA receptors are able to use calcium as a trigger for experience-dependent plasticity, most notably the phenomena known as long-term potentiation.

While NMDA receptors are gated by L-glutamate, these receptors also require the presence of another agonist, glycine, to achieve channel activation (Johnson and Ascher, 1987). Thus, L-glutamate or NMDA alone is insufficient to evoke an NMDA-receptor-mediated response. Likewise, glycine alone is insufficient to evoke an NMDA receptor response, but glycine together with L-glutamate results in potent receptor activation. In many preparations, the glycine binding site appears to be saturated, or nearly saturated, in the absence of exogenous glycine. Hence, the presence of endogenous glycine, or a related agonist, makes the receptor responsive to the addition of L-glutamate by itself. Because of the apparent tonic saturation of the glycine site, relatively few studies

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Table V.1 Ionotropic receptor pharmacology

Receptor	Agonists	Affinity (μM)	Antagonists	Affinity (μM)
NMDA				
NR1	Glycine	0.1	7-Chlorokynurenate	0.4
	D-Serine	0.4	5,7-DiCl-kynurenate	0.1
			L-689,560	0.006
NR2A-D	NMDA	2	D-AP5	2
	L-Aspartate	3	EAB-515	0.1
	L-Glutamate	0.3	D-CPPene	0.1
	L-CCG-IV	0.02	CGS-19755	1
Channel	MK-801	0.03		
	PCP	0.06		
	Ketamine	10		
AMPA				
Glutamate binding site	L-Glutamate	0.3	NBQX	0.06
	AMPA	0.05	DNQX	0.3
	Quisqualate	0.1	CNQX	0.3
	Kainate	10		
Positive modulator site	Aniracetam	–		
	Cyclothiazide	10		
Noncompetitive antagonist site			GYKI53655	5
Kainate				
Glutamate binding site	L-Glutamate	0.5	CNQX	1.5
	Kainate	0.05	NBQX	5
	Domoate	0.01	NS-102	0.6–10
	Quisqualate	0.2		
	AMPA	50		

L-CCG-IV, (2*S*1′*R*,2″*S*)-2-(Carboxycyclopropyl)glycine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D-2-amino-5-phosphonopentanoate; D-CPPene, D-4-(3-phosphonoprop-2-enyl) piperazine-2-carboxylic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; NBQX, 6-nitro-7-sulphamoyl[*f*]quinoxaline-2,3-dione.

have examined the role of cellular mechanisms that modulate glycine levels in the synapse. However, there is some evidence that both glycine and D-serine (also an agonist at the glycine binding site) may have their extracellular levels regulated at subsaturating concentrations, and hence may play a role in modulating the excitability levels of NMDA receptors (Danysz and Parsons, 1998).

Molecular Properties of NMDA Receptors

NMDA receptors are a multimeric complex composed of subunits derived from three related families: NR1, NR2 and NR3 subunits (Nakanishi, 1994; Seeburg *et al.*, 1995; Sucher *et al.*, 1995). Both the NR1 and NR2 subunits are required for receptor function. The NR1 subunit contains the glycine binding site, and the NR2 contains the L-glutamate binding site (Laube *et al.*, 1998). In contrast, the NR3 subunit appears to modulate receptor function in a limited number of situations. Multiple lines of evidence suggest that there are two NR1 subunits and two NR2 subunits in a single NMDA receptor complex. Thus NR1/NR2 complexes may exist as a tetramer (Laube *et al.*, 1998). However, the effect on the stoichiometry of including NR3 subunits is unknown.

In the early 1990s, the laboratories of Nakanishi and Mishina identified cDNA encoding NMDA receptor NR1 subunits from rat and mouse, respectively (Moriyoshi *et al.*, 1991; Yamazaki *et al.*, 1992). The corresponding protein, NR1 (also termed NMDAR1 for rat and $\zeta 1$ for mouse), is over 900 amino acids in length and displays 22–26% identity with AMPA and kainate receptor subunits. The NR1 gene consists of 22 exons; exons 5, 21 and 22 can be alternatively spliced, resulting in eight distinct NR1 isoforms (Hollman *et al.*, 1993; Sugihara *et al.*, 1992). Exon 22 includes a stop codon and hence has a different C-terminal than proteins that do not have exon 22.

Subsequent to the cloning of the NR1 subunit, the NR2 subunits were identified (Ikeda *et al.*, 1992; Ishii *et al.*, 1993; Kutsuwada *et al.*, 1992; Meguro *et al.*, 1992; Monyer *et al.*, 1994; Monyer *et al.*, 1992). The four members of the NR2 subunit family (NR2A–NR2D for rat and $\varepsilon 1$–$\varepsilon 4$ for mouse) are the products of four separate genes. The physiological and pharmacological properties of native and recombinant NMDA receptors vary with the specific NR2 subunit present in the heteromeric complex (Buller *et al.*, 1994; Monyer *et al.*, 1994; Monyer *et al.*, 1992; Williams, 1993). NMDA receptors containing NR2A subunits are potently inhibited by zinc. The NR3 was initially termed χ-1, (Ciabarra *et al.*, 1995; Sucher *et al.*, 1995). Among various glutamate receptor subunits, NR3 has highest identity with NR1 and NR2 subunits (27%). When coexpressed with NR1/NR2B in oocytes, it reduces the magnitude of current responses. Intriguingly, in the NR3 knockout mouse (Das *et al.*, 1998), NMDA-receptor-mediated responses are larger and spine density is increased.

Subunit Topology

When the first glutamate receptor was cloned, there was a high expectation that the receptor would be a member of the so-called ligand-gated ion channel superfamily (e.g. nicotinic, glycine and γ-aminobutyric acid A (GABA-A) receptor channels), which contain four hydrophobic transmembrane domains. Thus, when the initial sequence was identified, it was generally accepted that the four

apparent hydrophobic domains represented transmembrane domains in a pattern analogous to nicotinic and GABA receptors. For these receptors, both the N- and C-terminals are on the extracellular side of the cytoplasmic membrane, consistent with having four intervening transmembrane domains. Subsequent analysis of glutamate receptors revealed, however, that the second hydrophobic domain (M2) did not pass through the membrane but formed a loop within the membrane and had both ends of M2 on the cytoplasmic side of the membrane (Hollmann *et al.*, 1994). This model leaves three transmembrane domains (designated TM1, TM3 and TM4 — from the original nomenclature) with the C-terminal inside the cell (Figures V.1 and V.2).

The three transmembrane topology model of glutamate receptors is supported further by the identification of the glutamate binding site. X-ray crystallographic analysis shows that the glutamate binding site (of the related AMPA receptor) is a bilobed structure (Armstrong *et al.*, 1998). Glutamate binding causes the two lobes to approach each other and, presumably, to open the ion channel in the process (Armstrong and Gouaux, 2000). One lobe arises from the extracellular N-terminal peptide sequence that immediately precedes TM1; the other lobe is formed by the peptide sequence found between TM3 and TM4. These portions of the glutamate receptor display a modest sequence homology and strong three-dimensional structural similarity to bacterial amino acid binding sites. Thus, the three transmembrane topographic structure is important for placing the two lobes of the glutamate binding site on the extracellular surface of the cell.

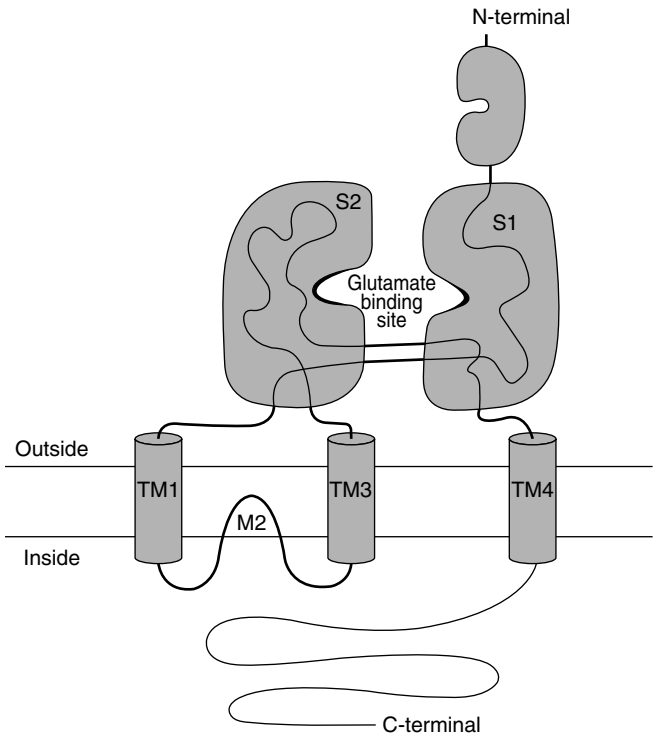

Figure V.1 Ionotropic glutamate receptor structure. Individual ionotropic glutamate receptors subunits have a multidomain structure with an initial N-terminal domain having homology to bacterial amino acid binding proteins (leucine-isoleucine-valine-binding protein, LIVBP). Glutamate (or glycine in the case of NR1 subunits) binds to a cleft between two domains, S1 and S2, which have homology to the bacterial amino acid binding protein lysine-arginine-ornithine-binding protein (LAOBP). There are three transmembrane domains (TM1, TM3, TM4) and one intramembrane loop (M2). In addition, there is an intracellular C-terminal, which can be relatively large and provide an important site for receptor regulation and signal transduction

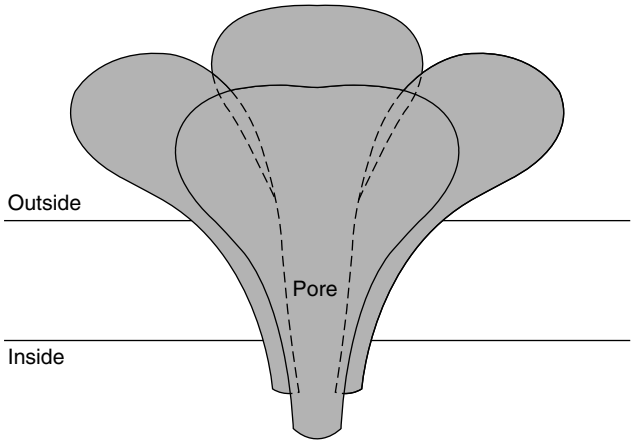

Figure V.2 The glutamate receptor complex is currently thought to be composed of four glutamate receptor subunits, with the ion channel pore formed by the TM1, M2, and TM3 portions of the four subunits

Protein–Protein Associations of the NMDA Receptor

The intracellular C-terminal tail the of NR1 and NR2 subunits appears to be quite important to the function of the receptor. NR2 subunits with truncated C-termini form functional ion channels, but their ability to be localized properly within the cell is impaired. Furthermore, since NR1 and NR2 C-termini bind to various signalling and cytoskeletal proteins, other downstream biochemical signals are probably initiated or modulated by the C-terminal domain. This may account for the fact that mice expressing truncated NR2 subunits act much like mice missing the subunit altogether (Sprenger *et al.*, 1998).

A key protein family that organizes many of these protein–protein interactions are the membrane-associated guanylate kinase (MAGUK) proteins, of which PSD-95 (SAP90) is the prototype. The PDZ domain-containing proteins related to PSD-95 are characterized by having three N-terminal PDZ domains followed by an SH3 (src homology 3) domain and a guanylate kinase (GK) homology domain (Sheng, 1996). Each of these domains mediates specific protein–protein interactions. Specific PDZ domains interact with specific sequences in the C-terminal of proteins, and specific SH3 domains bind to specific, proline-rich sequences in their target proteins. The GK domain is enzymatically inactive and binds to specific sequences in guanylate kinase associated proteins (GKAPs). The PSD-95 family includes PSD-95, SAP97, SAP102 and Chapsyn-110.

The C-terminus of the NR2 subunits end with the sequence S(L/I)ESDV, which preferentially interacts with the second PDZ domain (PDZ2) of PSD-95 (Kornau *et al.*, 1995), SAP102 (Lau *et al.*, 1996; Muller *et al.*, 1996) and other proteins. Further, NR1-3a has been reported to bind to PSD-95 (Kurschner *et al.*, 1998). In addition to the roles of clustering and anchoring, these scaffolding proteins allow for spatially ordered signal transduction systems. Hence, NMDA receptor activation can preferentially activate multiple calcium-activated processes by virtue of the localization of the calcium-responsive systems. For example, n-nitric oxide synthase (n-NOS) (Brenman *et al.*, 1996) and a calcium–calmodulin kinase II-sensitive neuronal ras-GAP (GTPase activating protein) (Chen *et al.*, 1998; Kim *et al.*, 1998) can also associate with PSD-95. Thus, the Ca^{++} current through the NMDA

receptor can selectively modulate nitric oxide (NO) production and ras effector pathways, such as mitogen activated protein (MAP) kinase.

The list of identified proteins that associate with glutamate receptors is growing rapidly. Other recently identified scaffolding proteins include channel-interacting PDZ domain protein (CIPP), which binds NR2s (Kurschner *et al.*, 1998); synaptic scaffolding molecule (S-SCAM), which binds NR2s (Hirao *et al.*, 1998); Cysteine-rich interactor of PD 23 (CRIPT), which binds to PSD-95's PDZ3 domain (Niethammer *et al.*, 1998); yotiao, which binds to NR1 subunits that have the C1 alternatively spliced cassette (Lin *et al.*, 1998); and α-actinin-2, which binds to NR1 and NR2s in a Ca^{++}-sensitive manner (Wyszynski *et al.*, 1997).

AMPA Receptors

With the use of nonselective glutamate receptor antagonists and selective NMDA receptor antagonists, it was discovered that ionotropic glutamate receptors, but not NMDA receptors, were responsible for mediating fast synaptic transmission in the major projection pathways of the CNS (Collingridge *et al.*, 1988; Monaghan *et al.*, 1989). However, it was unclear for many years whether these actions were mediated by AMPA and/or kainate receptors, hence the frequent use of the term 'non-NMDA receptors'. Indeed, at one point many scientists accepted the idea that AMPA and kainate were in fact the same receptor. However, the cloning of distinct AMPA and kainate receptor subunit families, together with the identification of AMPA-receptor-specific and kainate-receptor-specific antagonists, has now established firmly that there are two distinct receptor families corresponding to AMPA and kainate.

Four distinct genes code for the AMPA receptor subunits, GluR-A to GluR-D (also termed GluR1 to GluR4, respectively) (Hollmann *et al.*, 1989). Homomeric or heteromeric expression of the GluR1-4 subunits yields receptors with high affinity for AMPA and low affinity for kainate. The observation that kainate-evoked AMPA receptor responses do not desensitize (whereas AMPA-evoked responses desensitize rapidly) helps to account for why observed kainate-evoked responses are frequently mediated by AMPA instead of kainate receptors. In addition to the four gene products, alternative splicing and RNA editing increases significantly the diversity and complexity of AMPA receptors. Each of the AMPA receptors expresses one of two alternative splice cassettes, termed 'flip' and 'flop'. These two peptide segments result in differing desensitization properties, with the flop form displaying more rapid desensitization.

AMPA receptor channels composed of GluR-A (GluR1), GluR-C (GluR3) or GluR-D (GluR4) subunits are calcium permeable (Hollmann *et al.*, 1991). In contrast, the vast majority of native AMPA receptors do not display calcium permeability. When a GluR-B (GluR2) subunit is included in the receptor complex, the resulting receptor displays little calcium permeability. It is the presence of a single residue change in the M2 domain of GluR-B that inhibits calcium influx (Hume *et al.*, 1991; Verdoorn *et al.*, 1991). Interestingly, this single residue of the GluR-B subunit (arginine, R) is not coded for by the GluR-B gene. Instead, the GluR-B gene, like the other AMPA receptors, codes for a glutamine (Q). Using the process of RNA editing, the RNA sequence of GluR-B is altered to code for arginine.

A significant implication of these studies was the prediction of calcium-permeable native AMPA receptors. These have since been identified (Jonas *et al.*, 1994). A further implication is that cells that do not express the edited GluR-B subunit would be expected to be more vulnerable to calcium overload toxicity and, perhaps, calcium-mediated plasticity.

Pharmacological analysis of a large number of CNS projection pathways has led to the general conclusion that AMPA receptors represent the predominate fast excitatory neurotransmitter receptor in the CNS. In the phenomenon of long-term potentiation (LTP), the enhancement of AMPA receptor currents is responsible for the potentiated synaptic current. Recent studies have shown that a key step to this potentiation of AMPA receptor currents is the rapid delivery of new AMPA receptors to the cell surface (Luscher *et al.*, 1999; Luthi *et al.*, 1999). Currently, studies that characterize the differential regulation of the cellular localization of the various AMPA receptor subunits are emerging rapidly.

Kainate Receptors

Two related subfamilies of proteins comprise the mammalian kainate receptor subunits, GluR 5-7 and KA1 and KA2. The GluR5-7 (Bettler *et al.*, 1990; Bettler *et al.*, 1992; Egebjerg *et al.*, 1991) subfamily display 74–81% sequence similarity to each other and approximately 40% similarity to the AMPA receptor subunits. The KA-1/KA-2 subfamily (Herb *et al.*, 1992; Sakimura *et al.*, 1992; Werner *et al.*, 1991) have 68% sequence identity to each other and approximately 45% identity to the GluR5-7 subfamily. To obtain activity at the KA-1 and KA-2 subunits, the addition of at least one member of the GluR5-7 subfamily is necessary. Thus, native kainate receptors may exist as heteromeric combinations of subunits from the two subfamilies (Herb *et al.*, 1992).

The GluR5 subunit displays multiple forms due to alternative splicing. The sequence designated GluR5-1, in contrast to the GluR5-2 subunit, contains a 15-amino acid insertion on the N-terminal side of the first transmembrane domain (Bettler *et al.*, 1992). In addition, the GluR5-2 has three alternative splice variants (GluR5-2a, b and c) due to differing insertions near the C-terminal side. Both the GluR5 and GluR6 display RNA editing at the site homologous to the AMPA receptor's Q/R site. This modification also alters calcium permeability, but in a more complex manner that is also dependent upon additional RNA editing at sites in the TM1 domain (Seeburg, 1996).

Kainate Receptor Function

Kainate has been known for many years to be a potent convulsant and excitotoxin (for review, see Watkins, 1981). However, it has been possible only recently to establish a role for kainate receptors in synaptic transmission. The hippocampal mossy fibre synapse contains a very high density of kainate receptors (Foster *et al.*, 1981). In this pathway, kainate-receptor-mediated responses have been observed by including AMPA and NMDA receptor blockers and by stimulating the pathway at high frequencies (Castillo *et al.*, 1997; Vignes *et al.*, 1997). Another important synaptic action of kainate receptors may be the presynaptic inhibition of GABAergic neurotransmission (Rodriguez-Moreno *et al.*, 1997). The inhibition of GABAergic neurotransmission may account for the potent convulsant activity of kainic acid and the related seafood toxin domoic acid.

Protein–Protein Associations of Kainate and AMPA Receptors

PSD-95 and SAP102 have been shown to associate with both KA2 and GluR6 kainate receptor subunits, whereas SAP97 can associate with GluR6 but not KA2 (Garcia *et al.*, 1998). In contrast, AMPA receptors have limited interactions with the PSD/SAP proteins; SAP97 complexes with GluR 1, but not GluR2 or GluR3 (Leonard *et al.*, 1998).

AMPA receptors associate with the glutamate receptor interacting protein (GRIP) family of proteins (Dong *et al.*, 1997). GRIP contains seven PDZ domains and interacts with AMPA receptor subunits (GluR2) via its fourth and fifth PDZ domains. A related protein that contains six PDZ domains has been called AMPA

receptor binding protein (ABP) (Srivastava *et al.*, 1998). Interestingly, APB and GRIP can associate with each other, forming heteromultimeric complexes. In addition, other proteins such as the N-methylmalemide-sensitive factor (NSF) and soluble NSF attachment proteins (SNAP) have been shown to associate with, and modulate the function of, select AMPA receptor subunits (Lin *et al.*, 1998; Osten *et al.*, 1998).

METABOTROPIC GLUTAMATE RECEPTORS

Until the mid-1980s, glutamate was considered to exert its actions solely through ligand-gated ion channels. However, in 1987 Sugiyama and co-workers used the term 'metabotropic glutamate (mGlu) receptor' to describe a G-protein-coupled receptor (GPCR) activated by glutamate leading to phosphoinositide hydrolysis. Since then, numerous studies have been carried out examining the structure, anatomy, pharmacology, physiology and function of the mGlu receptors (for reviews, see, Anwyl, 1999; Cartmell and Schoepp, 2000; Conn and Pin, 1997; de Blasi *et al.*, 2001; Holscher *et al.*, 1999; Pin *et al.*, 1999; Pin and Duvoisin, 1995; Schoepp *et al.*, 1999). This work has resulted in the discovery of eight distinct mGlu receptor subtypes.

Classification of Metabotropic Glutamate Receptors

In mammals, eight genes encoding mGlu receptors have been identified so far. These receptors belong to the third family of GPCRs, a family that also includes $GABA_B$ receptors, calcium-sensing receptors and some putative pheromone and taste receptors (Bockaert and Pin, 1999). Within this family, the mGlu receptors can be subclassified into three groups based on their transduction mechanisms, sequence homology and pharmacology (Table V.2; Nakanishi, 1992). Group I receptors are comprised of mGlu1 and mGlu5, which are linked positively to phospholipase C (PLC). Activation of these receptors results in an increase of phosphoinositide (PI) turnover and consequently an increase in intracellular calcium concentration (Conn and Pin, 1997). In contrast, both group II mGlu (comprised of mGlu2 and mGlu3) and group III mGlu (comprised of mGlu4 and mGlu6-8) receptors are coupled negatively to adenylyl cyclase, so that upon activation they inhibit cyclic adenosine monophosphate (cAMP) production (Tanabe *et al.*, 1993). All three groups show some sequence homology (about 40%). However, the degree of homology rises to around 70% when receptors are compared within a group (Nakanishi, 1992). Finally, the three groups can be separated by their different pharmacological profiles.

Structure of Metabotropic Glutamate Receptors

mGlu receptors belong to the third family of GPCRs. As with all GPCRs, mGlu receptors have seven putative transmembrane domains. However, they share no sequence homology with GPCRs outside family three (Bockaert and Pin, 1999). All mGlu receptors have structural similarities, including a large N-terminal extracellular domain and a cytoplasmic carboxy-terminal domain that is variable in length. Splice variants of the mGlu receptors exist, so the carboxy-terminal domain can vary in length within a single subtype (e.g. the mGlu1 receptor has splice variants mGlu1a, b, c, d and e, of which only mGlu1a has a long carboxy-terminal domain). In addition, all mGlu receptors possess 19 cysteine residues that are located in the predicted extracellular domain and extracellular loops. These cysteine residues are thought to be important either in determining the three-dimensional structure of the receptor or for intramolecular signal transduction (Conn and Pin, 1997).

Glutamate Binding Site

Unlike the binding site for small ligands in most GPCRs, which is located in a pocket formed by the seven transmembrane domains, the binding site of mGlu receptors is extracellular and is composed of two globular domains with a hinge region. This site has been proposed to be the equivalent to the binding site of bacterial periplasmic binding proteins (O'Hara *et al.*, 1993). Recently, the crystal structure of the extracellular ligand binding site of the mGlu1 receptor has been elucidated in three states, in a complex with glutamate and two unliganded states (Kunishima *et al.*, 2000). The structure of the binding site is homodimeric, with the two monomers being connected by a disulphide bridge between cysteine residues. Each monomer is made up of two domains named LB1 and LB2; it is the LB1 domain that is predominantly involved in anchoring glutamate (Figure V.3). The bilobed structures are flexible and are able to adopt multiple open or closed conformations. Upon glutamate binding, the lobes are more likely to adopt the closed conformation and hence activate the associated G-protein.

Location of Metabotropic Glutamate Receptors

mGlu receptors are widespread in the mammalian CNS, where they are found notably in the hippocampus, cerebellum, spinal cord and on glial cells. However, mGlu receptors are not restricted to neuronal tissue, and they are also found in tissues as diverse as osteoblasts, ileum and heart tissue (see Skerry and Genever, 2001 for a review of glutamate receptors in non-neuronal tissues).

Table V.2 Classification of metabotropic glutamate receptor subtypes

Group	Subtype	Transduction mechanism	Agonist	Antagonist
I	mGlu1a–e	↑ PI hydrolysis	DHPG	CPCOOEt
	mGlu5a,b	↑ PI hydrolysis	DHPG	MPEP
II	mGlu2	↓ cAMP	(2*R*,4*R*)-APDC	LY341495
	mGlu3	↓ cAMP	(2*R*,4*R*)-APDC	LY341495
III	mGlu4a,b	↓ cAMP	(*S*)-AP4	CPPG
	mGlu6	↓ cAMP	(*S*)-AP4	CPPG
	mGlu7a,b	↓ cAMP	(*S*)-AP4	CPPG
	mGlu8a,b	↓ cAMP	(*S*)-AP4	CPPG

(*S*)-AP4, (*S*)-2-amino-4-phosphonobutanic acid; (2*R*,4*R*)-APDC, (2*R*,4*R*)-4-aminopyrrolidine-2-4-dicarboxylic acid; CPCOOEt, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester; CPPG, (*RS*)-α-cyclopropyl-4-phosphonophenylglycine; LY341495, (2*S*,1′*S*,2′)-2-(9-xanthylmethyl)-2-(2-carboxycyclopropyl)glycine; MPEP, 2-methyl-6-(phenylethynyl)pyridine.

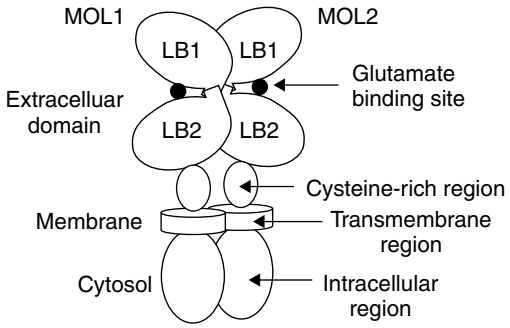

Figure V.3 Schematic diagram of the spatial arrangements of mGluR1 domains. MOL1 and MOL2 represent the two monomers, each of which has two domains (LB1 and LB2), together forming a bilobed structure. Glutamate (shown as a black circle) stabilizes the closed (activated) conformation of the bilobed domain

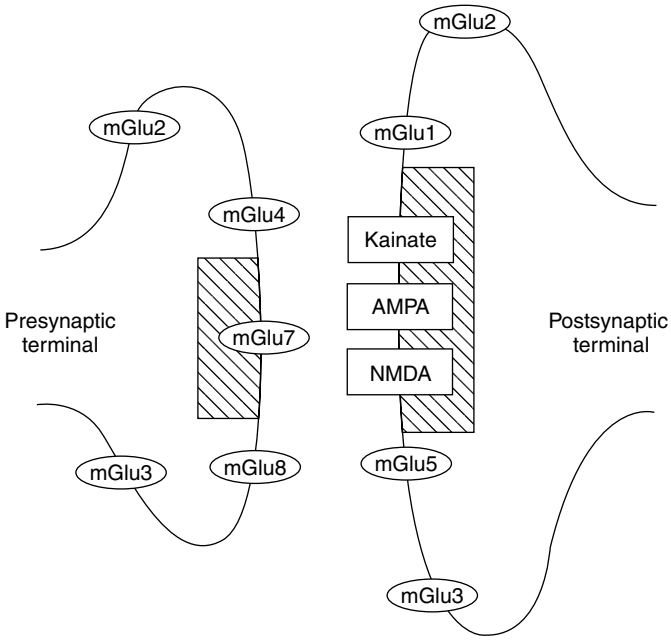

Figure V.4 Diagrammatic representation of the synaptic localization of glutamate receptors at a synapse. Group I mGlu receptors are generally located postsynaptically, away from the active zones (hatched areas). Some studies have indicated, however, that group I mGlu receptors may be localized presynaptically (Cartmell and Schoepp, 2000). Group II mGlu receptors are localized both pre- and postsynaptically, although they are located away from the active zones. Group III mGlu receptors are localized presynaptically, and the mGlu7 receptor is located within the active zone. The mGlu4 and mGlu8 receptors are not found within the active zones

Synaptic Location of Metabotropic Glutamate Receptors

The synaptic localization of the different mGlu receptor subtypes is important as this plays a major role in determining the physiological function of the receptor (Figure V.4). The majority of studies have shown that group I mGlu receptors are generally located postsynaptically and away from the active zones. In addition, immunogold localization studies of mGlu1a, b and c and mGlu5 receptors have shown them to be present at the highest density outside the membrane specializations (Baude *et al.*, 1993; Lujan *et al.*, 1997). In contrast to the group I mGlu receptors, most group III mGlu receptors (mGlu4a, 7a, 7b and 8) are presynaptic and are located in or near the active zones (Shigemoto *et al.*, 1997), the only exception seeming to be mGlu6, which appears to be expressed only in retinal bipolar cells. Group II mGlu receptors have been reported to be both presynaptic, in the presynaptic terminal of the hippocampus, and postsynaptic, on the periphery of longer membrane specializations (Petralia *et al.*, 1996; Shigemoto *et al.*, 1997). In addition, the mGlu3 receptor subtype is highly expressed in glial cells (Mineff and Valtschanoff, 1999).

Pharmacology of Metabotropic Glutamate Receptors

It is beyond the scope of this review to discuss in detail the numerous pharmacological agents acting at mGlu receptors that are now available. Therefore only a table of the major tools commonly used is presented (Table V.3); readers are directed to a comprehensive review for further detail (Schoepp *et al.*, 1999). An important aim is the development of subtype-selective agonists and antagonists for each of the eight mGlu receptors. Such agents are beginning to be developed with highly selective mGlu1 receptor antagonists, mGlu5 receptor agonists and antagonists, and mGlu8 receptor agonists now available (Clark *et al.*, 1997; Doherty *et al.*, 1997; Gasparini *et al.*, 1999; Litschig *et al.*, 1999; Thomas *et al.*, 2001).

Signal Transduction and Second-Messenger Systems

As stated earlier, the mGlu receptor subtypes utilize different signal transduction and second-messenger pathways. However, all the receptors can couple to G-proteins and have certain properties in common. Mutagenesis studies have shown that the second intracellular loop has a critical role in recognition of the G-protein, whereas the other intracellular domains are involved in controlling coupling efficacy. It has also been demonstrated that the extreme carboxy-terminus of the α-subunit of the G-protein is critical for the recognition of the receptor. This has led therefore to the idea that the carboxy-terminus of the G-protein α-subunit is also interacting in a cavity formed by the second and third intracellular loops (Francesconi and Duvoisin, 1998; Gomeza *et al.*, 1996).

Signal Transduction of Group I Metabotropic Glutamate Receptors

Most work examining the signal transduction of group I mGlu receptors has been carried out using cloned receptors expressed in cell lines. Depending on the cell type and mGlu receptor subtype studied, the signal transduction mechanisms can differ. However, in every expression system examined, group I mGlu receptors, including their splice variants, can stimulate PC and PI hydrolysis via coupling to G_{q11} (Figure V.5). Coupling to PI hydrolysis by group I mGlu receptors has also been demonstrated in many native systems, including cultured astrocytes, cultured neurons, synaptoneurosomes and brain slices (for a review, see Conn and Pin, 1997). In addition to inositol trisphosphate (IP_3) being formed upon PI hydrolysis, diacylglycerol (DAG) is also formed. DAG is lipid soluble and stays in the membrane, where it interacts with protein kinase C (PKC). Activated PKC causes phosphorylation of serine and threonine residues on a variety of intracellular proteins, including the group I mGlu receptors, causing them to desensitize (de Blasi *et al.*, 2001). Coupling of group I mGlu receptors to systems other than PI hydrolysis has also been reported in a number of studies. Both the mGlu1a and

Table V.3 Potencies of agonists (EC_{50} values) and antagonists (IC_{50} values) at individual metabotropic glutamate receptor subtypes expressed in cell lines (expressed in μM)

	Group I		Group II		Group III			
Compound	mGlu1	mGlu5	mGlu2	mGlu3	mGlu4	mGlu6	mGlu7	mGlu8
Group I agonists								
DHPG	6–60	0.7–20	>1000	>100	>1000	–	>1000	>1000
CHPG	>10000	750	–	–	–	–	–	–
Antagonists								
LY367385	8.8	>100	>100	–	–	–	–	–
CPCCOEt	6.5–23	>100	>100	–	>100	–	>100	>100
MPEP	>100	0.032	>100	–	>100	>100	>100	>100
Group II agonists								
(2*R*,4*R*)-APDC	>100	>100	0.4–10	0.4–5	>100	>100	>100	>100
LY354740	>100	>100	0.005–0.1	0.04–0.1	>100	3	>100	12 – 36
Antagonists								
LY341495	6.8	8.2	0.03	0.014	22	1.8	0.99	0.173
EGLU	–	–	94	–	–	–	–	–
Group III agonists								
(*S*)-AP4	>1000	>1000	>1000	>1000	0.2–1	0.2–0.9	160–1300	0.06–0.9
(*S*)-3,4-DCPG	IC_{50} = 32	>100	>100	>100	8.8	3.6	>100	0.031
Homo-AMPA	>1000	>1000	>1000	–	>1000	58	>5000	–
Antagonists								
CPPG	–	–	–	–	12(K_D)	–	–	0.18
MAP4	>500	–	15–2000	–	190	*Ag* 36–88	–	25

Data taken from Schoepp *et al.* (1999), Thomas *et al.* (2001), and Naples and Hampson (2001). –, not tested; Ag, agonist; (*S*)-AP4, (*S*)-2-amino-4-phosphonobutanoic acid; (2*R*,4*R*)-APDC, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid; CHPG, (*RS*)-2-chloro-5-hydroxyphenylglycine; CPCCOEt, 7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester; CPPG, (*RS*)-α-cyclopropyl-4-phosphonophenylglycine; (*S*)-3,4-DCPG, (*S*)-3,4-dicarboxyphenylglycine; DHPG, (*S*)-3,5-dihydroxyphenylglycine; EGLU, (*S*)-α-ethylglutamic acid; Homo-AMPA, (*S*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butanoic acid; LY341495, (2*S*,1′*S*,2′*S*)-2-(9-xanthylmethyl)-2-(2-carboxycyclopropyl)glycine; LY354740, (1*S*,2*S*,-5*R*,6*S*)-(+)-2-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY367385, (+)-4-carboxy-2-methylphenylglycine; MAP4, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid; MPEP, 2-methyl-6-(phenylethynyl)pyridine.

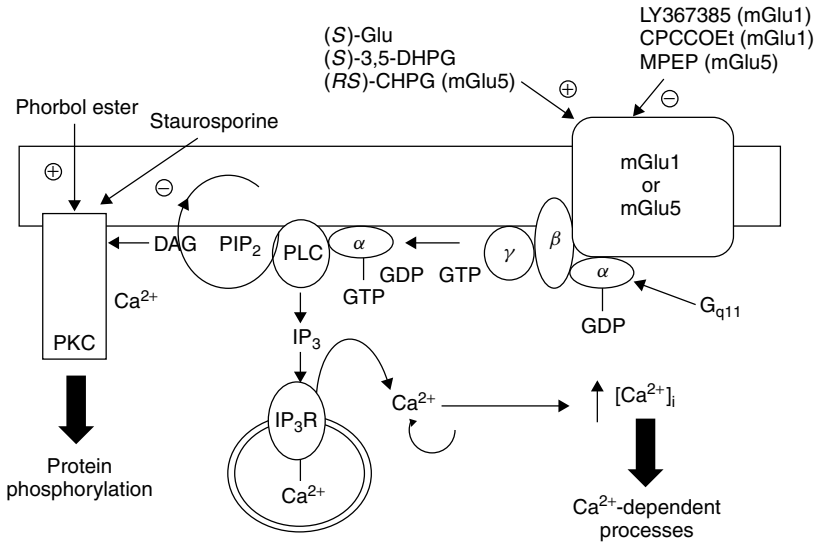

Figure V.5 A composite scheme for the molecular events that are initiated through activation of group I mGlu receptors. Upon receptor activation, the α-subunit of the G-protein activates PLC. This leads to IP_3 and DAG formation, which in turn cause Ca^{2+} release and PKC activation, respectively

mGlu5a receptors have been shown to increase cAMP formation, probably via direct coupling to adenylyl cyclase through G_s proteins (Pin and Duvoisin, 1995). Interestingly, in Chinese hamster ovary (CHO) cells, mGlu1a receptors but not mGlu5a receptors have been shown to increase cAMP formation (Abe *et al.*, 1992; Aramori and Nakanishi, 1992). The mGlu1a receptor has also been shown to couple to G_i protein, though this was using a mutated mGlu1a receptor (Francescori and Duvoisin, 1998). Additionally, when mGlu1a receptors are expressed in superior cervical ganglia neurons, they have been shown to inhibit M-type

potassium channels (Ikeda *et al.*, 1995). The mechanism of this inhibition is unclear, but it may involve direct coupling of the G-protein to the channel or it may be secondary to activation of PI hydrolysis.

Signal Transduction of Group II and Group III Metabotropic Glutamate Receptors

When cloned receptors are expressed in mammalian cells, both group II and group III mGlu receptors can, when activated, inhibit forskolin or G_s-coupled receptor-stimulated cAMP formation. This effect of cloned group II and III mGlu receptors is supported by studies using brain slices and neuronal cultures where inhibition of forskolin-stimulated cAMP formation was observed. In expression systems, both group II and group III mGlu receptors are pertussis toxin sensitive, indicating a role of a G_i- or G_o-type protein in their transduction pathways (Figure V.6). One difference between expression systems of group II and III mGlu receptor coupling is that the inhibition of adenylyl cyclase is often less efficient by group III mGlu receptors than by group II mGlu receptors (Conn and Pin, 1997). This difference may be due to either a lower expression of group III mGlu receptors in the plasma membrane or the inhibition of adenylyl cyclase not being the preferred transduction pathway of group III mGlu receptors. It is also worth noting that most studies of native group II and III mGlu receptors have examined the inhibition of cAMP formation induced by activating adenylyl cyclase using forskolin rather than using agonists of receptors that activate adenylyl cyclase via coupling to G_s-proteins. This method of stimulation means that the question of whether each of the group II and III mGlu receptors is coupled to inhibition of neurotransmitter-induced increases in cAMP accumulation in native systems remains debatable (Conn and Pin, 1997).

PLD-Coupled Metabotropic Glutamate Receptors

In addition to the eight mGlu receptors characterized to date, a body of evidence has been growing to suggest that a novel mGlu receptor exists that is coupled to phospholipase D (PLD). It has been demonstrated that the nonselective mGlu receptor agonist (1*S*, 3*R*)-1-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*R*)-ACPD) is able to stimulate PLD activity in neonatal and adult hippocampal slices. Whereas the PLD stimulation in neonatal hippocampal slices appears to be indirect via PKC activation, the PLD-coupled mGlu receptor in adult hippocampal slices does not. In addition, this PLD-coupled mGlu receptor does not correspond to the known pharmacological profile of any of the known PLC- or adenylyl cyclase-coupled mGlu receptors (Albani-Torregrossa *et al.*, 1999).

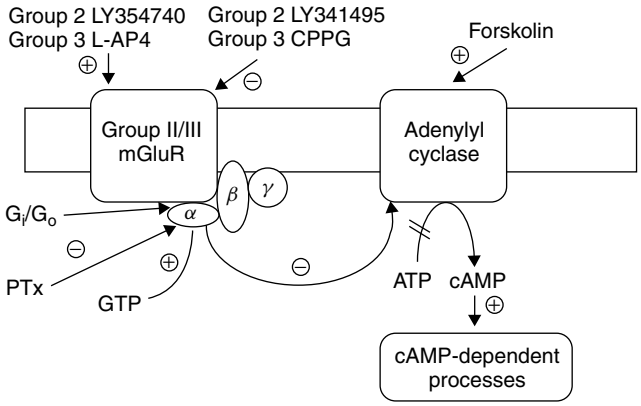

Figure V.6 A composite scheme for the molecular events that are initiated through activation of group II and III mGlu receptors. Upon receptor activation, the α-subunit of the pertussis toxin (PTx)-sensitive G-protein exerts an inhibitory effect on adenylyl cyclase, leading to a decrease in cAMP formation

G-Protein-Independent Signalling

In recent years, evidence has begun to accumulate indicating that mGlu receptors can elicit responses by G-protein-independent mechanisms (for a review, see Heuss and Gerber, 2000). G-protein-independent signalling can occur because the intracellular loops and free carboxy-termini of the mGlu receptors provide potential binding sites for protein interactions. In addition, several of the proteins that are able to bind to these sites exhibit enzymatic activity or are adapter proteins that can, for example, promote kinase binding to the receptor. Binding of these proteins would allow direct cross-talk of the receptor to other transduction pathways without the need for G-protein involvement. G-protein-independent signalling has been demonstrated for the mGlu1 receptor in CA3 pyramidal neurons in the hippocampus, where activation of the receptor produces an increase in a cationic conductance. The mGlu1-receptor-activated current is not blocked by a G-protein inhibitor but is blocked by an SRC tyrosine kinase inhibitor, showing that the response was G-protein-independent (Heuss *et al.*, 1999). It is not known what the link between the receptor and SRC kinase is, but a likely candidate is an arrestin-like protein (Heuss and Gerber, 2000).

Homer Proteins

Homer is a protein, the N-terminal of which interacts with the C-terminus of mGlu1a or mGlu5 receptors. Several types of Homer protein exist (1a,b,c, 2 and 3), and their expression is regulated by an immediate early gene. Homer 1b,c and 3 contain a coiled-coil domain that allows interaction with a group I mGlu receptor and another protein, e.g. the IP_3 receptor. This interaction leads to efficient coupling of the receptor to intracellular calcium release. In contrast to Homer 1b,c and 3, Homer 1a does not contain a coiled-coil domain. Without the coiled-coil domain, Homer 1a cannot couple the mGlu receptor with the IP_3 receptor; however, Homer 1a can still compete with the other Homer proteins for the binding site on the mGlu receptor, disrupting the coupling efficiency. Homer protein expression is dynamically responsive to physiological synaptic activity, with overexcitation leading to a higher expression of Homer 1a and therefore decreasing coupling efficiency. This means that Homer proteins can act as a mechanism controlling calcium release from intracellular stores (Brakeman *et al.*, 1997).

Physiology of Metabotropic Glutamate Receptors

The effects of mGlu receptor activation at the cellular level have been studied extensively using agonists and antagonists in various systems both *in vitro* and *in vivo*. In addition to pharmacological studies, anatomical studies have also been employed using mGlu receptor subtype-specific mRNA probes and antibodies. Finally, targeted disruptions of the mGlu receptor subtype genes have been used to elucidate synaptic and behavioural functions of the mGlu receptors.

Modulation of Cation Currents

Potassium channels are amongst the most common targets for modulation by mGlu receptors. An example of potassium channel modulation occurs in hippocampal pyramidal neurons, where activation

of mGlu receptors results in a reduction of a leak potassium conductance, the calcium-dependent slow after-hyperpolarization current (I_{AHP}), a slow non-inactivating voltage-dependent potassium current (I_M), and a slowly inactivating voltage-dependent potassium current (I_K) (Charpak *et al.*, 1990; Desai and Conn, 1991; Guerineau *et al.*, 1994; Luthi *et al.*, 1996). It has also been shown that mGlu receptor activation can have a direct inhibitory effect on neurons, likely by opening potassium channels. This has been shown in basolateral amygdala neurons, where mGlu receptor activation induces a hyperpolarization. In addition to modulating potassium channels, mGlu receptors can exert excitatory effects on neurons by activating nonselective cation currents, such as a Na^+/Ca^{2+} exchange (Linden *et al.*, 1994), a calcium-activated nonspecific cation current (Crepel *et al.*, 1994), and a calcium-independent nonspecific cation channel (Miller *et al.*, 1995).

Modulation of Voltage-Dependent Calcium Channels

All the three major subgroups of mGlu receptors can modulate calcium channels, mainly by inhibiting them, although in a few instances they have been shown to activate calcium currents. Inhibition of N-type calcium channels has been shown in cortical neurons, and is likely to be mediated by members of group I and group II mGlu receptors (Choi and Lovinger, 1996). Group II mGlu receptors have also been indicated in inhibition of N-type calcium channels in granule cells (Chavis *et al.*, 1995). Group III mGlu receptors have been shown to inhibit calcium currents in the olfactory bulb, retinal ganglia neurons, and a subpopulation of hippocampal neurons (Rothe *et al.*, 1994; Sahara and Westbrook, 1993; Trombley and Westbrook, 1992). Group I mGlu receptor activation has also been reported to increase N-type calcium currents in retinal ganglion neurons and L-type calcium currents in cerebellar granule cells (Chavis *et al.*, 1995; Rothe *et al.*, 1994).

Regulation of Synaptic Transmission by Metabotropic Glutamate Receptors

One of the most prominent physiological effects of mGlu receptor activation, consistent throughout the CNS, is a regulation of transmission at glutamatergic synapses. This effect is typically mediated by presynaptic mGlu receptors acting as autoreceptors (for a review, see Cartmell and Schoepp, 2000), with the most common response being a reduction of glutamate release mediated by group II and group III mGlu receptors. However, over the last decade it has become clear that multiple mGlu receptor subtypes of each of the three major groups can act as autoreceptors. Which receptor subtype acts as the autoreceptor is dependent on the preparation being studied, e.g. in adult hippocampal area CA1, both group I and group III mGlu receptors, but not group II mGlu receptors, act as autoreceptors leading to a reduction in glutamate release (Gereau and Conn, 1995). Immunocytochemical analysis of this area suggests that the subtypes mGlu5 and mGlu7 are responsible for eliciting the reduction in glutamate release. However, in the medial perforant pathway synapse, it is a group II mGlu receptor that is acting as the autoreceptor, again reducing glutamate release (Macek *et al.*, 1996; Ugolini and Bordi, 1995). In the neonatal rat spinal cord, both group II and group III mGlu receptors serve as autoreceptors (Cao *et al.*, 1995; Jane *et al.*, 1994), with recent evidence suggesting that the mGlu8 receptor is responsible at least partly for the group III mGlu receptor-mediated depression of synaptic transmission (Thomas *et al.*, 2001).

The mechanisms underlying the regulation of glutamate release by mGlu receptors acting as autoreceptors are not yet known. However, for receptors responsible for decreasing glutamate release, it seems likely that a reduction of the voltage-dependent calcium currents is one potential mechanism. Other mechanisms to decrease glutamate release that work without the regulation of voltage-dependent calcium currents may exist, such as an activation of presynaptic potassium currents (Sladeczek *et al.*, 1993). Enhancement of glutamate release by (*S*)-3,5-dihydioxy phenylglycine (DHPG) has also been shown under certain conditions (Cartmell and Schoepp, 2000). That the facilitation of glutamate release was still observed in mGlu1 knockout mice is suggestive that this is an mGlu5 receptor-mediated effect.

Metabotropic Glutamate Receptor Activation in Regulation of Release of Other Neurotransmitters

As well as regulating glutamate release, mGlu receptors also regulate the release of other neurotransmitters. As with glutamate, the release of other neurotransmitters can be up- or downregulated depending on the type of mGlu receptor present, the tissue preparation, the agonist used, and the stimulation conditions employed. It is beyond the scope of this chapter to detail the effects of mGlu receptor activation on all these neurotransmitters, and so GABA regulation will be considered as a representative example (for a review of the effects on other neurotransmitters, see Cartmell and Schoepp, 2000).

Presynaptic mGlu receptors have been shown to reduce GABA release and, therefore, to reduce inhibitory synaptic transmission. This occurs in several brain areas and, again, members from each of the three major groups of mGlu receptor can carry out this role. In the CA1 area of the hippocampus in mature rats, a subtype of the group I mGlu receptor regulates GABA release (Gereau and Conn, 1995), whereas group II mGlu receptors have been shown to reduce GABA release in the accessory olfactory bulb (Hayashi *et al.*, 1993), the hippocampal area CA3 in young rats (Poncer *et al.*, 1995), and the thalamus (Salt and Eaton, 1995). Also, in the thalamus activation of group III mGlu receptors has been shown to reduce GABA release (Salt and Eaton, 1995).

Metabotropic Glutamate Receptor-Induced Modulation of Ligand-Gated Ion Channels

A final way in which mGlu receptor activation can regulate synaptic transmission is by modulating currents through glutamate and GABA receptor channels. This modulation of ligand-gated ion channels allows selective modulation of specific components of the synaptic potentials. An example of mGlu receptor activation increasing currents is in the hippocampus, where activation leads to an enhancement of NMDA-receptor-mediated responses without modulating responses of non-NMDA ionotropic glutamate receptors. This effect appears to be mediated by a group I mGlu receptor (Fitzjohn *et al.*, 1996). A similar effect is observed in spinal cord dorsal horn neurons, although in this example mGlu receptor activation potentiates currents through both NMDA and non-NMDA glutamate receptors (Bleakman *et al.*, 1992). Negative modulation of ligand-gated ion channel mediated currents can also occur. In striatal neurons, mGlu receptor activation causes a decrease in the level of depolarization due to NMDA receptor activation (Colwell and Levine, 1994). Therefore, the effect of mGlu receptor activation on ligand-gated ion channels is variable and may play a unique role in regulating inhibitory and excitatory synaptic transmission.

Functions of Metabotropic Glutamate Receptors

As has been outlined above, there is a plethora of ways in which mGlu receptor activation can exert effects. These mechanisms can be separated broadly into excitatory effects, inhibitory effects, presynaptic effects and modulation of neurotransmitter release. As glutamate is the most common transmitter in the mammalian CNS, and as mGlu receptor activation can also modulate the release

of other transmitters, the potential functions of mGlu receptor activation are vast. mGlu receptors have been shown to be important in various motor circuits, including those of the spinal cord, basal ganglia and cerebellum, in pain transmission, and in many forms of synaptic plasticity (Anwyl, 1999; Conn *et al.*, 1994; Salt and Eaton, 1994; Young *et al.*, 1995). In addition, mGlu receptors have been indicated in many pathophysiological roles, such as strokes, epilepsy and neurodegenerative disorders (Bruno *et al.*, 1995; Buisson *et al.*, 1996; Conn *et al.*, 1994). The functional significance of mGlu receptors will be illustrated by an overview of their role in synaptic plasticity (for a more detailed review of the material presented in the following section, see Anwyl, 1999).

Synaptic Plasticity

Synaptic plasticity at glutamatergic synapses is characterized by long-term changes in the synaptic efficacy. LTP and long-term depression (LTD) are the two most common, most well-understood forms of synaptic plasticity. LTP is a long-lasting (hours *in vitro*, days or weeks *in vivo*) enhancement of synaptic transmission, and is a model for the study of the cellular processes that underlie learning and memory. Briefly, LTP in area CA1 of the hippocampus is induced by activation of an AMPA-receptor-associated sodium channel leading to membrane depolarization and removal of the magnesium block of the NMDA-receptor-associated ion channel. On activation of NMDA receptors, calcium enters the neuron and activates a number of kinases and lipases that are part of a cascade that consolidates the LTP. As it is the calcium entry through the NMDA channel that is critical for LTP, it is perhaps not surprising that group I mGlu receptors, which are coupled to PLC, enhance NMDA receptor activity, depolarize neurons and increase intracellular calcium concentration, should facilitate or even induce LTP. This is indeed seen, as group I mGlu receptor agonists such as DHPG or (1*S*,3*S*)-1-aminocyclopentane 1,3-dicarboxylic acid (ACPD) have been shown to enhance LTP and even induce LTP in the CA1 area of the hippocampus and dorsolateral septal nucleus (Zheng and Gallagher, 1992). The exact mechanism involved in LTP induction or potentiation by mGlu receptors is not known, but it is likely to be due to a release of calcium from internal stores and PKC activation. The use of mGlu receptor antagonists to block LTP induction has had mixed results depending on the antagonist used, stimulation conditions, and preparation studied. The variability may be due in part to the presence of an (*S*)-α-methyl-4-carboxyphenylglycine (MCPG)-sensitive receptor with an unknown pharmacological profile that is not one of the known mGlu receptors.

LTD is the counterpart of LTP and is used to describe a long-lasting depression of synaptic transmission. LTD of glutamatergic synapses has been found in many different brain areas. The best-described example of LTD is of the parallel fibre–Purkinje cell synapse that is induced by the coactivation of climbing and parallel fibres. Group I mGlu receptor activation alone is not sufficient to induce LTD at this synapse, although this can be achieved by coactivation of group I mGlu receptors and voltage-sensitive calcium channels.

GLUTAMATE TRANSPORTERS

It is becoming increasingly apparent that the overall functioning of an EAA synapse is governed not only by its constituent receptors but also by those proteins that influence the amount of L-glutamate in the synaptic environment. It is in this regard that attention has focused on two distinct uptake systems: the intracellular transporters that mediate the uptake of L-glutamate into synaptic vesicles, and the cellular excitatory amino acid transporters (EAATs) that effectively sequester glutamate into glia and neurons. In addition to subcellular localization, these systems are readily distinguishable in terms of their protein structure, energetics, and pharmacology (Bridges *et al.*, 1999; Ozkan and Ueda, 1998; Palacin *et al.*, 1998; Seal and Amara, 1999). Each also appears to hold considerable potential to modify EAA synaptic signalling as a target of either pathological mechanisms or therapeutic interventions.

Vesicular Glutamate Transporter

As is characteristic of classic neurotransmission, the release of L-glutamate into the synaptic cleft occurs as a consequence of the calcium-dependent fusion of synaptic vesicles containing the transmitter with the plasma membrane of the presynaptic terminal. The vesicular glutamate transport system (VGLUT; Figure V.7) is responsible for sequestering L-glutamate in these vesicle before release. In this respect, the VGLUT may represent a novel site at which excitatory transmission can be regulated.

Studies of glutamate uptake into isolated synaptic vesicles demonstrate that this transport system is clearly distinct from the cellular EAATs. Rather than ionic gradients of sodium and potassium, the transport of L-glutamate into the synaptic vesicle is driven by a proton gradient generated by a vacuolar type ATPase (V-ATPase) (Maycox *et al.*, 1990). V-ATPases are found on lysosomes, endosomes, and secretory and synaptic vesicles, and can be distinguished from mitochondrial and plasma membrane ATPases by specific inhibitors, such as Bafilomycin. The resulting electrochemical gradient, which is estimated to be capable of concentrating L-glutamate 10–100-fold within the synaptic vesicles, includes an electrical ($\Delta\Psi$, inside positive) as well as a chemical (ΔpH, inside acidic) component (Maycox *et al.*, 1990; Tabb *et al.*, 1992; Wolosker *et al.*, 1996). Comparisons of the relative contributions of $\Delta\Psi$ and ΔpH indicate that, like GABA, the accumulation of L-glutamate in synaptic vesicles appears to depend primarily on $\Delta\Psi$ rather than ΔpH, which predominates in the vesicular transport of monoamines and acetylcholine. Uptake of L-glutamate into synaptic vesicles also exhibits a dependence on chloride ions (Ozkan

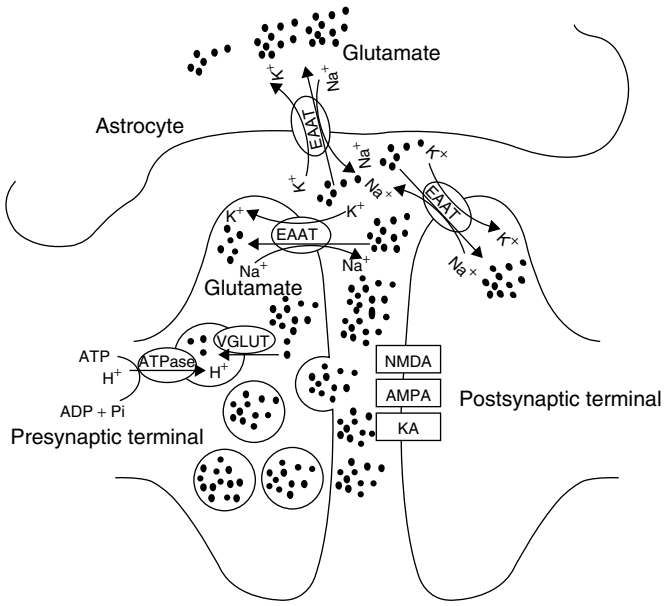

Figure V.7 Diagrammatic representation of the synaptic localization of the vesicular glutamate transporter (VGLUT) and the cellular sodium-dependent excitatory amino acid transporter (EAAT) (KA, kainic acid receptor)

and Ueda, 1998). Transport is stimulated by the concentrations of chloride in the 1–5-mM range and inhibited when levels increase above 20 mM. In addition to its ability to influence ΔpH as a consequence of its presence inside the synaptic vesicle, the inhibitory action of the anion channel blocker 4,4′diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) suggests the presence of a distinct site at which chloride may regulate VGLUT (Hartinger and Jahn, 1993).

Vesicular Glutamate Transporter Pharmacology

From a kinetic and pharmacological perspective, the VGLUT and EAAT systems share little beyond the basic ability to translocate L-glutamate. Uptake by the vesicular transporter is considered low affinity when compared with the EAATs, as the K_m values for L-glutamate uptake into synaptic vesicles typically fall within the 1–2-mM range (Ozkan and Ueda, 1998). Similarly, specificity differences between the two become readily apparent as soon as the activities of glutamate and aspartate are compared. In contrast to the EAATs, transport of glutamate through VGLUT is both blocked by D-glutamate and insensitive to inhibition by either L- or D-aspartate. Unfortunately, while specificity studies have identified numerous compounds that inhibit the vesicular accumulation of ^{3}H-L-glutamate, the inhibitors represent such a wide spectrum of molecules that a clear structure–activity model has yet to emerge. (This situation may be made worse by the fact that compounds can potentially also inhibit uptake indirectly, by disrupting the vesicular proton gradient.) The list of VGLUT blockers ranges from molecules that are clearly glutamate mimics, such as *erythro*-4-methyl-L-glutamate and 4-*methylene*-L-glutamate (Winter and Ueda, 1993), to those that exhibit little resemblance to the natural substrate, such as the ergot bromocriptine and the azo dyes Chicago Sky Blue and Evans Blue (Carlson *et al.*, 1989; Roseth *et al.*, 1995). This latter group appears to be amongst the most potent inhibitors yet identified, as the azo dyes block uptake with K_i values in the submicromolar range. More recent studies with the dye Rose Bengal suggest that VGLUT inhibitors may be capable of regulating levels of synaptically released glutamate (Ogita *et al.*, 2001). The list of VGLUT inhibitors also includes a number of glutamate analogues best known for their activity at other sites within the EAA system. For example, kynurenate and 7-Cl-kynurenate, which act as antagonists at the EAA ionotropic receptors and the glycine site on the NMDA receptor, respectively, both competitively inhibit VGLUT (Bartlett *et al.*, 1998). Similarly, *trans*-ACPD, which is recognized for its activity at EAA metabotropic receptors, not only inhibits the uptake of L-glutamate into synaptic vesicles but also appears to act as an alternative substrate (Winter and Ueda, 1993). Lastly, a family of inhibitory protein factors (IPF_α, IPF_β, IPF_γ) has also been identified that potently block L-glutamate uptake into isolated synaptic vesicles (Tamura *et al.*, 2001).

Molecular Biology of Vesicular Glutamate Transporters

The circumstances surrounding the molecular characterization of the VGLUT system are particularly fascinating, as the transporter was actually cloned several years before it was recognized as a vesicular transporter of L-glutamate (Bellocchio *et al.*, 2000; Otis, 2001; Takamori *et al.*, 2000). Originally identified as a consequence of upregulated mRNA expression in cerebellar granule cells treated with NMDA, this protein exhibited considerable sequence homology with known sodium-dependent inorganic phosphate transporters. As Northern analysis revealed its presence to be restricted to the brain, and heterologous expression in *Xenopus* oocytes produced an increase in sodium-dependent inorganic phosphate (P_i) uptake, the protein was referred to as BNPI (brain, Na-dependent P_i transporter). Its potential role in vesicular uptake was suggested by later immunocytochemical studies demonstrating a localization to presynaptic vesicles and genetic studies in *Caenorhabditis elegans* demonstrating that mutations in a BNPI-like protein compromised glutamate-mediated signalling. Confirmation that BNPI was indeed capable of mediating the uptake of L-glutamate followed shortly. Consistent with L-glutamate transport into isolated synaptic vesicles, uptake by BNPI demonstrated a dependence on both ATP and chloride, yielded K_m values in the low-millimolar range, and exhibited the appropriate pharmacological profile (Bellocchio *et al.*, 2000). Intriguingly, it was also reported that when BNPI was transfected into GABAergic neurons maintained in culture, the neurons exhibited a new glutamate-mediated postsynaptic signal when stimulated (Takamori *et al.*, 2000). The combination of these pharmacological data and the localization to synaptic vesicles prompted the renaming of BNPI to VGLUT1. Following the identification of VGLUT1, a second sodium-dependent transporter, DNPI, was also shown to mediate vesicular glutamate transport and consequently was renamed VGLUT2 (Fremeau *et al.*, 2001). The two vesicular transporters exhibit complementary distributions: VGLUT1 is preferentially expressed in glutamate terminals in the cortex, hippocampus and cerebellum, while VGLUT2 is present in thalamus, hypothalamus and brainstem. The identification of distinct VGLUT isoforms raises the interesting possibility that transporter expression may be a factor in determining the release properties of a particular synapse.

Cellular Excitatory Amino Acid Transporters

Although a number of uptake systems have been identified that can mediate the translocation of L-glutamate across the plasma membrane, those systems that are thought to play the most significant role in excitatory transmission are grouped together on the basis of a high affinity for L-glutamate (K_m values in the 5–50-μM range) and a dependence upon sodium ions (Gegelashvili and Schousboe, 1997b; Robinson, 1998; Seal and Amara, 1999; Takahashi *et al.*, 1997). These transmembrane proteins use sodium and potassium ionic gradients to drive the uptake of L-glutamate into neurons and glia, where it can be concentrated several thousand-fold above extracellular levels (Zerangue and Kavanaugh, 1996). Current stoichiometric models couple the intracellular translocation of one molecule of glutamate with the uptake of three Na^+ ions and one H^+ ion and the outward movement of one K^+ ion. As a consequence of the ability to effectively clear L-glutamate from the extracellular synaptic environment, the transporters have been postulated to play a number of critical roles in excitatory transmission, including signal termination, transmitter recycling, and the maintenance of L-glutamate levels below those that would result in excitotoxic damage. Of equal interest are studies suggesting that under pathological conditions, these transporters also have the capacity to participate in excitotoxic mechanisms as a primary site of efflux for the large intracellular pools of L-glutamate normally present in neurons and glia (Takahashi *et al.*, 1997).

A major advance in the characterization of these cellular transporters was the almost simultaneous cloning of three distinct proteins that, when expressed, exhibited kinetic and pharmacological properties consistent with high-affinity, sodium-dependent uptake: glutamate/aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) from rat brain, and excitatory amino acid carrier 1 (EAAC-1) from rabbit intestine (Kanai and Hediger, 1992; Pines *et al.*, 1992; Storck *et al.*, 1992). Shortly thereafter, the homologous counterparts for these three transporters were isolated from human tissue: EAAT1, EAAT2 and EAAT3, respectively (Arriza *et al.*, 1994). The subsequent identification of EAAT4 and EAAT5 brings the total number of glutamate transporters to at least five (Arriza *et al.*, 1997; Fairman *et al.*, 1995). The existence of these distinct EAAT subtypes is also consistent with more traditional uptake studies with CNS preparations (e.g. synaptosomes,

Table V.4 Sodium-dependent excitatory amino acid transporters

Transporter	CNS distribution	Alternative substrates	Non-substrate inhibitor
EAAT1 (GLAST, rat)	Cerebellar glia	L-Glutamate L-Aspartate D-Aspartate (2*S*,4*R*)-4-methylglutamate L-Serine-O-sulfate L-*trans*-2,4-Pyrrolidine dicarboxylate	*β-threo*-Benzyloxy-aspartate
EAAT2 (GLT1, rat)	Forebrain glia	L-Glutamate L-Aspartate D-Aspartate L-*trans*-2,4-Pyrrolidine dicarboxylate	Dihydrokainate *β-threo*-Benzyloxy-aspartate
EAAT3 (EAAC1, rabbit)	Cortical neurons	L-Glutamate L-Aspartate D-Aspartate L-*trans*-2,4-Pyrrolidine dicarboxylate	
EAAT4	Cerebellar Purkinje neurons	L-Glutamate L-Aspartate D-Aspartate L-*trans*-2,4-Pyrrolidine dicarboxylate L-α-Aminoadipate	
EAAT5	Retina	L-Glutamate L-Aspartate D-Aspartate	*β-threo*-Hydroxy-aspartate L-*trans*-2,4-Pyrrolidine dicarboxylate

tissue slices, primary cultures) where pharmacological, kinetic and anatomical differences pointed to heterogeneity within the uptake process. The EAATs appear to be part of a novel gene family that also includes the neutral amino acid transporters alanine serine cysteine transporters 1 and 2 (ASCT1 and ASCT2) (Palacin *et al.*, 1998). Sequence analysis reveals about 50% sequence homology among the EAATs, as well as the presence of a conserved heptapeptide sequence [AA(I/Q)FIAQ] in the C-terminal region of the transporter that may be a defining structural motif for the family. Further structural studies reveal a lack of a cleavable signal sequence, greater level of conservancy in the C-terminal half, and at least two sites for N-glycosylation. While there is general agreement on the presence of six highly conserved putative transmembrane domains in the NH_2-terminal portion of the protein, predictions as to the exact number of such domains in the C-terminal have been somewhat controversial. Consequently, structural models have been put forward containing 10–12 membrane-spanning domains. The topological structure is, however, distinct from the 12 transmembrane domains that typify the superfamily of the sodium- and chloride-dependent neurotransmitter transporters.

Anatomical and Cellular Distributions of the Excitatory Amino Acid Transporters

Cloning of the EAATs was soon followed by the development of specific antibodies and molecular probes with which to investigate the distribution of the transporters. Distribution studies in a number of species reveal that in addition to a presence in brain, EAAT1–4 can be variously detected in peripheral tissues (e.g. heart, muscle, placenta, lung, liver, kidney, intestine) (Gegelashvili and Schousboe, 1997a; Palacin *et al.*, 1998). Within the CNS, each of the transporters exhibits a distinctive combination of cellular and anatomical expression. EAAT1 and EAAT2 are considered principally to be glial transporters, with enriched levels of expression localized to cerebellar and forebrain regions, respectively. An exception to this patterns is found on the Bergmann glia of the cerebellar molecular layer, where high levels of EAAT2 are present. In contrast to EAAT1 and EAAT2, the expression of EAAT3 and EAAT4 is limited to neurons. EAAT3 is distributed generally throughout the brain, although higher levels of expression are typically found in cortical areas. The distribution of EAAT4, on the other hand, appears to be restricted to the Purkinje cells of the cerebellum. Lastly, EAAT5 stands apart from the other EAATs in that its expression appears to be localized to the retina. Table V.4 gives an overview of the sodium-dependent EAATs.

Pharmacology of the Excitatory Amino Acid Transporters

The existence of EAAT subtypes raises obvious questions as to pharmacological differences among the subtypes and the potential to identify subtype-selective inhibitors. Like many transporters, however, the pharmacological specificity of glutamate transport was initially delineated by quantifying the ability of a wide range of EAA analogues to inhibit the uptake of radiolabelled substrates (e.g. ^{3}H-L-glutamate, ^{3}H-D-aspartate) into a variety of CNS preparations, such as synaptosomes, tissue slices, and primary cultures of neurons or glia. While in retrospect these uptake assays were (and continue to be) confounded somewhat by transporter heterogeneity, the studies were instrumental in establishing glutamate uptake as sodium-dependent, high-affinity (i.e. K_m values in the micromolar range), and stereoselective (i.e. D-glutamate is inactive as an inhibitor) (Balcar and Johnston, 1972; Hertz *et al.*, 1978; Logan and Snyder, 1972; Roberts and Watkins, 1975). Early structure–activity studies demonstrated that competitive inhibitors of L-glutamate uptake are typically α-amino acids that possess a second acidic group two to four carbon units away from the α-carboxyl group. With the cloning of the various EAATs, selective expression systems could be used to evaluate the potential subtype-selective action of the inhibitors, many of which were identified in these early specificity studies. The *Xenopus* oocyte preparation has proven particularly useful in this regard. The combination of its suitability for electrophysiological studies and the electrogenic nature of EAAT-mediated uptake permits transport to be quantified by recording substrate-induced currents (Zerangue and Kavanaugh, 1996). This not only eliminates a reliance on radiolabelled substrates but also overcomes a serious limitation of classic competition assays, allowing substrates and non-substrate inhibitors (i.e. analogues that bind

to the substrate site on the transporter but are not translocated intracellularly) to be readily differentiated (Koch *et al.*, 1999). Indeed, many studies now report substrate activity in terms of the currents generated relative to the maximum current produced by L-glutamate (I_{max}), rather than the more traditional values used to quantify uptake (e.g. pmol min^{-1} mg protein).

Specificity studies have demonstrated that in addition to L-glutamate, each of the EAAT subtypes can also utilize L- and D-aspartate as substrates. Pharmacological differences among the EAATs begin to emerge when the activities of structurally modified or conformationally constrained analogues of L-glutamate were compared (Bridges *et al.*, 1999). Compounds that have been particularly useful in this regard include the pyrrolidine dicarboxylates (PDCs, e.g. L-*trans*-2,4-PDC, L-*trans*-2,3-PDC, L-*anti-endo*-3,4-methano-3,4-PDC), *β-threo* substituted aspartates (e.g. *β-threo*-benzyloxy-aspartate), methyl-substituted glutamates (e.g. (2*S*,4*R*)-4-methylglutamate), and the 2-(carboxycyclopropyl)glycines (CCGs, e.g. (2*S*,3*S*,4*R*)-2-CCG, (2*S*,3*R*,4*S*)-2-CCG) (Chamberlin and Bridges, 1993; Nakamura *et al.*, 1993; Shimamoto *et al.*, 1998; Vandenberg *et al.*, 1997). While the point has not yet been reached where selective inhibitors or substrates are readily available for each of the individual EAATs, a number of interesting pharmacological differences have been observed among the transporter subtypes (Bridges *et al.*, 1999). For example, EAAT2 is easily distinguished from the other transporters based on its sensitivity to the non-substrate inhibitors dihydrokainate and L-*trans*-2,3-PDC. Although not quite as straightforward as EAAT2, EAAT1 stands apart from the other transporters because of its ability to effectively utilize (2*S*,4*R*)-4-methylglutamate and L-serine-O-sulphate as substrates. Studies on EAAT3 and EAAT4 suggest that these systems may be differentiated from the other transporters based on an increased sensitivity to inhibition by L-aspartate-*β*-hydroxymate and L-*α*-aminoadipate, respectively. Lastly, EAAT5 is distinguished by the action of *β-threo*-hydroxy-aspartate and L-*trans*-2,4-PDC, which act as non-substrate inhibitors of this system but as alternative substrates all the other EAATs.

Physiological Roles of the Excitatory Amino Acid Transporters

Investigations into the roles of transport in excitatory transmission have focused primarily on two central issues: its contribution to shaping the postsynaptic signal, and its significance in protecting neurons from glutamate-mediated excitotoxic injury. By influencing the amount and/or time course of L-glutamate in extracellular environment accessible to EAA receptors, it is easy to envision how transport might contribute to either process. Estimations of binding rates obtained from EAAT1 expressed in oocytes suggest the values are comparable to those reported for binding to postsynaptic receptors (Wadiche and Kavanaugh, 1998). Consequently, if transporters are present in sufficient densities near the site of glutamate release, then they may compete with the receptors for transmitter. Experimental evidence with transport inhibitors suggests, however, that the contribution of uptake in shaping postsynaptic signals appears to vary among glutamatergic synapses (Barbour *et al.*, 1994; Diamond and Jahr, 1997; Isaacson and Nicoll, 1993; Sarantis *et al.*, 1993; Tong and Jahr, 1994). Thus, while studies with hippocampal neurons in cultures and Purkinje neurons in cerebellar slices both indicate that inhibition of transport does influence the excitatory postsynaptic signal, studies carried out with other model systems suggest that this is not always the case. In a related capacity, transporter density and activity may also be factors in determining the extent to which synaptically released glutamate can 'spill over' and activate receptors at sites distant to the location of release (Bergles *et al.*, 1999; Diamond and Jahr, 2000; Scanziani *et al.*, 1997).

The importance of regulating extracellular levels of L-glutamate takes on even greater significance when the potential excitotoxic properties of this neurotransmitter are considered. Accumulating evidence suggests that excessive activation of EAA receptors is a fundamental mechanism of neuropathology in acute CNS injury (e.g. stroke, spinal cord injury, head trauma) as well as chronic neurodegenerative disorders (e.g. amyotrophic lateral sclerosis, epilepsy, Huntington's disease, Alzheimer's disease) (Choi, 1994; Meldrum, 2000). In this respect, the transporters appear ideally suited to play a vital role in excitotoxic protection. That this is indeed the case is supported by *in vitro* and *in vivo* experiments in which the excitotoxic properties of L-glutamate are exacerbated when EAAs are administered to the CNS in combination with uptake blockers (McBean and Roberts, 1985; Robinson *et al.*, 1993). In a number of studies, transport inhibitors by themselves have also been observed to produce neuronal injury (Lievens *et al.*, 1997). For example, the direct administration of either *β-threo*-hydroxy-aspartate or L-*trans*-2,4-pyrrolidine to rat striatum produced significant neuropathology. In other studies, these same inhibitors produced a slow degeneration of motor neurons when added chronically to organotypic spinal cord cultures (Rothstein *et al.*, 1993). The loss of motor neurons in this model system is particularly intriguing in view of the reduction in transporter capacity observed in amyotrophic lateral sclerosis (see below). Also consistent with an inverse relationship between transporter function and vulnerability to glutamate-mediated damage are molecular studies demonstrating that chronic administration of antisense oligonucleotides for GLAST, GLT-1 or EAAC1 (homologues of EAAT1-3, respectively) in both *in vitro* and *in vivo* models induce neuronal injury consistent with excitotoxicity (Rothstein *et al.*, 1996). Equally compelling, mice carrying a homozygous deficiency for GLT-1 (EAAT2 homologue) exhibit lethal spontaneous seizures and increased susceptibility to cortical damage (Tanaka *et al.*, 1997).

Glutamate Transport in Neurological Disorders

In view of the evidence indicating that these transporters serve in a neuroprotective capacity, it is ironic that data are also coming forth that suggest these uptake systems may, under certain pathological conditions, actually participate in the processes of excitotoxicity as sites of cellular L-glutamate efflux (Takahashi *et al.*, 1997). Thus, CNS insults that severely compromise energy levels may lead to the collapse of the ion gradients that enable the cells to accumulate and maintain high intracellular concentrations of L-glutamate. This may, in turn, lead to a concentration-dependent efflux of L-glutamate into the extracellular space through the reverse action of the transporters. The end result would be increased levels of extracellular L-glutamate, decreased transport capacity, and a corresponding increase in the likelihood of excitotoxic pathology. Such a possibility is supported by experiments demonstrating that the efflux of EAAs observed following metabolic insults can be influenced by alternative substrates and non-substrate inhibitors of the EAATs (Koch *et al.*, 1999; Longuemare and Swanson, 1995). It is much more difficult, however, to determine whether the process of transporter reversal occurs *in vivo* and to what extent it contributes to neuronal injury.

To a large degree, a similar problem arises in trying to assess the significance of changes observed to occur in glutamate transport systems in a variety of neurological disorders. For example, both increases and decreases in transport capacity have been reported to occur in schizophrenia (Aparicio-Legarza *et al.*, 1997; Simpson *et al.*, 1998; Smith *et al.*, 2001). In the case of neurodegenerative diseases, glutamate uptake appears to be compromised in both Alzheimer's disease and amyotrophic lateral sclerosis (ALS). It is probably within ALS that the strongest connection has been made between transport and a disease process (Rothstein, 1996). Studies at the functional level have demonstrated that activity is

attenuated, while those at the molecular level indicate that the loss of activity may be linked to aberrant mRNA processing (Lin *et al.*, 1998). A complexity, however, that remains with all such studies is the difficulty in assessing not only the specific pathological contribution of compromised transport but also whether observed losses in transport are a primary or secondary result of the disease. The latter is particularly likely, as several studies have demonstrated that the EAATs are especially sensitive to inactivation by the reactive oxygen species (Trotti *et al.*, 1998). Given the wide range of CNS disorders to which increased oxidative stress is believed to contribute (e.g. stroke, spinal cord injury, chronic neurodegenerative diseases, and even ageing), it should not be surprising to find excitotoxicity linked to losses in glutamate transport as a common feature of CNS pathology.

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VI

Peptidergic Transmitter Systems

Miklós Palkovits

INTRODUCTION

The past decades of research on peptides have provided convincing evidences that neuronal peptides play an important role in the nervous system as neurotransmitters, neuromodulators and neurohormones. The identification and localization of neuropeptides in the brain have been greatly facilitated by the development of biochemical, immunohistochemical and *in situ* hybridization histochemical techniques. A variety of neuropeptides and peptide receptors has been isolated, characterized and localized in the central nervous system (CNS). This chapter gives a brief summary of the topography of peptidergic neurons in major brain areas, as well as their participation in central regulatory mechanisms. Instead of the overwhelming list of the original references for each neuropeptides listed below, we refer to review articles for information and references (Hökfelt *et al.*, 1986; Hökfelt *et al.*, 1987; Palkovits, 1987a; Palkovits, 1987b; Palkovits, 1996; Palkovits, 1999).

BRAIN-BORN NEUROPEPTIDES

The classification of neuropeptides in different groups is rather arbitrary. The *hypothalamic neuropeptides* are synthesized mainly in the hypothalamus and act partly as neurohormones at the pituitary level (releasing and release-inhibiting hormones) or in the periphery (posterior pituitary hormones: vasopressin and oxytocin). Since many of them have been identified in neurons of other brain areas, the terminology does not relate exclusively to their hypothalamic topography. The *pituitary peptides* are characteristically of pituitary origin, but they are also synthesized in neuronal cells, especially in the hypothalamus. Their concentrations in the brain, however, are in orders of magnitude less than in the pituitary. The third group includes the *opioid peptides*, which are distributed widely in the CNS, some of them in high concentrations in the hypothalamus. The fourth group is the *brain-born gastrointestinal or brain–gut peptides*. These were discovered in the gastrointestinal tract, but they are also expressed by neurons in the central and peripheral nervous systems. The *other neuropeptides* are classified in the artificially constructed fifth group; many of the neuropeptides in this group participate in the central control of blood pressure and food intake.

Hypothalamic Neuropeptides

These neuropeptides are involved intimately in regulating anterior pituitary functions or acting as neurohormones on the periphery. All of them are highly concentrated in the median eminence (Table VI.1).

Luteinizing Hormone-Releasing Hormone or Gonadotropin-Releasing Hormone

The decapeptide luteinizing hormone-releasing hormone (LH-RH) has been identified in hypothalamic extracts. This is a signalling molecule that regulates reproduction in all vertebrates. Recently, genes encoding two other LH-RH forms have been discovered that are identical in all species (Fernand and White, 1999).

LH-RH is synthesized mainly in preoptic and anterior hypothalamic neurons in the rat and cat, and in the arcuate nucleus of birds, dogs, monkey and human brains. In the rat, the majority of LH-RH fibres travel along the fibres of the medial forebrain bundle in the lateral hypothalamic area and enter the medial basal hypothalamus through the lateral retrochiasmatic area. This small area, at the caudal edge of the optic chiasm, serves as a gate for a number of peptidergic and aminergic fibres to reach the median eminence (Palkovits, 1984). In the external layer of the median eminence, LH-RH is secreted into portal vessels transported to the anterior pituitary, where it stimulates secretion of luteinizing hormone and follicle-stimulating hormone from pituitary gonadotrophs.

LH-RH fibres from the medial preoptic neurons innervate the organum vasculosum laminae terminalis (OVLT), one of the circumventricular organs where no blood–brain barrier exists. LH-RH neurons and fibres in the accessory olfactory bulb, medial olfactory tract and the septum represent the migration path of LH-RH neurons during the prenatal life.

Thyrotropin-Releasing Hormone

Thyrotropin-releasing hormone (TRH) stimulates anterior pituitary cells to produce and release thyrotrophin hormone and acts as a neurotransmitter/neuromodulator in the brain. TRH secretion is also regulated by neurotransmitters, such as noradrenaline, somatostatin and opioids, as well as by stressful stimuli, including acute cold exposure. This tripeptide is distributed widely in the CNS. Neuronal perikarya were demonstrated mainly in the hypothalamus and the medulla oblongata. In the hypothalamus, TRH is synthesized in the parvicellular portion of the paraventricular nucleus (PVN), the preoptic suprachiasmatic nucleus, the dorsomedial nucleus and the perifornical nucleus, the lateral hypothalamus, and the premamillary region. In the medulla oblongata, TRH immunoreactivity occurs in neurons of the raphe nuclei in coexistence with substance P and serotonin. These neurons project to the spinal cord and participate in pain inhibition. The densest concentration of TRH fibres and terminals is in the median eminence (mainly of PVN origin) and in the OVLT. TRH-immunoreactive axons and terminals form relatively dense networks in the amygdala, the lateral septal nucleus, and the dorsomedial medulla oblongata, mainly in the nucleus of the solitary tract (NTS).

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Table VI.1 Neuropeptides in the median eminence

Neuropeptide	Concentration (ng mg^{-1} protein)	Compared with brain average	Origin of fibres	
			External layer	Internal layer
LH-RH	10–100	Highest	MPO	
TRH	10–100	Highest	PVN	
CRH	10–100	Highest	PVN	PVN
GRH	10–100	Highest	NA	
Somatostatin	100–1000	Highest	NPE	
Vasopressin	100–1000	Highest	PVN	PVN, SON
Oxytocin	100–1000	Highest	PVN	PVN, SON
POMC	1–10	Higher	NA	
Enkephalins	1–10	Average	PVN	PVN, SON
Dynorphins	0.1–1	Average	PVN	PVN, SON
Substance P	1–10	Average	NA	
NPY	100–1000	Higher	NA	
VIP/PHI-27	0.1–1	Lower	PVN	
PACAP		Higher	PVN	PVN, SON
CCK	1–10	Average	PVN	PVN
GRP/bombesin	1–10	Higher	NA	
Galanin	10–100	Highest	NA	PVN
Neurotensin	1–10	Higher	PVN	
Angiotensin II		Highest		PVN, SON
Apelin		Highest	NA	PVN
ANP	1–10	Highest	NA	
CGRP		Average	NA	
CART		Average	NA	

MPO, medical preoptic nucleus; NA, arcuate nucleus; NPE, hypothalamic periventricular nucleus; PVN, paraventricular nucleus; SON, supraoptic nucleus.

Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH) represents the major stress hormone in the brain. It stimulates the release of adrenocorticotropic hormone (ACTH) from the corticotroph cells of the anterior pituitary and acts as a neurotransmitter in the CNS. This neuropeptide shows a wide distribution in the hypothalamus and extrahypothalamic brain. The PVN contains a high number of CRH-immunopositive cells, mainly in the medial and anterior parvicellular subdivisions of the nucleus (exclusive sources of the CRH fibbers in the median eminence). CRH cells merge into the posterolateral parvicellular subdivision as far caudal as the posterior edge of the nucleus (neurons from here project to spinal and medullary autonomic centres). CRH fibres leave the PVN in a lateral direction. One set of fibres turns around the fornix and approaches the median eminence, while others travel through the lateral hypothalamus and the ventrolateral corner of the lower brain stem to parasympathetic (vagal nuclei) and sympathetic (thoracic spinal cord) preganglionic neurons.

The presence of various numbers of CRH cells has been reported in other hypothalamic regions, mainly in the medial preoptic, anterior hypothalamic, dorsomedial, and perifornical nuclei (Table VI.2). A number of CRH-immunoreactive cells were demonstrated in the central nucleus of the amygdala, the bed nucleus of the stria terminalis (NIST; only its lateral subdivision), the lateral parabrachial nucleus, and the NTS. Considerable amounts of CRH are synthesized in inferior olivary neurons and transported through the olivocerebellar tract to the cerebellum (CRH coexists with aspartate in the climbing fibres).

The densest accumulation of CRH-immunoreactive terminals is seen in the external layer of the median eminence. The concentration of CRH in the median eminence measured by radioimmunoassay is almost two orders of magnitude higher than that of other brain nuclei (Table VI.1). Moderate to dense networks of CRH fibres and terminals are present in several brain areas and nuclei.

Recently, a potent mammalian CRH-related neuropeptide, urocortin II, was discovered and shown to be bound with high affinity to type 2 CRH receptors. This 38-amino acid peptide is expressed in the paraventricular and arcuate nuclei of the hypothalamus and in the locus coeruleus. Initial functional studies are consistent with urocortin II involvement in central autonomic and appetitive controls (Reyes *et al.*, 2001).

Growth Hormone-Releasing Hormone

Two forms of growth hormone-releasing hormone (GRH), GRH-40 and GRH-44, have been isolated from a tumour of human pancreatic islet cells causing acromegaly. Both forms stimulate growth hormone release from the anterior pituitary. It is known that full biological activity of these peptides resides in the first 29 amino acids.

GRH-synthesizing cells are found almost exclusively in the medial basal hypothalamus (in the arcuate and ventromedial nuclei) with a major projection to the median eminence–pituitary stalk. Scattered GRH-immunopositive fibres can be traced to several hypothalamic regions, mainly to the periventricular nucleus (they form synaptic contact with somatostatin neurons here) and the lateral hypothalamic area.

Somatostatin

Somatostatin was first identified in the hypothalamus, where it is highly concentrated in neuronal cells. This peptide is also produced in a wide variety of neurons throughout the CNS and in various cell types in peripheral organs. Besides its inhibitory effect on growth hormone release and thyroid-stimulating hormone secretion in the anterior pituitary, somatostatin acts as a neurotransmitter in the brain and appears to regulate the activity of pancreatic islet, gastrointestinal and immune cell functions in the periphery. These

Table VI.2 Presence of peptide-expressing neurons in neuropeptide-rich brain areas

Nucleus/region	LH-RH	TRH	CRH	Somatostatin	GRH	Vasopressin	Oxytocin	ACTH/β-endorphin	α-MSH	Enkephalins	Dynorphins	Substance P	NPY	VIP-PHI27	CCK	Galanin	Neurotensin	Angiotensin II	ANP	CGRP	CART
Lateral septal										+	+	+	+	+	+	+	+				+
Central amygdala		+	+						+	+	+	+	+	+	+	+				+	
NIST										+	+	⊕	+	+	+	+	+	+	+		
Medial preoptic	⊕	+	+							+	+	+	+		+	+	+		+	+	
Periventricular		+		⊕						+	+		+		⊕	+	+		⊕		+
Paraventricular		⊕	⊕	+		⊕	⊕			⊕	+		+	+	+	+	+	+		+	+
Dorsomedial				+						+	+	+	+		+	⊕	+			+	
Perifornical		+							+	+	+					+	+	⊕		+	
Arcuate				+	⊕			⊕	⊕	+	+	+	⊕		+	⊕	+		+	+	⊕
Thalamic PVN										+						+	+			+	
Parabrachial			+								+		+		+	+	+			+	
NTS			+							+	+	+	⊕	+	+	+	+			+	+
Dorsal horn										+		+	+				+				

Higher number of peptide-expressing neurons is indicated by encircled crosses.

actions are mediated by five different subtypes of G-protein-coupled somatostatin receptors (Patel, 1999).

The majority of somatostin-synthesizing neurons are present in the preoptic and hypothalamic periventricular nuclei along the third ventricle. These cells project to the external layer of the median eminence. Axon collaterals of these cells reach GRH-containing neurons in the medial basal hypothalamus and establish synaptic contacts with these cells (Horváth *et al.*, 1989).

Somatostatin-immunostained perikarya are found scattered in the cerebral cortex, hippocampus, olfactory bulb and tubercle, striatum, nucleus accumbens, lateral septal nucleus and amygdaloid nuclei. In the lower brainstem, somatostatin cells were demonstrated in the periaqueductal central grey and the reticular formation. A number of cells in the dorsal horn (lamina II) and the intermediolateral cell column of the thoracic spinal cord also synthesize somatostatin.

Somatostatin fibres and terminals are present throughout the entire CNS in varying density, but by far the highest concentration is in the median eminence (Table VI.1). The major somatostatin-containing neuronal systems include the hypothalamic pathway connecting the preoptic and hypothalamic periventricular neurons with the limbic system, cortical interneurons, and amygdala-limbic connections through the stria terminalis.

Somatostatin is regarded as a phylogenetically ancient multigene family of peptides with two bioactive products: somatostatin-1–14 and somatostatin-1–28. These forms are synthesized from a large pre-prosomatostatin precursor molecule. Recently, a novel somatostatin-like gene, *cortistatin*, has been discovered that gives rise to two cleavage products, cortistatin-14 and cortistatin-29 (Patel, 1999; Spier and de Lecea, 2000). In contrast to the wide distribution of somatostatin in the brain, cortistatin is restricted to the cerebral cortex and the hippocampus (Spier and de Lecea, 2000).

Prolactin-Releasing and -Inhibiting Factors

Much physiological evidence supports the existence of the peptides Prolactin-releasing factor (PRF) and Prolactin-inhibiting factor (PIF) in the hypothalamus, but neither PRF nor PIF has been identified yet. Many neuropeptides (TRH, oxytocin, β-endorphin, vasoactive intestinal peptide (VIP)) have been shown to elevate prolactin release. In contrast, hypothalamic dopamine and γ-aminobutyric acid (GABA) may act as PIFs.

Vasopressin

Vasopressin was one of the first neuropeptides (along with oxytocin) to be isolated in the hypothalamus. Cells are located mainly in magnocellular neurosecretory nuclei: two-thirds of the supraoptic neurons and about half of the paraventricular magnocellular neurons synthesize vasopressin. Evidence has been furnished on the synthesis of vasopressin in parvicellular PVN cells coexpressing with CRH, especially in response to stressful stimuli. A distinct group of vasopressin-synthesizing cells is present in the suprachiasmatic nucleus.

Vasopressin fibres from the supraoptic and paraventricular nuclei, in addition to oxytocin fibres, constitute the hypothalamo-hypophyseal neurosecretory tract. This passes through the lateral retrochiasmatic area and the internal layer of the median eminence–pituitary stalk on the way into the posterior pituitary, where vasopressin is stored until its release into the general circulation.

Solitary vasopressin-immunoreactive fibres from the hypothalamic nuclei can be traced in different forebrain and limbic areas. Long, descending vasopressin fibres to the lower brainstem and spinal cord have been demonstrated by immunohistochemical techniques. These axons appear to arise in the PVN.

Oxytocin

This peptide is synthesized only by neurons in the magnocellular hypothalamic nuclei, in almost equal numbers in the supraoptic, paraventricular and accessory magnocellular nuclei. In neurons of these nuclei, oxytocin is coexpressed with cholecystokinin (CCK) and enkephalins. Oxytocin fibres constitute a substantial component of the hypothalamo-hypophyseal neurosecretory tract, which terminates in the posterior pituitary. Descending oxytocin fibres to the medullary and spinal autonomic centres are exclusively of paraventricular origin. They form a relatively strong terminal network in the NTS, the caudal ventrolateral medulla and the

spinal cord (substantia gelatinosa in the dorsal horn and the intermediolateral cell column).

Brain-Born Pituitary Neuropeptides

The presence of anterior pituitary hormones, such as luteinizing hormone (LH), thyrotropin, growth hormone (GH) and prolactin, has been reported in rat forebrain structures as revealed by radioimmunoassay and immunohistochemistry. Their origin in the brain is supported by their invariable presence after hypophysectomy.

Three brain-born pituitary peptides arise from a common 31-kDa precursor molecule, ***pro-opiomelanocortin (POMC)***: ACTH, β-endorphin and α-melanocyte-stimulating hormone (α-MSH). (Although, β-endorphin is one of the opioid peptides, because of its origin from the POMC molecule and colocalization with ACTH and α-MSH, it is classified into this group of neuropeptides.) The ACTH molecule is cleaved to α-MSH and Corticotropin-like intermediate lobe peptide (CLIP) (ACTH-18–39). The vast majority of ACTH immunoreactivity in arcuate neurons is actually CLIP.

The POMC peptides colocalize in the same neurons of the medial basal hypothalamus, almost exclusively in the arcuate and ventral premamillary nuclei. Some additional ACTH and β-endorphin neurons have also been found in the NTS.

ACTH- and β-endorphin-containing fibres and terminals are found in several hypothalamic (mainly in the PVN) and extrahypothalamic nuclei. Pronounced β-endorphin fibre labelling is present in the amygdala, thalamic PVN, midbrain central grey, raphe nuclei, lateral parabrachial nucleus, and in high concentration in the NTS.

Melanocyte-Stimulating Hormones

The presence of α-, β- and γ-MSH has been shown in the CNS of rats by biochemical and immunohistochemical techniques. Two sets of *α-MSH*-containing cell groups are present in the hypothalamus: the first in the arcuate nucleus (in POMC neurons with ACTH and β-endorphin), and the second (immunostained only for α-MSH) in the dorsal part of the lateral hypothalamic area, the perifornical nucleus and the ventral portion of the zona incerta. (The distribution area of these neurons overlaps the area where orexin and melanin-concentrating hormone neurons are localized; see p. 90) This group of cells appears to project exclusively to the cerebral cortex, hippocampus, olfactory bulb and striatum.

α-MSH exerts an anorexigenic action in the brain. It has been demonstrated recently that leptin has a stimulatory effect on the production of α-MSH.

γ-MSH-containing cells were identified in the arcuate nucleus, the dorsolateral tegmentum of the pons, and the NTS.

Brain-Born Opioid Peptides

Five groups of opioid peptides have been identified in the CNS: (1) enkephalins (Met- and Leu-enkephalin, Met-enkephalin-Arg-Phe, Met-enkephalin-Arg-Gly-Leu), (2) β-endorphin, (3) the dynorphins (dynorphin A, dynorphin 1–8, dynorphin B, α-neo-endorphin, β-neo-endorphin), (4) endomorphins and (5) nociceptin (orphanin FQ). They distribute in the brain in individual topographical patterns. The opioid neuropeptides frequently coexist with other neuropeptides or neurotransmitters. Besides their potent antinociceptive actions, they appear to participate in regulation of secretion of pituitary hormones, and are involved in autonomic regulations.

Enkephalins

Met- and Leu-enkephalins are distributed widely in the CNS. Enkephalin-containing perikarya were demonstrated in all major brain regions, including the cerebral cortex (high density in the cingulate and piriform cortex), hippocampus, striatum, hypothalamus (ventromedial, arcuate and paraventricular nuclei), amygdala, and NIST. In the lower brainstem, enkephalin-containing cells occur in the periaqueductal central grey, raphe nuclei, parabrachial nucleus, spinal trigeminal nucleus and NTS. In the dorsal horn of the spinal cord (lamina III), enkephalin is synthesized in inhibitory interneurons.

The most abundant enkephalinergic networks are seen in the hypothalamic ventromedial nucleus and several extrahypothalamic regions (globus pallidus, nucleus accumbens, central grey, NTS, spinal trigeminal nucleus, dorsal horn). In the hypothalamus, enkephalin-expressing neurons are coexpressed with CRH (in the PVN) and oxytocin (in the supraoptic nucleus). Enkephalins are coexpressed with almost all of the classical neurotransmitters in particular brain areas: with acetylcholine in the spinal cord, with dopamine in the arcuate nucleus, with noradrenaline in the locus coeruleus, with serotonin in medullary raphe nuclei, and with GABA in the caudate nucleus.

Met-enkephalin-Arg-Phe was originally isolated from the adrenal gland, but it is also distributed widely in the brain. Its topographical distribution pattern in the brain is similar but not identical with that of met- and leu-enkephalins.

β-Endorphin

As a member of the POMC-derived peptides, endorphin was discussed earlier.

Dynorphins

The five dynorphin-related peptides are derived from a common precursor molecule, pro-neo-endorphin/dynorphin. The distribution patterns of dynorphin A and α-neo-endorphin are identical, while those of the other three dynorphins are individual. They establish high analgesic activities at both hypothalamic (action on β-endorphin neurons) and spinal cord levels.

The hypothalamus contains one of the highest concentrations of dynorphins in the CNS. The most prominent cell types containing dynorphins are the magnocellular hypothalamic neurons (coexisting with vasopressin) and neurons in the arcuate nucleus, where they are likely to coexist with dopamine. In addition, dynorphin-containing perikarya are expressed in the striatum, dentate gyrus, central amygdaloid nucleus and lower brainstem (substantia nigra, central grey matter, parabrachial, spinal trigeminal and solitary tract nuclei). Dynorphins are expressed in lamina I and II neurons of the dorsal horn.

Among the several brain regions where dynorphins are present in nerve terminals, five regions are prominently rich in these opioid peptides: nucleus accumbens, globus pallidus, hippocampus CA3 area, hypothalamic paraventricular nucleus, and pars reticulata of the substantia nigra.

Endomorphins

Endomorphins (endomorphin 1 (EM1) and endomorphin 2 (EM2)) are endogenous tetrapeptides with high affinity and selectivity for the μ-opiate receptor and potent analgesic activities. Both are synthesized by neurons in the hypothalamus (dorsomedial and posterior nuclei), but only EM1 perikarya are present in the nucleus of the solitary tract (Martin-Schild *et al.*, 1999). EM1-like immunoreactivity is distributed widely and densely in the brain, while EM2 is more restricted to the superficial laminae of the dorsal horn and the spinal trigeminal nucleus. Dense networks of EM1-like immunoreactive fibres and terminals were detected in the lateral parabrachial and solitary tract nuclei.

Moderate EM1 immunostaining was present in the frontal cortex, nucleus accumbens, NIST, several hypothalamic and amygdaloid nuclei, medial septal-diagonal band complex, periaqueductal central grey, locus coeruleus, and lamina I of the dorsal horn. The neuroanatomical distributions of these two peptides suggest that endomorphins participate in modulating nociceptive and autonomic nervous system processes (Martin-Schild *et al.*, 1999).

Nociceptin

Nociceptin/orphanin FQ (N/OFQ) is a heptadecapeptide opioid-like endogenous agonist of the opioid receptor homologue, N/OFQ receptor. This peptide and its precursor, pre-proN/OFQ, exhibit structural features suggestive of the opioid peptides. N/OFQ, its precursor protein and its receptor are distributed widely throughout the CNS. There are several regions where N/OFQ cells colocalize with other opioid peptides. N/OFQ cells are present in numerous neuronal circuits and systems, supporting its possible involvement in many behavioural, autonomic and physiological functions, including nociception (Neal *et al.*, 1999; Houtani *et al.*, 2000).

Brain-Born Gastrointestinal or Brain–Gut Neuropeptides

Substance P

The undecapeptide substance P is the main member of the tachykinin peptide family. The pre-protachykinin A precursor molecule contains sequences encoding substance P and neurokinin A, while pre-protachykinin B encodes neurokinin B. All of these are present in the central and peripheral nervous systems and in gut endocrine cells.

The major substance P pathways and systems in the brain are extrahypothalamic. This neuropeptide is strongly involved in pain conduction. Neurons are present in dorsal root ganglion cells (coexpressed with glutamate and calcitonin gene-related peptide (CGRP)) and also in the secondary nociceptive neurons in the dorsal horn and the spinal trigeminal nucleus.

Several other brain areas contain substance P-synthesizing neurons. Substance P represents one of the major transmitters in the striato-nigral connections. Small- to medium-sized cells in the caudate-putamen project to the globus pallidus and the substantia nigra, where substance P facilitates dopaminergic neurons. Substance P neurons are present in other telencephalic regions (amygdala, nucleus of the diagonal band, lateral septal nucleus, NIST), but cerebral cortical areas are devoid of substance P cells. One of the limbic pathways, the habenulo-interpeduncular tract, contains substance P fibres in colocalization with acetylcholine.

Almost all of the hypothalamic nuclei contain substance P neurons. The moderate amount of substance P in the median eminence is of arcuate nucleus origin.

Substance P is frequently coexpressed with other neuropeptides and serotonin (substance P–CCK in central grey neurons, substance P–TRH–serotonin in the ventromedial medulla oblongata). Axons of the medullary substance P–TRH–serotonin neurons constitute the major antinociceptive pathway in the CNS, from the brainstem to the spinal dorsal horn.

Neuropeptide Y

Neuropeptide Y (NPY) is a 36-amino acid peptide. It shares important sequence homology with another peptide isolated from porcine intestine, peptide YY. These two peptides share important similarities with the pancreatic polypeptides. NPY is a potent stimulator of feeding behaviour, influences the activity of the gastrointestinal and cardiovascular systems, and participates in body temperature regulation.

NPY is one of the most abundant neuropeptides in the rat brain. Several NPY-containing perikarya have been demonstrated in the cerebral cortex (especially in the entorhinal cortex), hippocampus, striatum and amygdala. In the hypothalamus, the arcuate nucleus contains a fairly large set of NPY neurons. These neurons appear to play a key role in the central regulation of food intake. A group of arcuate NPY cells project to the PVN and lateral hypothalamic neurons, while other NPY cells innervate sympathetic preganglionic neurons in the thoracic spinal cord. These spinal cord-projecting NPY cells coexpress cocaine- and amphetamine-regulated transcript (CART) peptide (Elias *et al.*, 1998).

NPY is coexpressed in lower-brainstem noradrenergic neurons, mainly in the A1 and A2 noradrenergic cell groups of the medulla oblongata (Everitt *et al.*, 1984). Some of the fibres in the ascending ventral noradrenergic bundle also contain NPY. These fibres may innervate hypothalamic and limbic (septal and amygdaloid) nuclei. A high degree of coexpression has also been demonstrated between NPY and somatostatin, particularly in neurons of the cerebral cortex, hippocampus and striatum.

Vasoactive Intestinal Polypeptide

VIP has been designated as a member of the glucagon–secretin peptide family. It is related structurally to PHI-27 (peptide histidine isoleucine of 27 amino acids). VIP has a wide range of effects on the cardiovascular, respiratory and gastrointestinal systems.

Numerous VIP-immunoreactive neurons have been demonstrated in the brain, especially in the cerebral cortex (bipolar neurons in layers II–IV). They are frequently coexpressed with acetylcholine. Evidence from surgical isolation of cortical areas suggests that VIP cells in the cortex are local interneurons. VIP perikarya have also been found in the hippocampus, olfactory bulb, claustrum and amygdala. In the hypothalamus, the suprachiasmatic nucleus is the major source of VIP neurons. Axons leave the nucleus in a dorsal direction and terminate in the periventricular and anterior hypothalamic nuclei. A number of VIP cells have been found in the periaqueductal central grey, dorsal raphe nucleus and NTS.

Numerous VIP axons and terminals have been demonstrated throughout the entire CNS, mainly in forebrain areas.

PHI-27

This peptide has an NH_2-terminal histidine and a COOH-terminal isoleucine amine and 27 amino acid residues. It has considerable structural similarities to VIP, and they have a common precursor molecule. PHI-27 is highly concentrated in the suprachiasmatic nucleus, and is coexpressed with CRH and enkephalin in same parvicellular PVN neurons. Axons from these neurons run into the median eminence and form a dense network around the capillaries in the external layer. It has been suggested that these neurons are involved in the control of prolactin, ACTH and growth hormone secretion in the anterior pituitary (Hökfelt *et al.*, 1983).

Pituitary Adenylate Cyclase Activating Peptide

The neuropeptide Pituitary adenylate cyclase activating peptide (PACAP) belongs to the VIP–secretin–glucagon family. It is a 38-amino acid residue peptide with 68% homology with VIP. As indicated by its name, PACAP is a potent stimulator of pituitary adenylate cyclase. PACAP-containing neurons occur in all major brain regions (cerebral cortex, thalamus, amygdala, nucleus accumbens), but the highest density of PACAP-immunoreactive cells and fibres is found in hypothalamic nuclei (Arimura and Shioda, 1995). The supraoptic and paraventricular nuclei are the major sources of PACAP-immunostained fibres in

the median eminence. PACAP-synthesizing cells have been demonstrated in the medulla oblongata, vagal nuclei and lateral reticular nucleus.

Cholecystokinin

CCK is a 33-amino acid residue peptide. Among different forms of CCK, CCK-8 appears to be the main representative in the brain. It has a strong analgesic effect, acting in the brainstem, thalamus and cerebral cortex by stimulating opioid receptors in these regions. CCK also facilitates sleep and reduces food intake.

CCK is distributed widely in the brain. Several bi- and multipolar but not pyramidal CCK-immunopositive cells are seen in the cerebral cortex, mainly in laminae II and III, having a similar but not identical pattern to VIP. CCK cells are distributed widely in the hippocampus; mainly interneurons (basket cells) but few pyramidal cells express CCK. CCK is coexpressed with GABA in cortical and hippocampal neurons.

CCK perikarya were recognized in the NIST and the medial and cortical amygdaloid nuclei. In the hypothalamus, CCK neurons occur in the magnocellular neurons (coexpressed with oxytocin) and in the periventricular and dorsomedial nuclei. Two groups of CCK-containing cells are seen in the midbrain: one in the substantia nigra-ventral tegmental area (coexpressing with dopamine) and another in the central grey matter. CCK-immunopositive cells are also located in the central grey (lamina X) of the spinal cord.

Several CCK-containing neuronal pathways have been demonstrated in the brain: (1) descending corticostriatal projections in the external capsule from the piriform and prefrontal cortex to the caudate-putamen; (2) hippocampal-septal projections through the fornix and the fimbria; (3) parabrachial CCK neurons project to the hypothalamic ventromedial nucleus (Záborszky *et al.*, 1984); (4) CCK–dopamine fibres from the substantia nigra and the ventral tegmental area ascend in the medial forebrain bundle to the nucleus accumbens, olfactory tubercle, NIST and central amygdaloid nucleus; and (5) evidence for periaqueductal central grey-spinal cord CCK projections has also been reported.

Gastrin-Releasing Peptide/Bombesin

Gastrin-releasing peptide (GRP) is homologous at its carboxy terminus to the tetradecapeptide bombesin, which can be isolated from amphibian skin. They share many physiological activities, therefore GRP has been proposed as a mammalian counterpart of amphibian bombesin. This neuropeptide is the most potent modulator of thermoregulation, acting on the preoptic thermoregulator centre. It also increases blood pressure, inhibits feeding and acts as an antidiuretic.

GRP/bombesin is highly concentrated in the hypothalamus, especially in the arcuate nucleus. These cells project to the parvicellular PVN and may participate in the central control of food intake. High densities of GRP/bombesin-immunoreactive terminals are found in nociceptive brain areas: this peptide and substance P codistribute in the marginal layers of the dorsal horn and the spinal trigeminal nucleus.

Galanin

This peptide was originally isolated from the upper small intestine. It is a 29-amino acid residue peptide that derives from the precursor molecule, pre-progalanin. Galanin stimulates plasma growth hormone and prolactin levels, inhibits insulin secretion, and is involved in the central regulation of food intake.

Galanin shows a wide distribution in the brain, with a high density in the hypothalamus, including the median eminence (Table VI.1). The expression of galanin in primary sensory neurons in dorsal root and Gasserian ganglia has been demonstrated by immunostaining and *in situ* hybridization. Galanin coexists with oxytocin in the PVN and with acetylcholine in the septo-hippocampal system.

The presence of other gastrointestinal peptides, such as secretin, motilin and glucagon, in brain nuclei has been shown by immunostainings (see the reviews listed in the introduction to this chapter).

Other Neuropeptides

A number of neuropeptides in this group (neurotensin, bradykinin, atrial natriuretic polypeptide, angiotensin II) have a common feature: they all participate in central baroreceptor mechanisms.

Neurotensin

Neurotensin was first isolated and sequenced from the hypothalamus on the basis of its strong cardiovascular effect. This 13-amino acid residue peptide is synthesized from the large neurotensin/neuromedin precursor molecule. A large amount of neurotensin is present in endocrine cells of the gastrointestinal mucosa, and its activity there is ten times higher than in the brain. (This is one of the reasons why neurotensin is frequently classified into the brain–gut neuropeptides family.) Neurotensin reduces body temperature, locomotor activity and food intake.

The presence of neurotensin-immunostained perikarya can be demonstrated in several brain areas. Neurotensin is highly concentrated in the caudate nucleus, nucleus accumbens, globus pallidus and mamillary body. In the hypothalamus, neurotensin-synthesizing perikarya have been shown in the arcuate, periventricular and medial preoptic nuclei, in the periventricular subdivision of the PVN, and in the lateral hypothalamic area. Neurotensin fibres are concentrated densely in the external layer of the median eminence, where neurotensin is released into the hypophysial portal circulation and stimulates secretion of prolactin at the level of the anterior pituitary (Rostene and Alexander, 1997). In the pituitary, neurotensin occurs in subsets of gonadotrophs and thyrotrophs.

Many lower-brainstem regions contain dense neurotensin networks of fibres and terminals, such as the ventral tegmental area, parabrachial nucleus, marginal layer of the spinal trigeminal nucleus, and NTS. In the spinal cord, neurotensin fibres are concentrated in the lamina II of the dorsal horn. Neurotensin is coexpressed with dopamine in the substantia nigra and the arcuate nucleus, and with noradrenaline in the NTS.

Angiotensins

All components of the renin–angiotensin system (renin, angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin I, II, III, IV, angiotensin 1–7, and angiotensin receptors) have been demonstrated in the brain. The two major effective peptides (angiotensin II and III) increase blood pressure, drinking behaviour, salt appetite, and the release of anterior and posterior pituitary hormones.

Angiotensin II-immunoreactive perikarya are visualized in the hypothalamus (mainly in the preoptic, paraventricular, supraoptic, suprachiasmatic, perifornical and premamillary nuclei). Substantial numbers of angiotensin II-expressing cells are also present in the subfornical organ, lateral septal, thalamic paraventricular and central amygdaloid nuclei. Two major angiotensin II pathways have been described: (1) interconnections between the subfornical organ and the median preoptic, paraventricular and supraoptic nuclei, and (2) a pathway connecting the hypothalamus to the medulla oblongata (Lenkei *et al.*, 1997).

The effects of angiotensin II and III are mediated through interactions with specific angiotensin receptors (AT1A, AT1B, AT2). The

highest densities of AT1A and AT2 receptors have been demonstrated in the hypothalamus (median preoptic and paraventricular nuclei, retrochiasmatic area), the medulla oblongata, and the circumventricular organs, including the organum vasculosum laminae terminalis, subfornical organ and area postrema (Lenkei *et al.*, 1997). These circumventricular organs may serve as open gates for the circulating angiotensin to enter the brain and influence the activity of hypothalamic vasopressin and atrial natriuretic polypeptide (ANP) neurons.

Apelin

The peptide apelin originates from a larger precursor pre-proapelin molecule. It has recently been isolated and identified as the endogenous ligand of the human orphan G-protein-coupled receptor, APJ (putative receptor protein related to the angiotensin receptor AT1). Apelin-containing perikarya are visualized in the preoptic region, supraoptic and paraventricular nuclei, and (in the highest density) arcuate nucleus. Apelin cells are also seen in the pons and the medulla oblongata. Apelin-immunoreactive nerve fibres and terminals appear in many hypothalamic nuclei and the internal layer of the median eminence. They are present in circumventricular organs, paraventricular thalamic nucleus, periaqueductal central grey matter, dorsal raphe nucleus, parabrachial and Barrington nuclei in the pons, and in the NTS, lateral reticular, prepositus hypoglossal and spinal trigeminal nuclei in the medulla oblongata (Reaux *et al.*, 2002). The topographical distribution of apelinergic neurons in the brain suggests a multiple role for apelin, especially in the central control of feeding behaviours, pituitary hormone release and circadian rhythms.

Atrial Natriuretic Polypeptides

The atrial natriuretic peptides (ANP, cANP, brain natriuretic peptide [BNP]) are found in high concentrations in the brain (hypothalamus, limbic system, brainstem), where they regulate fluid balance, thirst and drinking behaviour, and cardiovascular functions, and inhibit the release of vasopressin. They are dominantly synthesized by neurons of the preoptic and hypothalamic periventricular nuclei. These cells are connected synaptically with subfornical angiotensin II, as well as with supraoptic and paraventricular vasopressinergic neurons. Reportedly, periventricular ANP neurons project downwards to innervate medullary baroreceptor neurons in the NTS.

Bradykinin

The presence of the nonapeptide bradykinin in neurons of the CNS was demonstrated by immunohistochemistry. This neuropeptide regulates blood pressure and acts as one of the most potent pain-transmitting substances in the brain. Bradykinin-containing perikarya are mainly present in the periaqueductal central grey and the posterior and lateral hypothalamus. Fibres are seen in the lateral hypothalamus, preoptic area and periaqueductal central grey.

Calcitonin Gene-Related Peptide

CGRP originates from a gene common with calcitonin. This 37-amino acid peptide acts on central and peripheral brain tissues via distinct receptor mechanisms. CGRP inhibits growth hormone release, feeding and gastric acid secretion, and participates in conduction of pain signals. CGRP is coexpressed with glutamate and substance P in sensory ganglion cells (in dorsal root and Gasserian ganglia). Very dense CGRP-immunoreactive terminal networks are seen in the marginal layers of the spinal dorsal horn and spinal trigeminal nucleus. Besides nociceptive areas, CGRP is distributed widely in the entire CNS. In the hypothalamus, CGRP is synthesized by neurons in the dorsomedial, perifornical and ventral premamillary nuclei and the retrochiasmatic and lateral hypothalamic areas.

Cocaine- and Amphetamine-Regulated Transcript (CART) Peptide

In situ hybridization studies have revealed that CART mRNA is localized in neuronal groups involved in a large variety of neural, endocrine and autonomic functions, including the regulation of feeding behaviour (Broberger, 1999). CART-expressing neurons are found in the paraventricular, arcuate and dorsomedial hypothalamic nuclei, and in the lateral hypothalamic area, olfactory bulb, lateral septal nucleus and medial posterodorsal amygdaloid nucleus (Koylu *et al.*, 1997). CART is coexpressed in a percentage of NPY neurons in the arcuate nucleus. Axons of these cells descend to the spinal cord and innervate preganglionic sympathetic neurons (Elias *et al.*, 1998). CART peptide is coexpressed with TRH in the PVN, and with melanin-concentrating hormone (MCH) in lateral hypothalamic neurons (Broberger, 1999).

Orexin

Two orexin (hypocretin) peptides (orexin-33 (hypocretin-1) and orexin-28 (hypocretin-2)) have been isolated. Orexin is synthesized exclusively in the lateral hypothalamus and adjacent areas (Broberger *et al.*, 1998). These peptides have a prominent role in sleep–wake regulation by actions on basal forebrain structures (medial preoptic area, medial septal nucleus, substantia innominata).

Melanin-Concentrating Hormone

MCH-expressing cells occupy the dorsal part of the caudal hypothalamus, including a portion of the dorsomedial nucleus, the perifornical nucleus and the lateral hypothalamic area (Broberger *et al.*, 1998). A smaller population of MCH neurons exist in the olfactory tubercle and pontine tegmentum. MCH neurons in the lateral hypothalamic area receive inputs from arcuate NPY and agouti-related protein (AgRP) neurons (Broberger *et al.*, 1998). These neurons participate in the hypothalamic neuronal circuit that controls food intake.

Agouti-Related Protein

AgRP, a melanocortin receptor antagonist, is a potent orexigenic peptide. It is coexpressed with NPY in the arcuate nucleus, but not all NPY neurons express AgRP. Axons of these neurons project to the lateral hypothalamus and establish synaptic contacts with orexin- and MCH-expressing cells, whereby AgRP is considered to be an important component of the central neuronal circuit that controls food intake (Broberger *et al.*, 1998). The expression of AgRP in arcuate neurons is suppressed by leptin.

Tuberoinfundibular Peptide of 39 Residues

Tuberoinfundibular peptide of 39 residues (TIP39) peptide was recently purified from bovine hypothalamus based on selective parathyroid hormone-2 receptor activation (Usdin *et al.*, 2000). TIP39 is expressed in the caudal hypothalamus (subparafascicular area) and in the pons (caudal paralemniscal nucleus). Among other functional activities, this peptide may participate in pain perception.

NEUROPEPTIDE-RICH AREAS IN THE CENTRAL NERVOUS SYSTEM

Neuropeptides in general are present in almost all major brain areas, but there are high variations regarding their concentrations and cell/terminal densities. In this section, we introduce brain regions and nuclei where the expression or concentration of neuropeptides is higher then the average in the brain (Table VI.2). The region that is most rich in neuropeptides in the brain is the median eminence. Almost, all of the known brain-born neuropeptides are present in the median eminence (Table VI.1).

Cerebral Cortex

The 'classical' neurotransmitters (GABA, glutamate, acetylcholine) dominate in both local and projecting cortical neurons. Neuropeptides in cerebral cortical neurons are represented by somatostatin, NPY, VIP and CCK. These neuropeptides are expressed by neurons located mainly in layers II–IV. Their cell density here is not particularly high, but they occur in each neocortical and most of the limbic cortical areas such that their total number in the cortex is much higher then that in the extracortical brain.

Limbic System

Hippocampus

Several neuropeptides are present in the hippocampus, almost all of them in interneurons. VIP, PHI-27 and CRH are coexpressed in the same neurons in the molecular and granule cell layers throughout the hippocampal formation, including the dentate gyrus, subicular complex and entorhinal area. Within the pyramidal layer, the basket cells contain these neuropeptides. In addition, CCK, somatostatin, NPY, substance P, enkephalins and dynorphins have been localized in the hippocampus.

Septum

The lateral septal nucleus is rich in neuropeptides (Table VI.2). They are present in both perikarya and nerve terminals.

Amygdala

In general, the amygdala is rich in neuropeptidergic neurons. Somatostatin, VIP and CCK neurons are present in the lateral and basal amygdaloid nuclei. In addition, substance P, enkephalin and neurotensin have been demonstrated in the cortical and medial nuclei. Neuronal perikarya of the central amygdaloid nucleus contain one of the richest assortments of neuropeptides in the brain (Table VI.2). These cells express CRH, somatostatin, neurotensin, substance P, CCK, enkephalin and dynorphins. At least ten other neuropeptides have been immunostained in networks of neuronal fibres and terminals innervating central amygdaloid cells.

The NIST serves as a relay nucleus for amygdaloid efferent (and partly afferent) fibres. This nucleus is rich in neuropeptides in both perikarya and nerve terminals. It contains all the neuropeptides in the neurons found in the central and medial amygdaloid nuclei (Table VI.2).

Thalamus

The paraventricular thalamic nucleus is the only thalamic nucleus that contains neuropeptides in both high numbers and high concentration (Table VI.2).

Hypothalamus

The hypothalamic-pituitary interactions, and control of the production and release of pituitary hormone, represent the most significant function of the hypothalamic neuropeptides. They also play a significant role in other mechanisms controlling the autonomic nervous system, temperature regulation, biological rhythms and behavioural responses.

Peptidergic Neuronal Perikarya

Preoptic Area

Several neuropeptides are synthesized by preoptic neurons (Table VI.2). In the rat, LH-RH is mainly present in the medial preoptic nucleus. This nucleus is one of the major centres of temperature regulation. Neuropeptides here, such as CRH, NPY, neurotensin and CCK, may participate in this regulatory mechanism. The periventricular preoptic nucleus is one of the major pools of brain-born ANP. Neurons here receive inputs from subfornical angiotensin neurons and project to vasopressinergic neurons in the supraoptic and paraventricular nuclei.

Anterior Hypothalamus

The PVN serves as an integrator of endocrine, autonomic and behavioural functions, having neuronal interconnections with most of the limbic and autonomic brain areas. Several neuropeptides (Table VI.2) are synthesized in the magno- or parvicellular neurons of this nucleus. Many of the neuropeptides, including CRH, enkephalin, VIP/PHI-27, neurotensin, vasopressin, oxytocin and galanin, are coexpressed in the same PVN neurons or are colocalized in the subdivisions of the nucleus (Hökfelt *et al.*, 1983; Sawchenko *et al.*, 1984; Ceccatelli *et al.*, 1989).

Besides vasopressin and oxytocin, the magnocellular neurons in the supraoptic nucleus synthesize other neuropeptides (CCK, dynorphins, angiotensin II, apelin, galanin).

The suprachiasmatic nucleus serves as a biological clock, regulating sleep–wake and other body rhythms. Several kinds of neuropeptide-synthesizing neurons are distributed in this nucleus. VIP, vasopressin and somatostin are colocalized here, occupying different portions of the nucleus.

The hypothalamic periventricular nucleus is the major source of the neurohormonal somatostatin in the hypothalamus. These cells project directly to the median eminence, while through their axon collaterals they may influence the activity of hypothalamic GRH neurons. This nucleus, like the preoptic periventricular nucleus, is also rich in ANP and CCK (Table VI.2).

Middle Hypothalamus

The arcuate nucleus contains the major POMC neuronal pool in the CNS. Besides POMC, arcuate neurons synthesize several other neuropeptides (Table VI.2). The major projection area for arcuate neurons is the external layer of the median eminence. Along several intrahypothalamic targets, arcuate neurons innervate vagal and spinal autonomic preganglionic neurons. A significant portion of the descending arcuate fibres contain POMC and CART peptide (Palkovits *et al.*, 1987c; Elias *et al.*, 1998).

Several peptidergic neurons have been visualized in the dorsomedial and perifornical nuclei (Table VI.2). Many of these peptidergic neurons (cells those express angiotensin II, α-MSH, MCH, orexin/hypocretin, CART) are strongly involved in the central regulation of food and water intake. They do not respect the anatomical borders of the above two nuclei, but are scattered through the dorsal part of the middle hypothalamus from the third ventricle into the lateral hypothalamic area. These neurons receive strong NPY and AgRP innervation from the arcuate nucleus.

Posterior Hypothalamus
A wide variety of neuropeptides are synthesized by premamillary, supramammillary and posterior hypothalamic neurons (Lantos *et al.*, 1995).

Peptidergic Pathways

Neuropeptides in the Median Eminence–Pituitary Stalk
Almost all of the neuropeptides discovered in the brain are present in nerve fibres and terminals in the median eminence (Table VI.1). The majority are synthesized in hypothalamic neurons.

Internal Layer
Fibres arising mainly in the supraoptic, paraventricular and accessory magnocellular nuclei run through the internal layer–pituitary stalk to the posterior pituitary. Scattered fibres from other nuclei join this tract (Palkovits, 1986). Most of these fibres contain vasopressin and oxytocin, but other neuropeptides, such as CRH, enkephalins, dynorphins, CCK, neurotensin, galanin, substance P, angiotensin II and apelin, have also been demonstrated among the fibres in the hypothalamo-hypophyseal tract and the posterior pituitary. These neuropeptides may colocalize with either vasopressin or oxytocin. POMC-containing fibres from the arcuate nucleus do not project to the posterior lobe but terminate around the subependymal plexus in the internal layer.

External Layer
A number of peptidergic fibres terminate in the pericapillary space of the external layer. According to their origin and pathway to the external layer, they can be classified into two major groups: (1) tuberoinfundibular fibres arise in the arcuate nuclei and, in small numbers, the periventricular, ventromedial and ventral premamillary nuclei. These neurons may synthesize GRH, POMC, galanin, substance P, neurotensin and apelin; (2) fibres from the parvicellular PVN (TRH, CRH vasopressin, enkephalin, dynorphin, CCK, apelin and ANP), the medial preoptic nuclei (LH-RH) and the periventricular nuclei (somatostatin, ANP) travel through the lateral retrochiasmatic area and reach the median eminence from an anterolateral direction. Neuropeptides in the external layer may have two different physiological roles. First, after their release into the pericapillary space, they reach the portal blood and are transported to the anterior pituitary, where they exert their neurohormonal effects on pituitary cells. Second, some of the neuropeptides act locally in the external layer as neuromodulators by stimulating or inhibiting the release of neurohormonal peptides into the portal circulation.

Descending Peptidergic Fibres from the Hypothalamus
The majority of the descending hypothalamic fibres are peptidergic (Sawchenko and Swanson, 1982; Cechetto and Saper, 1988; Moga *et al.*, 1990). Vasopressin and oxytocin are the most prominent peptidergic neurotransmitters to the lower brainstem and the spinal cord, but several other neuropeptides (enkephalin, dynorphins, neurotensin, POMC, CRH, angiotensin II, galanin) also participate in this system. Besides the PVN (Sawchenko and Swanson, 1982), descending fibres arise in the arcuate, perifornical, dorsomedial and medial preoptic nuclei, and in the dorsal part of the lateral hypothalamus.

Peptidergic Afferents to the Hypothalamus
Several peptidergic afferents to the hypothalamus arise in the lower brainstem: NPY inputs from A1 and A2 catecholaminergic cell groups, where NPY is coexpressed with noradrenaline project to the PVN (Everitt *et al.*, 1984; Sawchenko *et al.*, 1985). Carrying viscero-sensory signals, peptidergic (neurotensin, somatostatin, enkephalin, dynorphin, NPY) neurons in the NTS project to the hypothalamus, mainly to the PVN (Riche *et al.*, 1990; Sawchenko *et al.*, 1990).

Lower Brainstem

Parabrachial Nuclei

The lateral parabrachial nucleus is considered as a secondary viscero-sensory centre in the brainstem with strong afferent inputs from the NTS and projections to the hypothalamus and the limbic system, especially to the NIST and the central nucleus of the amygdala. In contrast to the medial parabrachial and Kölliker–Fuse nuclei (the other two components of the parabrachial cell group), the lateral parabrachial is rich in peptide-synthesizing neurons (Table VI.2), and CRH-, enkephalin-, dynorphin-, CGRP- and CART-containing nerve fibres form a dense network in this nucleus.

Nucleus of the Solitary Tract

A great variety of neuropeptide-synthesizing neurons are present in the nucleus of the solitary tract, which serves as the primary autonomic (viscero-sensory) centre in the lower brainstem (Table VI.2). Neurons in this nucleus receive viscero-sensory inputs mainly through the glossopharyngeal and vagal nerves (CCK, somatostatin, enkephalins) and somatosensory input through descending trigeminal fibres (CGRP), and fibres from hypothalamic and limbic (mainly amygdaloid) nuclei terminate here.

Spinal Cord

Substance P and CGRP are the major neuropeptides in primary sensory neurons in the spinal cord. Both of them are coexpressed with glutamate in dorsal root ganglionic neurons. In addition, somatostatin, VIP, dynorphin, bombesin, galanin and endomorphin are synthesized in the dorsal root ganglion. Strong networks of substance P and CGRP nerve terminals are seen in the marginal layers of the dorsal horn. Enkephalin is one of the major neuropeptides in the inhibitory interneurons in the lamina III of the dorsal horn. Enkephalin acts on μ-receptors, inhibiting the release of substance P and other transmitters from the primary afferents. The activity of these inhibitory enkephalin neurons is controlled by descending inputs from substance P-, TRH- and serotonin-containing neurons in the ventromedial medulla and noradrenergic neurons in the ventrolateral medulla (A5 catecholaminergic cell group).

PEPTIDERGIC TRANSMISSION

To be considered as a neurotransmitter, a neuropeptide should meet eight criteria: (1) it should be present in the presynaptic neurons; (2) the peptide precursor and the synthetic enzyme should be present in the neuron; (3) the peptide should be released from the neuron by afferent stimulation; (4) effects of direct application of the neuropeptide to the synapse should be identical to those produced by stimulating nerve afferents; (5) specific neuropeptide receptors should be present on the presynaptic neurons; (6) interactions of neuropeptides with their receptors should induce changes in postsynaptic membrane permeability; (7) specific inactivating mechanisms should exist that stop interactions between neuropeptides and their receptors; and (8) agonists should mimic the action of the neuropeptide and antagonists should block its effects (McGeer *et al.*, 1987). Neuropeptides listed in this chapter meet many of these criteria.

Coexpression and Colocalization of Neuropeptides

During the past two decades, it has become evident that we cannot find any homogeneous cell groups in the CNS that contain only a single neuropeptide. Furthermore, it is hard to find any neuronal cell in the brain that may express only one neuropeptide. In certain brain areas, such as the hypothalamic and amygdaloid nuclei, lateral septal nucleus, NIST, parabrachial nuclei, and nucleus of the solitary tract, a high number of different neuropeptides can be visualized in neuronal perikarya (Table VI.2). They may be synthesized by different cell types of the nucleus (colocalization), but frequently several neuropeptides are synthesized by the same neuronal cell (coexpression). (Unfortunately, these two terms are, incorrectly, used interchangeably. When using the word 'colocalization', it should be made clear whether neuropeptides are colocalized in separate neurons in a brain nucleus or colocalized in the same neuronal cell. In the latter case, 'coexpression' is the correct term.)

The degree of convergence of neuropeptidergic fibres in brain regions is very high. None of the cell groups in the brain receives single peptidergic input: various peptidergic fibres of different origins enter a brain nucleus or an area and form terminal networks there. A similar degree of neuronal divergence has been revealed by immunohistochemistry: a single peptidergic neuron can innervate hundreds, and in certain cases thousands, of other neurons.

Neurohormones/Neuromodulators/Neurotransmitters

Depending on the site of action, neuropeptides can act as neurotransmitters, neurohormones or both. Brain neuropeptides may be transported by axons to the nerve terminals and released into the blood circulation, thus exerting their effects as hormones on the pituitary or in the periphery. Since they are synthesized in neuronal cells, neuropeptides in this case may be called 'neurohormones'. Brain neuropeptides may also be transported by axons into the synapses and bind to receptors on the postsynaptic neuronal cells, influencing their activity as neurotransmitters. In certain cases, a neuropeptide may act as both neurohormone and neurotransmitter, e.g. the axon of a hypothalamic neuronal cell terminates in the median eminence and releases its peptide into the portal blood (neurohormonal action), while its axon collaterals terminate on other hypothalamic neurons (neurotransmitter action); this is the case for the GRH–somatostatin interaction at hypothalamic level (Horváth *et al.*, 1989).

Neuropeptides may act at presynaptic levels, stimulating or inhibiting the release of other neuropeptides or neurotransmitters. When neuropeptides bind nonsynaptic receptors and act at presynaptic neurons, they are considered to have neuromodulator activity. As neuromodulators, neuropeptides can alter the release of neurotransmitters from the nerve terminals of presynaptic neurons, and regulate receptor sensitivity, G-protein coupling and the activation of second-messenger systems in postsynaptic neurons, altering the responses of these cells to synaptic stimulation. Neuropeptides may act as both neurotransmitters and neuromodulators (possible action of cotransmitters at pre- and postsynaptic receptors).

Neuropeptide Receptors

After the neuropeptide is released into the synapse, it binds to its receptor(s) on the surface membrane of the postsynaptic neurons. In general, neuropeptides bind to G-protein-coupled membrane receptors to regulate the levels of cyclic adenosine monophosphate (cAMP) and other second messengers (cyclic guanosine monophosphate (cGMP), inositol-phospholipid, calcium-calmodulin) in pre- and postsynaptic target cells. In the cAMP system, neuropeptide receptors may be bound to stimulatory or inhibitory transducer proteins, and may stimulate (like TRH and VIP) or inhibit (like somatostatin and opioid peptides) cAMP synthesis.

Each neuropeptide may have more than one type of receptor or receptor subtypes. Most of the peptide receptors have a wide distribution throughout the brain. Overall, the correspondence between neuropeptides and their related peptide receptors in the brain is quite good. In several cases, however, there is an apparent mismatch between their distribution that needs further studies to elucidate it. The detailed description of the topographical distribution of peptide receptors in the brain is beyond the scope of this chapter. Instead, we refer to related chapters in two excellent handbooks (Björklund *et al.*, 1990; Björklund *et al.*, 1992).

GENERAL REMARKS

There are more then 60 neuropeptides in the mammalian nervous system that have been identified, characterized and cloned. These peptides bind receptors that belong to the G-protein-coupled receptor supergene family. All of these peptides may act as neurohormones or neurotransmitters, or exert a modulatory effect on neurotransmission. The increasing arsenal of powerful new techniques, such as subtractive hybridization approach, orphan receptor strategy, combinatorial chemistry and molecular modelling, helps researchers to identify new neurotransmitters. Studies on known and newly discovered neuropeptides will increase our knowledge about functional brain mechanisms, help us to understand molecular psychiatry and psychology, and, as a final goal, help us to understand neurological disorders.

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VII

Neuroendocrinology

David A. Gutman and Charles B. Nemeroff

INTRODUCTION

The occurrence of psychiatric symptoms such as thought disturbances and depressed mood in patients with primary endocrine disorders is common. In addition, a significant percentage of patients with psychiatric disorders demonstrate a consistent pattern of endocrine dysfunction. Our understanding of the neurobiology of depression and other psychiatric disorders has been aided tremendously by a systematic analysis of the neuroendocrine axes and the actions of neurohormones in the pituitary gland and throughout the central nervous system (CNS). The occurrence of prominent psychiatric symptoms in patients with primary endocrine disorders, including Cushing's disease and primary hypothyroidism, provided a rationale for exploring the connection between hormones and both affective and cognitive function. In fact, disorders of neuroendocrine dysregulation in subpopulations of psychiatric patients are among the most consistent neurobiological findings in all of biological psychiatry.

Bleuler was among the first investigators to investigate systematically the association between hormones, mood and behaviour. He first demonstrated that patients with primary endocrine disorders have higher-than-expected psychiatric morbidity, which often resolved after correcting the primary hormonal abnormality. Work over the past 25 years has demonstrated clearly that the CNS tightly regulates endocrine gland secretion and, further, that neurons are influenced directly by hormones.

The concept that neurons are capable of synthesizing and releasing hormones initially sparked a controversy in endocrinology and neuroscience when first introduced in the 1950s; namely, is it possible that certain neurons subserve endocrine functions? Two major findings fuelled this debate. First, neurohistologists working with mammalian as well as lower-vertebrate and invertebrate species made several key observations. Led by a husband-and-wife team, the Scharrers, early researchers documented by both light and electron microscopy the presence of neurons that had all the characteristics of previously studied endocrine cells. These neurons stained positive with the Gomori stain, which was believed to be specific to endocrine tissues; further, they contained granules or vesicles containing known endocrine substances. The second key area of research centred around the brain's control of the secretion of pituitary trophic hormones. These trophic hormones were long known to control the secretion of peripheral target endocrine hormones, e.g. thyroid hormone, gonadal steroids and adrenal steroids. These interactions were particularly compelling because of the earlier identification of an extremely important neuroendocrine system, namely the magnocellular cells of the paraventricular nucleus (PVN) of the hypothalamus, which synthesize vasopressin and oxytocin. These two nonapeptides were shown to be transported from PVN cell bodies down the axon to nerve terminals located in the posterior pituitary (neurohypophysis), and released in response to appropriate physiologic stimuli. Vasopressin, also known as antidiuretic hormone (ADH), is a critical regulator of fluid balance, and oxytocin regulates the milk-letdown reflex during breastfeeding.

The ability of neurons to function as true endocrine tissues has now been clearly established. Neural tissue can both synthesize and release substances known as (neuro)hormones directly into the circulatory system; these (neuro)hormones have effects at sites far removed from the brain. One important example noted above is the action of vasopressin on the kidney. Although early in the development of the emerging discipline of neuroendocrinology it seemed important to document the ability of neurons to function as neuroendocrine cells, particularly those in the CNS, classification of specific chemical messengers as endocrine or neuronal or neuroendocrine soon lost its heuristic value. It is now recognized that the same substance can act as a neurotransmitter and a hormone depending on its location within the CNS and periphery. A good example of this is adrenaline, which functions as a classical hormone in the adrenal medulla but as a conventional neurotransmitter in the mammalian CNS. Similarly, it has been demonstrated that corticotropin-releasing factor (CRF) functions as a true peptide hormone in its role as a hypothalamic-hypophysiotropic factor in promoting the release of adrenocorticotropin (ACTH) from the anterior pituitary, yet it also functions as a 'conventional' neurotransmitter in cortical and limbic areas. Thus, the field now seeks to elucidate the role of particular chemical messengers in particular brain regions or endocrine axes.

The traditional endocrine and hormonal functions for several peptides discussed above have been well established, but many of these substances may also possess paracrine roles as well, i.e. secretion of these substances from one cell acts upon proximal cells. These paracrine interactions remain largely unexplored. The importance of these paracrine effects has been demonstrated in the gastrointestinal tract, where several peptides that act as hormones or neurotransmitter substances at other sites, including the CNS, have influences on local cellular function. Examples include vasoactive intestinal peptide (VIP), cholecystokinin (CCK) and somatostatin.

OVERVIEW OF COMPONENTS AND CONTROL MECHANISMS

The hypothalamic-pituitary-end-organ axes are generally organized in an hierarchical fashion (Figure VII.1). A large percentage of the neuroendocrine abnormalities in patients with psychiatric disorders are related to disturbances of target hormone feedback. A generic description is outlined here. More comprehensive reviews on this topic are available (e.g. Levine, 2000). In general, the hypothalamus contains neurons that synthesize and release factors that either promote or inhibit the release of anterior pituitary hormones, so-called release or release-inhibiting factors. These peptide hormones,

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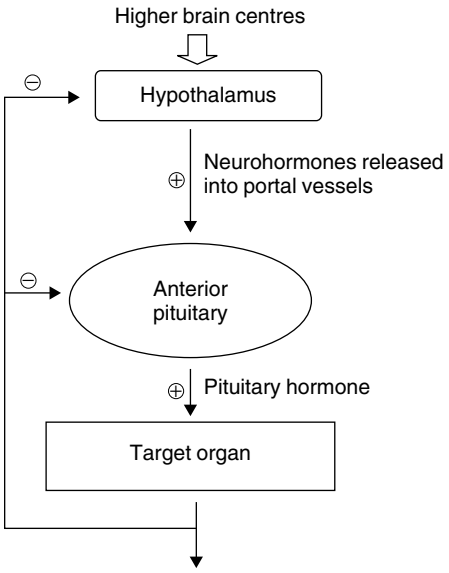

Figure VII.1 Overview of the common organizational motif of the neuroendocrine axes. The neurosecretion of hypothalamic factors into hypophyseal portal vessels is regulated by a set point of activity from higher brain centres. Neurohormones released from the hypothalamus into hypophyseal portal vessels in turn stimulate cells in the pituitary. These adenohypophyseal hormones then regulate the hormone output from the end organ. The end organ then exerts negative feedback effects at the pituitary and hypothalamus to prevent further neurohormone and pituitary hormone release via long-loop negative feedback. Short-loop negative feedback may also occur where pituitary hormones feed back directly on hypothalamic neurons to prevent further neurohormone release

Table VII.1 Major releasing factors and their hormonal targets

Neurohormone/releasing factor	Hormone stimulated
CRF	ACTH
TRH	TSH
GnRH	FSH
SRIF	GH
GHRH	GH
AVP	ACTH prolactin
Oxytocin	Prolactin

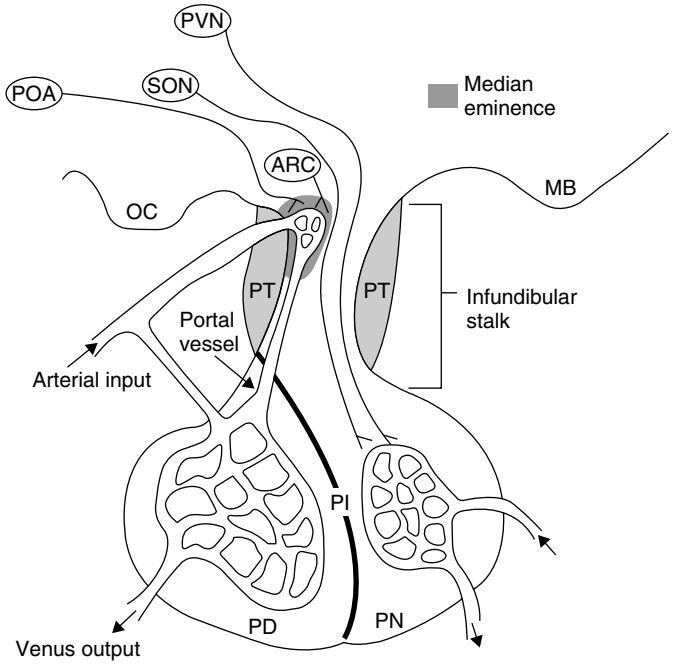

Figure VII.2 The neurovascular anatomy of the hypothalamic-pituitary axis ARC, arcuate nucleus; MB, mammillary body; OC, Optic chiasm; PD, pars distalis; PI, pars intermedia; PN, pars nervosa; POA, preoptic area; PT, pars tuberalis; SON, supraoptic nucleus

as summarized in Table VII.1, are synthesized by transcription of the DNA sequence for the peptide prohormone. After translation in the endoplasmic reticulum, these prohormones are processed during axonal transport and packaged into vesicles destined for the nerve terminals. These now biologically active peptides are then released following appropriate physiological stimuli from the median eminence, the most ventral portion of the hypothalamus, and secreted into the primary plexus of the hypothalamo-hypophyseal portal vessels (Figure VII.2). These peptides are transported in high concentrations to the sinusoids of the anterior pituitary (adenohypophysis), where they bind to specific membrane receptors on their targets, the pituitary trophic-hormone-producing cells. Activation of these receptors promotes or inhibits the release of pituitary trophic hormones into the systemic circulation. The increase or decrease in the plasma concentrations of these pituitary trophic hormones produces a corresponding increase or decrease in their respective end-organ hormone secretion. The hormones of the end-organ axes, such as gonadal and adrenal steroids, feed back on both pituitary and hypothalamic cells to prevent further release, often referred to as long-loop negative feedback. Short-loop negative feedback circuits have also been identified in which pituitary hormones feedback directly on hypothalamic neurons to prevent further release of hypothalamic-releasing factors.

Disturbances in the feedback regulation of the hypothalamic-pituitary-end-organ axes are of considerable interest in psychiatry. The common occurrence of psychiatric symptoms in many primary endocrine disorders, such as hypothyroidism and Cushing's syndrome, served as an impetus for investigation into the regulation of neuroendocrine systems in psychiatric disease states, such as depression, schizophrenia and bipolar disorder. Thus, a large part of psychoneuroendocrinology has focused on identifying changes in basal levels of pituitary and end-organ hormones in patients with psychiatric disorders. For many of the axes discussed below, tests have been developed to assess the functional status of these feedback systems. In these so-called stimulation tests, hypothalamic and/or pituitary-derived factors or their synthetic analogues are administered exogenously, and the hormonal response to this challenge is assessed. For example, in the standard CRF stimulation test, a 1-$\mu g\,kg^{-1}$ dose of CRF is administered intravenously, and the ACTH and cortisol response is measured over a period of 2 or 3 h. This test is a very sensitive measure of hypothalamic-pituitary-adrenal (HPA) axis activity, and changes in the magnitude and/or duration of the response relative to normal control values are characteristic of one or another type of dysregulation of the HPA axis.

Limitations of Stimulation Tests

Such studies as outlined above provide valuable information, but a brief discussion of some inherent limitations is warranted before a detailed review of the literature is presented. Normal

circadian rhythms and the pulsatile release of many of the hypothalamic-pituitary-end-organ axes components are often not taken into account when these stimulation tests are designed. Further, differences in assay sensitivity, gender, inclusion criteria for patients used in studies, and severity of symptoms in the target patient population studied can potentially generate confounding or at least quite variable results. Nevertheless, a great deal about the neurobiology of psychiatric disorders has been discovered through such experiments.

Although used less commonly today, a strategy that was often utilized in the 1970s and 1980s was based on the perception that the neuroendocrine axes served as a window into CNS function. Peripheral neuroendocrine markers were often used to assess indirectly CNS function because the brain was relatively inaccessible for study, with the exception of cerebrospinal fluid (CSF) and post-mortem studies. With the emergence of the monoamine theories of mood disorders and schizophrenia, many investigators attempted to draw conclusions about the activity of noradrenergic, serotonergic and dopaminergic circuits in patients with various psychiatric disorders by measuring the basal and stimulated secretion of pituitary and end-organ hormones in plasma. Although these approaches have severe limitations, they have been useful in elucidating the pathophysiology of mood and anxiety disorders and, to a lesser extent, schizophrenia.

In summary, neuroendocrinology broadly encompasses the following:

- The neural regulation of the secretion of peripheral, target-organ hormones, pituitary trophic hormones, and hypothalamic-hypophysiotropic hormones.
- The effects of each of the hormones that comprise the various endocrine axes on the CNS, e.g. the effects of synthetic glucocorticoids on memory processes.
- Study of alterations in the activity of the various endocrine axes in major psychiatric disorders and, conversely, the behavioural consequences of endocrinopathies.

THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Dysregulation of the HPA axis has frequently been reported in patients with psychiatric disorders, and is among the most robustly demonstrated neurobiological changes among psychiatric patients. The primary regulator of this axis is CRF, also known as corticotropin-releasing hormone (CRH), a 41-amino-acid-containing peptide synthesized in parvocellular neurons located primarily in the PVN of the hypothalamus. CRF-containing cells in the PVN receive input from a variety of brain nuclei, including the amygdala, bed nucleus of the stria terminalis, and other brainstem nuclei (Hauger and Dautzenberg, 2000). These CRF-containing neurons in turn project to nerve terminals in the median eminence (Swanson *et al.*, 1983); when CRF is released into the hypophyseal portal system it activates CRF receptors on corticotrophs in the anterior pituitary to promote the synthesis of pro-opiomelanocortin (POMC) and the release of its post-translational products, ACTH, β-endorphin and others (Figure VII.3). Arginine-vasopressin (AVP) also promotes the release of ACTH from the anterior pituitary, though CRF is necessary for AVP to exert this effect. Chronic stress can also upregulate AVP expression in the PVN, where under these conditions it may be coexpressed in CRF-containing neurons (Hauger and Dautzenberg, 2000). ACTH released from the anterior pituitary in turn stimulates the production and release of cortisol, the primary glucocorticoid in humans, from the adrenal cortex.

The concentration of circulating glucocorticoids is modulated via long-loop negative feedback. An increase in circulating glucocorticoids inhibits hypothalamic CRF gene expression and ACTH secretion from the pituitary. This in turn prevents further glucocorticoid release. The HPA axis also undergoes a circadian rhythmicity in humans, where serum cortisol levels peak immediately before awakening and reach a nadir in the evening.

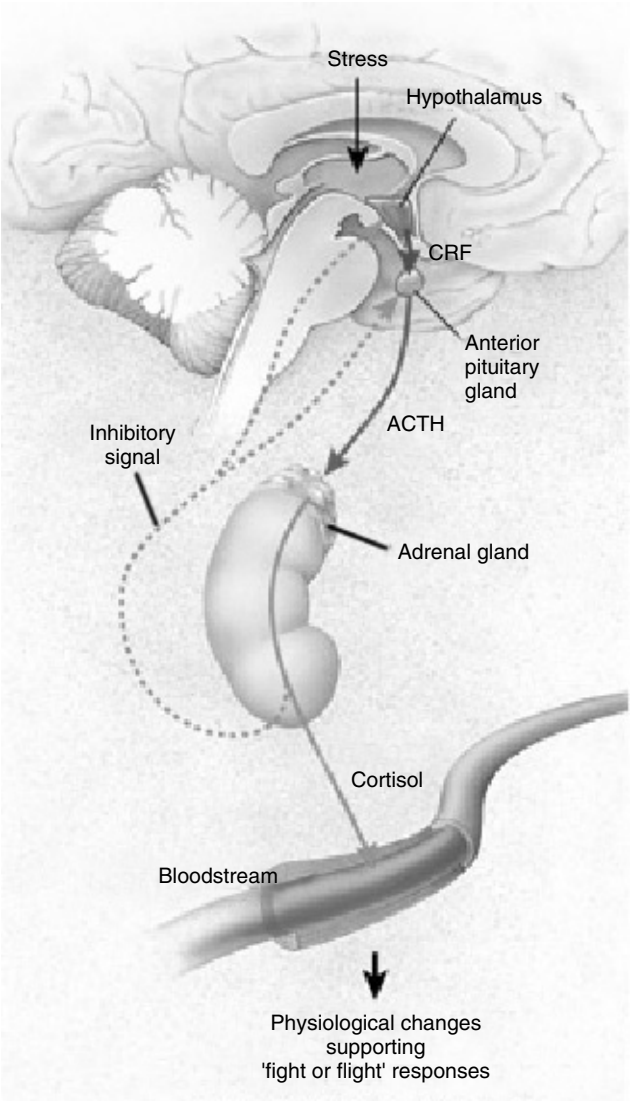

Figure VII.3 Overview of the feedback mechanisms of the HPA axis. Following relevant stimuli, including stress, CRF is released from the hypothalamus into hypophyseal portal vessels, where it is transported in high concentrations to the pituitary gland. CRF then promotes the release of ACTH, which in turn promotes the release of cortisol from the adrenal glands. Cortisol acts as an inhibitory signal at both the hypothalamus and pituitary, preventing further CRF and ACTH release, respectively. Mounting evidence suggests that chronic overactivity of the axis, and particularly overproduction of CRF, may contribute to the pathophysiology of depression. Reproduced with permission of Tomo Narashima from Nemeroff, C.B., 1998. *Scientific American*, June, 47

The biological effects of glucocorticoids are regulated by two cytosolic receptors: the glucocorticoid receptor and the mineralocorticoid receptor, both of which belong to a large superfamily of steroid hormone receptors. Because the mineralocorticoid receptor has a much higher affinity for glucocorticoids than does the glucocorticoid receptor, mineralocorticoid receptor binding sites may be saturated with glucocorticoids under physiological conditions.

In contrast, the occupancy of glucocorticoids receptor binding sites changes in response to changes in circulating glucocorticoid levels. The main genomic affects of glucocorticoids are mediated by glucocorticoids receptor binding to glucocorticoid response elements (GREs) in the promoter regions of specific genes. Glucocorticoid receptors may also inhibit or enhance the actions of other transcription factors, such as AP-1, NF-κB and CREB, by direct protein–protein interactions (Nestler *et al.*, 2001).

The Biology of Corticotropin-Releasing Factor

Although Saffran and Schally identified a crude extract that promoted the release of ACTH from the pituitary in 1955 (Saffran *et al.*, 1955), it was not until 1981 that CRF was isolated and chemically characterized. Working with extracts derived from 500 000 sheep hypothalami, Vale and colleagues at the Salk institute isolated, synthesized and elucidated the structure of CRF (Vale *et al.*, 1981). This discovery led to the availability of synthetic CRF, which allowed a comprehensive assessment of the HPA axis to proceed. It is now clear that CRF coordinates the endocrine, immune, autonomic and behavioural responses of mammals to stress. The regulation of CRF transcription is under control of a number of promoter elements. A cyclic adenosine monophosphate (cAMP) response element (CRE) is located in the 5′-flanking region of the human CRF gene, consistent with evidence that protein kinase A (PKA) activity regulates CFR gene expression. A glucocorticoid response element (GRE) is also located in the 5′-flanking region of the CRF gene, which is apparently the substrate where glucocorticoids act to inhibit CRF gene transcription (Hauger and Dautzenberg, 2000).

Two CRF-receptor subtypes, CRF_1 and CRF_2, with distinct anatomical localization and receptor pharmacology have been identified (Chalmers *et al.*, 1996; Lovenberg *et al.*, 1995; Grigoriadis *et al.*, 1996; Chang *et al.*, 1993; Chen *et al.*, 1993) in rats and humans. Both receptors are G-protein-coupled receptors and are coupled positively to adenylyl cyclase via G_s. In addition, a putative CRF_3 receptor has been identified recently in catfish (Arai *et al.*, 2001). The CRF_1 receptor is predominantly expressed in the pituitary, cerebellum and neocortex in the rat (Primus *et al.*, 1997). A growing body of evidence from animal studies has shown that the CRF_1 receptors may specifically mediate some of the anxiogenic-like behaviours observed after administration of CRF (Heinrichs *et al.*, 1997). The CRF_2 receptor family is composed of two primary splice variants, CRF_{2A} and CRF_{2B}. The CRF_{2A} receptor is more prevalent in subcortical regions, such as the ventromedial hypothalamus, lateral septum and dorsal raphe nucleus, whereas CRF_{2B} is expressed more abundantly in the periphery. A structurally related member of the CRF peptide family, urocortin, has also been identified in the mammalian brain. The endogenous neuropeptide urocortin has equally high affinity for both the CRF_1 and CRF_2 receptor subtypes (Vaughan *et al.*, 1995), whereas CRF displays a higher affinity at CRF_1 receptors than it does at CRF_2 receptors. The newly discovered urocortin II shows high selectivity for CRF_{2A} receptors, although its anatomic localization does not correlate precisely with the distribution of the CRF_{2A} receptor (Reyes *et al.*, 2001). With the discovery of a new ligand and a putative third receptor in the CRF family, much of the pharmacology and functional interactions between these ligands and receptors remains to be discovered.

The Effects of Changes in Glucocorticoid Availability

A deficiency of endogenous glucocorticoids produces overt clinical symptoms, including weakness, fatigue, hypoglycaemia, hyponatraemia, hyperkalaemia, fever, diarrhoea, nausea and shock. This condition, also known as Addison's disease, is caused most often by autoimmune destruction of the adrenal cortex. However, it is important to note that abrupt withdrawal from exogenous corticosteroids or ACTH can also induce an Addisonian crisis, because the exogenous administration of these compounds suppresses endogenous HPA axis activity. This is why tapering of the dose of adrenal steroids is essential before discontinuation. Glucocorticoid deficiency may also produce mild to severe depression or, less commonly, psychosis.

Excessive glucocorticoid secretion leads to a number of characteristic symptoms, including moon face, plethoric appearance, truncal obesity, purple abdominal striae, hypertension, protein depletion and signs of glucose intolerance or overt diabetes mellitus. Psychiatric symptoms, specifically depression and anxiety, are also associated with glucocorticoid excess. Cognitive impairment, especially decrements in memory function and attention, are also common and may be due to the direct effects of corticosteroids on the hippocampal formation (Sadock and Sadock, 2000).

The most common form of non-iatrogenic hypercortisolism is due to an ACTH-secreting pituitary adenoma, also known as Cushing's disease. Harvey Cushing, for whom the disease is named, first documented the occurrences of psychiatric symptoms, particularly depression, in 1913 in his first description of the illness. Other causes of hypercortisolism are often referred to as Cushing's syndrome. Since Dr Cushing's initial description, the occurrence of depression in Cushing's syndrome has been well documented (Spillane, 1951; Zeiger *et al.*, 1993).

Hypothalamic-Pituitary-Adrenal Axis Abnormalities in Depression

The occurrence of depression and other psychiatric symptoms in both Cushing's disease and Addison's disease served as an impetus for researchers to scrutinize HPA axis abnormalities in depression and other psychiatric disorders. Most investigators would agree that one of the most venerable findings in all of psychiatry is the hyperactivity of the HPA axis observed in a significant subset of patients with major depression (Table VII.2). Based on the work of research groups led by Board, Bunney and Hamburg, as well as by Carroll, Sachar, Stokes and Besser, literally thousands of studies have been conducted in this area.

The earliest studies in this field demonstrated elevated plasma cortisol concentrations in depressed patients (Carpenter and Bunney, 1971; Gibbons and McHugh, 1962). Other markers of hypercortisolism that have been demonstrated reliably in depressed patients include elevated 24-hour urinary-free cortisol concentrations and increased levels of cortisol metabolites in urine (Sachar *et al.*, 1970). One commonly used test to measure HPA axis function is the dexamethasone suppression test (DST). In this test, 1 mg of dexamethasone is given at 11 p.m.; blood is then drawn at 8 a.m. the following morning and cortisol levels are measured.

Table VII.2 HPA axis changes demonstrated in depression

↑ CRF in cerebrospinal fluid[a]
↓ ACTH to CRF stimulation
↓ Density of CRF receptors in frontal cortex of suicide victims
Enlarged pituitary gland in depressed patients[a]
Adrenal gland enlargement in suicide victims and depressed patients
↑ Plasma cortisol during depression[a]
↑ Urinary free cortisol concentrations[a]
Non-suppression of plasma cortisol and ACTH after dexamethasone administration[a]

[a]State-dependent.

Dexamethasone is a synthetic steroid similar to cortisol that suppresses ACTH secretion and subsequently cortisol release in healthy volunteers. Non-suppression of plasma glucocorticoid levels following the administration of dexamethasone is common in depression. The rate of cortisol non-suppression after dexamethasone administration generally correlates with the severity of depression (Evans and Nemeroff, 1987). In fact, nearly all patients with major depression with psychotic features exhibit DST non-suppression (Arana *et al.*, 1985; Evans and Nemeroff, 1983). Since Carroll's initial reports (Carroll *et al.*, 1968a; Carroll *et al.*, 1968b) and subsequent claims for diagnostic utility (Carroll, 1982), the DST has generated considerable controversy as to its diagnostic utility (Arana and Mossman, 1988). Diagnostic issues notwithstanding, the overwhelming conclusion from a myriad of studies demonstrates that a sizeable percentage of depressed patients exhibit HPA axis hyperactivity.

Another method used to assess HPA axis activity is the CRF stimulation test, which became available shortly after the synthesis of CRF. In this paradigm, CRF is administered intravenously (usually a $1\,\mu g\,kg^{-1}$ dose), and the ensuing ACTH and cortisol response is measured at 30-minute intervals over a 2–3-hour period (Hermus *et al.*, 1984). Numerous studies have now demonstrated a blunted ACTH and β-endorphin response to exogenously administered ovine CRF (oCRF) or human CRF (hCRF) in depressed patients when compared with non-depressed subjects, although the cortisol response in depressed patients and non-depressed control subjects did not differ consistently (Amsterdam *et al.*, 1988; Gold *et al.*, 1984; Holsboer *et al.*, 1984a; Kathol *et al.*, 1989; Young *et al.*, 1990). The attenuated ACTH response to CRF is presumably due to either chronic hypersecretion of CRF from nerve terminals in the median eminence, which results in downregulation of CRF receptors in the anterior pituitary, and/or to the chronic hypercortisolaemia. This receptor downregulation results in a reduced responsivity of the anterior pituitary to CRF, as has been demonstrated in laboratory animals (Aguilera *et al.*, 1986; Holmes *et al.*, 1987; Wynn *et al.*, 1983; Wynn *et al.*, 1984; Wynn *et al.*, 1988). Following recovery from depression, the documented disturbances in the HPA axis generally remit.

A combined dexamethasone/CRF test has also been developed. In this test, 1.5 mg of dexamethasone is administered orally at night (11 p.m.), and subjects receive an intravenous bolus of $100\,\mu g$ of hCRF at 3 p.m. the following day. Patients with HPA axis dysfunction, which is frequently encountered in depression, display a paradoxically increased release of ACTH and cortisol relative to controls. These abnormalities disappear following remission of depression, and normalization of HPA axis function seems to precede full clinical remission (Holsboer, 2000; Heuser *et al.*, 1994). The combined dexamethasone/CRF test appears to have much higher sensitivity for detecting subtle alterations in HPA axis function, and approximately 80% of patients with major depression exhibit an abnormal response to the dexamethasone/CRF test. In contrast, only approximately 44% of patients with major depression demonstrate an abnormal response when the DST is administered alone (Holsboer, 2000; Heuser *et al.*, 1994). Furthermore, otherwise healthy individuals with first-degree relatives with an affective illness, which greatly increases their own risk for psychiatric disorders, demonstrated cortisol and ACTH responses to the dexamethasone/CRF test that were higher than those of a control group but less than those of patients currently suffering from major depression. This suggests that a genetically transmittable defect in corticosteroid receptor function may render these individuals more susceptible to developing affective disorders (Holsboer *et al.*, 1995).

Structural changes in the components of the HPA axis have also been documented in depressed patients. Perhaps in part due to the trophic effects of CRF, pituitary gland enlargement has been documented in depressed patients as measured by magnetic resonance imaging (MRI) (Krishnan *et al.*, 1991). Enlargement of the adrenal glands, presumably due to ACTH hypersecretion, has been demonstrated repeatedly in both depressed patients *post mortem* (Nemeroff *et al.*, 1992; Amsterdam *et al.*, 1987) and suicide victims (Dorovini-Zis and Zis, 1987). It is reasonable to hypothesize that the normal plasma cortisol response to CRF seen in depressed patients is due to adrenocortical hypertrophy, in light of the blunted ACTH and β-endorphin responses to CRF seen in these same patients (Gold *et al.*, 1984; Kathol *et al.*, 1989; Amsterdam *et al.*, 1987; Gold *et al.*, 1986; Holsboer *et al.*, 1984b). Presumably, although the ACTH response to CRF is decreased in depressed patients, the enlarged adrenal cortex may secrete relatively greater quantities of cortisol when compared with control subjects in response to a given amount of ACTH. There are reports of increased cortisol responses to pharmacological doses of ACTH that support this hypothesis (Amsterdam *et al.*, 1983; Jaeckle *et al.*, 1987; Kalin *et al.*, 1982; Krishnan *et al.*, 1990; Linkowski *et al.*, 1985), although discordant findings have also been reported (Heim *et al.*, 2001).

The studies discussed thus far have focused primarily on dysregulations of the HPA axis, but, CRF controls not only the neuroendocrine but also the autonomic, immune and behavioural responses to stress in mammals. Moreover, results from clinical studies, and a rich body of literature conducted primarily in rodents and lower primates, have indicated the importance of CRF at extrahypothalamic sites. In rodents, primates and humans, CRF and its receptors have been localized heterogeneously in a variety of regions, including the amygdala, thalamus, hippocampus and prefrontal cortex (Suda *et al.*, 1984; Sanchez *et al.*, 1999; Van Pett *et al.*, 2000; Charlton *et al.*, 1987). These brain regions are important in regulating many aspects of the mammalian stress response and in regulating affect. The presence of CRF receptors in both the dorsal raphe and locus coeruleus, the major serotonergic- and noradrenergic-containing regions in the brain, respectively, also deserves comment. Because most available antidepressants, including the tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRI), are believed to work via modulation of noradrenergic and/or serotonergic systems, the neuroanatomical proximity of CRF and monoaminergic systems suggests a possible site of interaction between CRF systems and antidepressants.

Involvement of extrahypothalamic CRF systems in the pathophysiology of depression is suggested by numerous studies showing elevated CRF concentration in the CSF of patients suffering from depression (Banki *et al.*, 1987; Arato *et al.*, 1989; France *et al.*, 1988; Nemeroff, 1988; Risch *et al.*, 1992), although discrepant results have been reported (Roy *et al.*, 1987). Elevated CSF CRF levels have also been detected in depressed people who committed suicide (Arato *et al.*, 1989). A reduction in concentration of CRF in CSF has been reported in healthy volunteers treated with the tricyclic antidepressant desipramine (Veith *et al.*, 1993), providing further evidence of a possible interconnection between antidepressants, noradrenergic neurons and CRF systems. Similar effects have been reported with electroconvulsive therapy (ECT) in depressed patients (Nemeroff *et al.*, 1991).

Depressed patients who are non-suppressors on the DST also have significantly higher levels of CSF CRF than depressed patients with normal DST results. Presumably, the elevated CSF concentrations of CRF are due to CNS CRF hypersecretion (Post *et al.*, 1982), which may be acting at sites throughout the brain and contributing to many of the behaviours characteristic of depression. A reduction in the density of CRF receptors in the frontal cortex has also been reported in the frontal cortex of suicide victims (Nemeroff *et al.*, 1988). Presumably, hypersecretion of CRF results in a downregulation of CRF receptors in the frontal cortex.

While the exact mechanism contributing to CRF hyperactivity remains obscure, studies from our group and others have documented long-term persistent increases in HPA axis activity and

extrahypothalamic CRF neuronal activity after exposure to early untoward life events, e.g. neglect and child abuse, respectively, in laboratory animals (rat and non-human primates) and patients (Holsboer *et al.*, 1995; Nemeroff, 1999; Holsboer *et al.*, 1987; Coplan *et al.*, 1996). Early-life stress apparently sensitizes permanently the HPA axis and leads to a greater risk of developing depression later in life. In one schema, early sensitization of CRF systems results in heightened responses to stress later in life. To measure HPA responsivity to stress, the Trier Social Stress Test (TSST) was developed. This laboratory paradigm involves a simulated 10-minute public speech and a mental arithmetic task. The TSST has been validated as a potent activator of the HPA axis in humans (Kirschbaum *et al.*, 1993). Recently, our group has reported increased plasma ACTH and cortisol concentrations, presumably due to hypersecretion of CRF, after exposure to the TSST in women (both depressed and non-depressed) who were exposed to severe physical and emotional trauma as children (Heim *et al.*, 2000). These data provide evidence for functional hyperactivity of CRF systems that may be influenced by early adverse life events.

Space constraints do not permit an extensive review of the preclinical literature, but several additional points are worth noting. Numerous studies have documented that when CRF is injected directly into the CNS of laboratory animals, it produces effects reminiscent of the cardinal symptoms of depression, including decreased libido, reduced appetite and weight loss, sleep disturbances and neophobia. Indeed, newly developed CRF_1 receptor antagonists represent a novel putative class of antidepressants. Such compounds show activity in nearly every preclinical screen for antidepressants and anxiolytics currently employed. Recently, a small open-label study examining the effectiveness of R121919, a CRF_1 receptor antagonist, in major depression was completed (Zobel *et al.*, 2000). Severity measures of both anxiety and depression were reduced in the depressed patients. Although this drug is no longer in clinical development, it is clear that CRF_1 antagonists may represent a new class of psychotherapeutic agents to treat anxiety and affective disorders.

Hypothalamic-Pituitary-Adrenal Axis Alterations in Other Psychiatric Disorders

Patients with other psychiatric disorders also exhibit HPA axis dysregulation, although the vast majority of the available data is concerned with HPA axis alterations in depression. When depression is comorbid with a variety of other disorders, such as multiple sclerosis, Alzheimer's disease, multi-infarct dementia, Huntington's disease and others, both CRF hypersecretion and HPA axis hyperactivity are common. In contrast, HPA axis dysfunction has rarely been reported in schizophrenia. Consistent with the role of CRF in both depression-like and anxiety-like behaviours in preclinical animal studies, increased CSF CRF concentrations have been reported in post-traumatic stress disorder (PTSD) (Bremner *et al.*, 1997). A recent, elegant study using indwelling cannula in the lumbar space, allowing repeated sampling of CSF several hours after the initial, and presumably stressful, lumbar puncture, demonstrated elevated CSF CRF levels in PTSD combat veterans (Baker, *et al.*, 1999). In contrast, low serum cortisol and urinary free cortisol levels have been detected repeatedly, yet unexpectedly, in PTSD. One possible mechanism that has been proposed by Yehuda and colleagues suggests heightened negative feedback within the HPA axis in chronic PTSD patients (Yehuda *et al.*, 1996). Finally, CRF neuronal degeneration is now well known to occur in the cerebral cortex of patients with Alzheimer's disease with a compensatory upregulation of CRF receptor number, and this effect precedes the better-studied cholinergic neuronal involvement (Bissette, 1998).

OVERVIEW OF HYPOTHALAMIC-PITUITARY-THYROID AXIS COMPONENTS AND FUNCTION

The thyroid gland, composed of two central lobes connected by an isthmus, synthesizes the hormones thyroxine (T_4) and triiodothyronine (T_3). These iodine-containing compounds serve as global regulators of the body's metabolic rate, and are also critical for brain development. The release and synthesis of these hormones is controlled ultimately by signals from the CNS.

The hypothalamic-pituitary-thyroid (HPT) axis is composed of three main parts, as its name suggests. The tripeptide thyrotropin-releasing hormone (TRH) (pGlu-His-Pro-NH_2) is synthesized predominantly in the paraventricular nucleus in the hypothalamus and stored in nerve terminals in the median eminence, where it is released into the vessels of the hypothalamo-hypophyseal portal system (Figure VII.4). TRH is then transported to the sinusoids in the anterior pituitary, where it binds to thyrotrophs and releases the peptide thyroid stimulating hormone (TSH) into the systemic circulation. TRH is distributed heterogeneously in the brain, which strongly suggests a role for this peptide as a neurotransmitter as well as a releasing hormone. Thus, TRH itself can produce direct effects on the CNS independent of its actions on pituitary thyrotrophs. The HPT axis exhibits an ultradian rhythm, where TSH secretion, and consequently T_3 and T_4 levels, rise in the afternoon and evening, peak some time after midnight, and decline throughout the day (Veldhuis, 2000).

TSH is a 28-kDA glycoprotein composed of two non-covalently linked protein chains, TSH-α and TSH-β. The α subunit is identical to the α subunit contained in other pituitary hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropin. Upon release from the pituitary, TSH circulates through the blood and exerts its effects via binding to the TSH receptor in the thyroid, a G-protein-coupled receptor that stimulates the activation of adenylate cyclase.

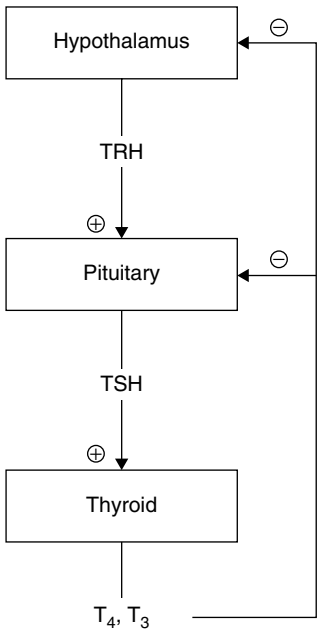

Figure VII.4 Overview of the feedback system of the HPT axis. TRH from the hypothalamus stimulates TSH from the pituitary, which stimulates thyroid hormone release. As circulating thyroid hormone levels increase, they inhibit further release of TSH and TRH. Other hypothalamic-pituitary-end-organ axes exhibit similar feedback control mechanisms

Upon stimulation by TSH, the thyroid gland releases the iodinated amino acids T_3 and T_4. Of the two hormones, T_3 is much more physiologically active. Although debate still exists in the literature, T_4 is often considered a prohormone that becomes active after monodeiodination in peripheral tissues. Thyroid hormones influence gene expression via two major thyroid hormone receptors, TRα-1 and TRβ-1, which in turn bind to specific DNA elements known as thyroid response elements (TREs) located in the promoter regions of a diverse number of genes. These receptors can function as homodimers or bind with other nuclear factors, such as thyroid hormone receptor auxiliary proteins, as heterodimers to modulate the transcription of target genes (Nestler, 2001). T_3 directly regulates the HPT axis by inhibiting TSH release and gene expression in the pituitary and TRH gene expression in the hypothalamus (DeVito, 2000). This is characteristic of the end-product negative feedback seen in the hypothalamic-pituitary-end-organ axes. In the circulation, these hormones are primarily bound to a carrier protein, thyroglobulin, although it is the unbound forms of these hormones that are metabolically active. Thyroid hormones have numerous effects on metabolism, and increase heat production, oxygen consumption, lipid metabolism, intestinal absorption of carbohydrates, cardiac function and the activity of the Na^+/K^+-ATPase. All of these functions are consistent with increasing metabolic rate.

Disorders of the Hypothalamic-Pituitary-Thyroid Axis

Disorders of the HPT axis lead to numerous psychiatric manifestations, ranging from mild depression to overt psychosis. Numerous conditions can lead to hypothyroid states, also known as myxoedema, including CNS causes of decreased TSH or TRH secretion, severe iodine deficiency, thyroid surgery, drugs and autoimmune disorders. The most common cause of hypothyroidism is Hashimoto's thyroiditis, which is due to autoimmune destruction of thyroid tissue. Regardless of the aetiology, hypothyroidism leads to a number of clinical manifestations, including slowed mentation, forgetfulness, decreased hearing, cold intolerance and ataxia. Decreased energy, weight gain, depression, cognitive impairment or overt psychosis ('myxoedema madness') may also result. Due to the overlapping symptoms with clinical depression, thyroid hormone deficiency must be ruled out when evaluating patients with depression.

Hypothyroidism is frequently subclassified into the following groups:

- *Grade 1* hypothyroidism is classic primary hypothyroidism (increased TSH), decreased peripheral thyroid hormone (T_3 and T_4) concentrations, and an increased TSH response to TRH.
- *Grade 2* hypothyroidism is characterized by normal, basal thyroid hormone concentrations, but an increase in basal TSH concentrations and an exaggerated TSH response to TRH.
- *Grade 3* hypothyroidism can be detected only by a TRH-stimulation test. Basal thyroid hormone and TSH concentrations are normal, but the TSH response to TRH is exaggerated.
- *Grade 4* hypothyroidism is defined as normal findings on the three thyroid axis function tests noted above, but the patients have the abnormal presence of anti-thyroid antibodies.

Without treatment, most patients will progress from grade 4 to grade 1 hypothyroidism.

The first treatments for hypothyroidism became available in the 1890s; prior to this, many patients with this condition spent their final days in psychiatric hospitals. One of the earliest descriptions of the effects of treatment with thyroid extracts was reported by Shaw and Stansfield in 1892. These physicians studied the effects of thyroid extracts in a patient suffering from severe thyroid deficiency secondary to trauma to her thyroid gland. Within 10 weeks following treatment with a sheep thyroid extract, the mental signs associated with myxoedema disappeared in this patient and she was discharged (Shaw, 1892). Stansfield followed this patient's progress for several months, and five months after the last injection of thyroid extract symptoms of hypothyroidism began to recur. Following ingestion of additional thyroid extracts, the symptoms were ameliorated again. These results demonstrated clearly the profound psychiatric effects of thyroid deficiency, and provided an early demonstration that treatment of primary endocrine abnormalities can resolve the psychiatric manifestations of the disease (DeVito, 2000).

The first prospective study that scrutinized psychiatric comorbidity in patients with hypothyroidism was carried out by Whybrow and colleagues (1969). In this seminal study, five of the seven patients manifested symptoms of depression at the time of the evaluation, while six of the seven displayed cognitive impairment. Interestingly, of the four patients with depression who were followed, thyroid replacement alone ameliorated the symptoms of depression in all. In a later study, Jain (1972) studied 30 hypothyroid patients; in this study, 13 (43%) of the patients had a clinical depression, ten (33%) had symptoms of anxiety, and eight (27%) were confused. Furthermore, these symptoms were improved or resolved following treatment of the thyroid condition alone. These early studies demonstrated clearly that hypothyroid states have pronounced psychiatric manifestations, predominantly depression and dementia, that can be reversed following thyroid hormone replacement. Later studies have demonstrated varying degrees of cognitive disturbance in up to 48% of psychiatrically ill hypothyroid cases (Boswell, 2001), and approximately 50% of unselected hypothyroid patients have symptoms characteristic of depression (Boswell, 2001). Anxiety symptoms are also common, occurring in up to 30% of unselected patients. Mania and hypomanic states have been reported rarely in hypothyroid patients. Finally, although psychosis is the most commonly reported symptom in the literature on hypothyroidism (52.9%), it accounts for only approximately 5% of the psychiatric morbidity in an unselected sample (Boswell, 2001), presumably due to reporting bias.

Hypothalamic-Pituitary-Thyroid Axis Dysfunction in Patients with Primary Psychiatric Disorders

Excluding patients with primary endocrine disorders, considerable amounts of data have revealed an elevated rate of HPT axis dysfunction, predominantly hypothyroidism, in patients with major depression (Table VII.3). In 1972, research groups led by Prange and Kastin demonstrated that approximately 25% of patients with major depression exhibit a blunted TSH response to TRH (Prange *et al.*, 1972; Kastin *et al.*, 1972). Presumably, this is due to hypersecretion of TRH from the median eminence, which leads to TRH receptor downregulation in the anterior pituitary, resulting in reduced sensitivity of the pituitary to exogenous TRH. This hypothesis seems plausible in light of evidence showing elevated TRH concentrations in the CSF of drug-free depressed patients (Banki *et al.*, 1988). Depressed patients have also been shown to have an increased occurrence of symptomless autoimmune thyroiditis (SAT), defined by the abnormal presence of anti-thyroglobulin and/or antimicrosomal thyroid antibodies consistent with grade 4 hypothyroidism (Nemeroff *et al.*, 1985).

Recently, Duval *et al.* (1996) performed a standard TSH stimulation test at both 8 a.m. and 11 p.m. in depressed patients and normal controls. The difference between the ΔTSH from 11 p.m. and the ΔTSH at the 8 a.m. time point was defined as $\Delta\Delta$TSH. These researchers demonstrated that depressed patients had a much lower $\Delta\Delta$TSH than did controls. Normal HPT axis function returned following remission from depression, but patients who did not respond to antidepressant medication continued to show blunted $\Delta\Delta$TSH. This suggests that treatment with antidepressants *per se* is not responsible for the improvement in HPT axis function.

Table VII.3 HPT axis alterations in depression

↑ CSF TRH in depressed patients
↓ Nocturnal plasma TSH
Blunted TSH in response to TRH stimulation (state-dependent)
Exaggerated TSH response to TRH stimulation
↓ ΔΔTSH (difference between 11 p.m. ΔTSH and 8 a.m. ΔTSH after TRH administration)
Presence of anti-thyroglobulin and/or antimicrosomal thyroid antibodies

Further, patients with the lowest pretreatment evening thyrotropin secretion also had the lowest rate of antidepressant response. This new methodology may serve as a more sensitive method to detect changes in HPT axis function.

Interestingly, Post's group recently measured both cerebral blood flow and cerebral glucose metabolism using positron emission tomography (PET) in both clinically depressed and bipolar patients. Both measures of cerebral activity were correlated inversely with serum TSH levels, and the authors suggested that HPT axis function contributes to primary and secondary mood disorders (Marangell *et al.*, 1997b). Also, the current literature has clearly demonstrated elevated TRH release in some depressed patients, but it is unknown if this is a causative factor in depression. The same group proposed that elevated TRH levels might instead be a compensatory response to depression. In fact, they reported that a lumbar intrathecal infusion of 500 μg of TRH into medication-free inpatients with depression produced a clinically robust, but short-lived, improvement in mood and suicidality (Marangell *et al.*, 1997a). Although this work is preliminary, it does suggest that the development of a systemically administered TRH receptor agonist may represent a novel class of antidepressant agents.

Bipolar Disorder and Hypothalamic-Pituitary-Thyroid Axis Abnormalities

HPT axis abnormalities have also been reported in bipolar disorders. Both elevated basal plasma concentrations of TSH and an exaggerated TSH response to TRH have been demonstrated (Haggerty *et al.*, 1987; Loosen and Prange, 1982). There is also evidence that bipolar patients with the rapid cycling subtype have a higher prevalence rate of hypothyroidism (grades I, II and III) than bipolar patients who do not (Bauer *et al.*, 1990; Cowdry *et al.*, 1983). A blunted or absent evening surge of plasma TSH, a blunted TSH response to TRH (Sack *et al.*, 1988; Souetre *et al.*, 1988), and the presence of anti-thyroid microsomal and/or anti-thyroglobulin antibodies (Lazarus *et al.*, 1986; Myers *et al.*, 1985) have also been demonstrated in bipolar patients.

Treatment of Hypothyroid States

As noted above, thyroid hormone extracts from sheep or cattle were the first treatments used that demonstrated efficacy in ameliorating the signs and symptoms of hypothyroidism. Several synthetic derivatives were introduced in the 1960s that quickly replaced desiccated thyroid tissue for the treatment of patients with thyroid disease. Among these are levothyroxine, synthetic forms of thyroxine (T_4) and liothyronine, and the synthetic levo-rotary isomer of triiodothyronine (T_3). Moreover, due in part to the seminal work carried out by Prange and collaborators in the USA in the 1960s, the use of thyroid hormones in augmenting antidepressant response in depression was established.

Hyperthyroid States

Although a number of conditions, including pituitary adenomas, can lead to hyperthyroid states, the most common non-iatrogenic cause of thyroid hormone excess is Graves' disease. In Graves' disease, the body generates an autoantibody to the TSH receptor, which directly stimulates thyroid follicular cells to secrete excessive amounts of T_3 and T_4. In this state, the normal negative feedback that T_3 and T_4 usually exert on TRH and TSH release is disrupted. The clinical manifestations of thyroid hormone excess are exaggerations of the normal physiological effects of T_3 and T_4, including diaphoresis, heat intolerance, fatigue, dyspnoea, palpitations, weakness (especially in proximal muscles), weight loss despite an increased appetite, hyperdefecation, increased psychomotor activity, and visual complaints. Psychiatric manifestations are also common, including anxiety (13% of unselected cases), depression (28% of patients), and cognitive changes (approximately 7% of patients). Psychotic manifestations and mania are less common, occurring in only 2% of unselected cases. Overall psychiatric morbidity is much less common in hyperthyroid states relative to hypothyroid states (Boswell *et al.*, 2001).

Hypothalamic-Pituitary-Thyroid Axis: Conclusions

Overall, there is clear evidence linking psychiatric symptomatology and thyroid disorders that extends back over 100 years. The observations that hypothyroid patients exhibit symptoms reminiscent of major depression led to a search for thyroid axis abnormalities in patients with affective illness. The efficacy of thyroid augmentation in the treatment of depression (Dording, 2000) and other affective disorders provides further evidence linking HPT axis function and psychiatric illness (Prange, 1996). Although work over the past 40 years has demonstrated a number of HPT axis abnormalities in depressed and bipolar patients, the aetiological connection between these findings remains elusive.

THE HYPOTHALAMIC-PITUITARY-GONAD AXIS

The overall organization of the hypothalamic-pituitary-gonad (HPG) axis is similar to the other major neuroendocrine axes. A 'pulse' generator in the arcuate nucleus of the hypothalamus controls gonadotropin-releasing hormone (GnRH) secretion, which occurs in a pulsatile fashion (Knobil, 1990) at intervals of 60–100 minutes (Nestler *et al.*, 2001). GnRH, previously known as luteinizing hormone-releasing hormone (LHRH), is released into the portal circulation connecting the hypothalamus and anterior pituitary, where it binds to gonadotrophs and promotes the release of LH and FSH into the systemic circulation (Midgley and Jaffe, 1971). These hormones then bind to Leydig cells in the testes to promote testosterone synthesis and secretion from Leydig cells or in the ovaries to promote oestrogen secretion. In females, FSH also promotes the development of ovarian follicles and the synthesis and secretion of androgen-binding proteins and inhibin. Inhibin acts directly on the anterior pituitary to inhibit FSH secretion without affecting LH release. In both sexes, testosterone/oestradiol generated by the testes/ovaries feeds back on the pituitary and hypothalamus to inhibit further FSH, LH and GnRH release. Gonadal steroids, in a similar fashion as glucocorticoids, modulates gene transcription. Gonadal steroids can bind to androgen or oestrogen response elements located in the regulatory regions of specific genes and directly modulate the expression of those genes. Gonadal steroids may also interact with transcription factors such as AP-1 or CREB, in turn influencing the expression of the genes controlled by those transcription factors (Nestler *et al.*, 2001).

Despite the significantly higher rates of depression in women, data on HPG abnormalities in psychiatric disorders remain remarkably limited. Early studies showed no differences in plasma concentrations of LH and FSH in depressed postmenopausal women compared with non-depressed matched control subjects (Nathan *et al.*, 1995). However, a later studied showed decreased plasma LH concentrations in depressed postmenopausal women compared with matched controls (Brambilla *et al.*, 1990). In a more recent study, significantly lower oestradiol levels were detected in women with depression, but the blood levels of other reproductive hormones fell within the normal range (Young *et al.*, 2000). Because oestradiol affects a number of neurotransmitter systems, including noradrenaline and serotonin, these results merit further study.

The response to exogenous administration of GnRH in depressed patients has also been investigated. Normal LH and FSH responses to a high dose of GnRH (250 μg) have been reported in male depressed and female depressed (pre- and postmenopausal) patients (Winokur *et al.*, 1982), whereas a decreased LH response to a lower dose of GnRH (150 μg) has been reported in pre- and postmenopausal depressed patients (Brambilla *et al.*, 1990). Unden and colleagues (1988) observed no change in basal or TRH/LHRH-stimulated LH concentrations in a depressed cohort including both sexes, although depressed males with an abnormal DST response showed a significantly higher increase in FSH compared with the controls.

The prevalence of mood disorders in women, including premenstrual syndrome and post-partum depression, also deserves mention. Premenstrual dysphoric disorder (PMDD) is a cyclic recurrence of symptoms that are both somatic (oedema, fatigue, breast tenderness, headaches) and psychological (depression, irritability, affective liability). The symptoms start following ovulation and disappear within the first day or two of menses, followed by a symptom-free interval between menses and the next ovulation. Although some of these symptoms may occur in all women, in some cases (5–10%), symptoms may be severe enough to interfere with normal functioning, leading to the diagnosis of PMDD (Altshuler *et al.*, 1995). GnRH agonists that produce a 'clinical ovariectomy' by downregulation of GnRH receptors in the pituitary and reduced gonadotropin secretions have been shown to be an effective treatment for premenstrual syndrome (PMS), suggesting that the HPG axis is involved in the manifestation of symptoms (Freeman *et al.*, 1997). However, significant variations in HPG axis function have yet to be identified in women especially susceptible to PMS.

Post-partum mood disorders are also common, occurring in approximately 10% of women after childbirth. Both post-partum depression and the less frequent post-partum psychosis occur with highest prevalence in the first three months after childbirth (Wisner and Stowe, 1997). The timing of these syndromes would suggest that neuroendocrine dysregulation may contribute to the expression of such disorders, but no major abnormalities in HPG axis function were detected in a prospective investigation of post-partum disorders (O'Hara *et al.*, 1990). Additional research on the HPG axis in depression and other mood states is needed.

THE HYPOTHALAMIC-PROLACTIN AXIS

Unlike other anterior pituitary hormones, prolactin release is regulated via tonic inhibition by prolactin-inhibitory factor (PIF), which was later determined to be dopamine. Dopamine neurons in the tuberoinfundibular system of the hypothalamus directly inhibit prolactin release. Prolactin can also inhibit its own release by a short-loop negative feedback to the hypothalamus. Prolactin primarily regulates the behavioural aspects of reproduction and infant care. Serum prolactin levels are normally low through life in males. Basal prolactin levels increase in females following parturition, and suckling stimulates prolactin release. Prolactin itself stimulates breast growth and milk synthesis. TRH, oxytocin, serotonin, oestrogen and other neuroregulators have prolactin-releasing factor (PRF) activity (Fink, 2000).

Excess circulating prolactin can lead to a number of clinical symptoms. The most common causes of hyperprolactinaemia are tumours, usually microadenomas of pituitary lactotrophs, or following treatment with conventional antipsychotic medications because of their potent blockade of dopamine receptors. Hyperprolactinaemia often leads to reduced testosterone secretion in men and a decreased libido in both men and women. Patients may also complain of depression, stress intolerance, anxiety and increased irritability, which usually resolve following treatments that reduce serum prolactin levels. Despite these effects, alterations in the hypothalamic-prolactin axis have not been demonstrated clearly in psychiatric disorders (Nicholas *et al.*, 1998). Because prolactin release is inhibited by dopamine, the prolactin response to infusions of dopaminergic agonists has also been used to estimate CNS dopaminergic tone, although it likely only reflects hypothalamic dopamine neuronal function.

Although abnormalities in prolactin secretion have not been demonstrated clearly in depression *per se*, a large number of reports have used provocative tests of prolactin secretion in patients with psychiatric disorders (for a review, see Van de Kar, 1989). Briefly, these tests use agents that increase serotonergic transmission, e.g. L-tryptophan, 5-hydroxytryptophan (5-HTP) and fenfluramine, among others. In general, the prolactin response to agents that increase serotonergic activity is blunted in depression (Mann *et al.*, 1995; Golden *et al.*, 1992), as well as in patients with cluster-B personality disorders (Coccaro *et al.*, 1997). These data suggest that the blunted prolactin response is mediated by alteration in 5-HT_{1A} receptor responsiveness and that serotonergic transmission in these patients is dysfunctional.

OXYTOCIN AND VASOPRESSIN

Oxytocin and arginine-vasopressin (AVP), also known as antidiuretic hormone (ADH), are nonapeptides synthesized in the magnocellular neurons of the paraventricular nucleus of the hypothalamus and released directly into the bloodstream from axon terminals in the posterior pituitary. This is in contrast to the hypothalamic-releasing factors we have discussed thus far, which are released in the portal system from the median eminence, and distinct from the anterior pituitary hormones that are released following the activation of pituicytes by the releasing factors synthesized in the hypothalamus.

AVP has prominent roles in controlling fluid balance via its effects on the kidney, in regulating blood pressure by its vasoconstrictive effects on blood vessels, and can directly promote the sensation of thirst. AVP also promotes the release of ACTH from the anterior pituitary in the presence of CRF and is released following stressful stimuli (Insel, 1997). In humans, oxytocin is involved predominantly in controlling smooth muscle contraction during parturition (myometrium) and during breastfeeding by mediating milk letdown in lactating mothers. In rodents, oxytocin promotes a number of reproductive (grooming, arousal, lordosis, orgasm, nesting, birthing) and maternal behaviours.

Although there are marked species differences in the effects of oxytocin, central infusion of this peptide in females of a monogamous prairie vole species promotes lifelong pair bonding in the absence of mating. Furthermore, pair bonding in this species, which normally accompanies mating, can be blocked by oxytocin antagonists, thus implicating oxytocin's key role in the expression this lifelong behaviour in some species. Rodent studies have also demonstrated that AVP has a pair-bonding function in males, analogous to the pair-bond-promoting behaviours induced by oxytocin administration in females. AVP promotes monogamy and paternal behaviour in some male prairie vole species. These studies

have led some researchers to speculate that oxytocin and AVP may play a role in psychiatric disorders characterized by disrupted affiliative behaviours, such as Asperger's disease and autism (Insel, 1997). Clearly, more work is needed in order to better understand the function of these two hormones in the human brain.

THE PITUITARY GROWTH HORMONE AXIS

Growth hormone (GH) is synthesized and secreted from somatotrophs located in the anterior pituitary. Its release is unique in that it is controlled by two peptide hypothalamic-hypophysiotropic hormones, growth-hormone-releasing factor (GHRF), also known as growth-hormone-releasing hormone (GHRH) and somatostatin, also known as growth hormone-release-inhibiting hormone (GHIH) or somatotropin release-inhibitory factor (SRIF). Somatotropin was first isolated from ovine hypothalamus in 1974. It is a tetradecapeptide, containing a disulphide bridge linking the two cysteine residues. Somatostatin is released predominantly from the periventricular and paraventricular nucleus of the hypothalamus; it inhibits GH release. Somatostatin has a wide extrahypothalamic distribution in brain regions, including the cerebral cortex, hippocampus and amygdala.

GHRF was characterized and sequenced in 1981 after considerable difficulty. The long-postulated GHRF was discovered several years after the elucidation of the structure of somatostatin, from extracts of an ectopic tumour associated with acromegaly. GHRF is a 44-amino-acid peptide, and has the most limited CNS distribution of all the hypothalamic-releasing hormones that have been identified. GHRF-containing neurons are concentrated in the infundibular and arcuate nuclei of the hypothalamus and stimulate the synthesis and release of GH. Dopamine, noradrenaline and serotonin innervate GHRF-containing neurons to modulate GH release. Both GHRF and SRIF are released from the median eminence into the hypothalamo-hypophyseal portal system, where they act on somatotrophs in the anterior pituitary to regulate GH release. Negative feedback is provided by GH, which stimulates somatostatin release, preventing further GH release. The GH axis is unique in that it does not have a single target endocrine gland; instead, GH acts directly on targets, including bone, muscle and liver. GH also stimulates the release of somatomedin from the liver and insulin-like growth factors.

GH is released in a pulsatile fashion, with highest release occurring around the time of sleep onset and extending into the first non-REM (rapid eye movement) period of sleep (Finkelstein *et al.*, 1972). A variety of stressors, including starvation, exertion and emotional stress, also promote GH release (Nestler *et al.*, 2001). GH is necessary for the longitudinal bone growth that occurs during late childhood; accordingly, GH levels are high in children, reach their peak during adolescence, and decline throughout adulthood. In addition to its effects on the long bones, GH has predominantly anabolic effects and leads to increased muscle mass and decreased body fat.

GH release to a variety of stimuli, including L-dopa, a dopamine precursor (Boyd *et al.*, 1970), apomorphine, a centrally active dopamine agonist (Fink, 2000), and the serotonin precursors L-tryptophan (Muller *et al.*, 1974) and 5-HTP (Imura *et al.*, 1973), has been demonstrated. Several findings indicate dysregulation of GH secretion in depression (Table VII.4). Studies have demonstrated a blunted nocturnal GH surge in depression (Schilkrut *et al.*, 1975), whereas daylight GH secretion seems to be exaggerated in both unipolar and bipolar depressed patients (Mendlewicz *et al.*, 1985). A number of studies have also demonstrated a blunted GH response to the α-adrenergic agonist clonidine in depressed patients (Siever *et al.*, 1982a; Charney *et al.*, 1982). Siever *et al.* (1982b) demonstrated that the blunted GH response to clonidine was not related to age or sex, and this study provided evidence that the diminished GH response to clonidine may be secondary to decreased α_2-adrenergic receptor sensitivity in depression. Using a GHRF stimulation test, our group later demonstrated a slight exaggeration of GH response to GHRF in depressed patients compared with controls, although this group difference was mainly attributable to three of the 19 depressed patients who exhibited markedly high GH responses to GHRF (Krishnan *et al.*, 1988a). Others, however, have reported a blunted GH response to GHRH in depressed patients. Thus, it is unclear whether the blunted GH response to clonidine seen in depression is due to a pituitary defect in GH secretion, further implicating a subsensitivity of α-adrenergic receptors in depression, or to a GHRH deficit. Recently, a diminished GH response to clonidine was demonstrated in children and adolescents at high risk for major depressive disorder. When considered with evidence demonstrating GH dysregulation in childhood depression (Ryan *et al.*, 1994), this suggests that the blunted GH response seen in high-risk adolescents may represent a trait marker for depression in children and adolescents (Birmaher *et al.*, 2000). Arguably, the blunted GH response to clonidine seen in depression may be the most reproducible and specific finding in the biology of affective disorders.

A GHRH stimulation test has also been developed and studied in depressed patients. Two groups have shown a blunted GH response to GHRH in depressed patients (Lesch *et al.*, 1987a; Lesch *et al.*, 1987b; Risch, 1991). However, Krishnan and colleagues (Krishnan *et al.*, 1988a; Krishnan *et al.*, 1988b) found minimal differences in serum GH response to GHRH between depressed and control patients. A comprehensive review of GHRH stimulation tests in depression, anorexia nervosa, bulimia, panic disorder, schizophrenia and Alzheimer's disease was conducted; the authors concluded that the results of this test are not always consistent and in some cases are contradictory (Skare *et al.*, 1994). Factors including the variability of GHRH-stimulated GH among controls, lack of standard outcome measures, and age- and gender-related effects may account for some of this variability. Further studies using GHRH will help develop a standard stimulation test to clarify further the response to GHRH in depression and other psychiatric disorders.

Several studies have demonstrated decreased SRIF levels in the CSF of patients suffering depression (Agren and Lundqvist, 1984; Gerner and Yamada, 1982), dementia, schizophrenia (Bissette *et al.*, 1986) and Alzheimer's disease (Molchan *et al.*, 1993; Bissette *et al.*, 1998). Somatostatin concentrations are also markedly elevated in the basal ganglia of patients with Huntington's disease (Nemeroff *et al.*, 1983), although the implications of this finding are unknown. Somatostatin also inhibits the release of both CRF and ACTH (Brown *et al.*, 1984; Heisler *et al.*, 1982; Richardson and Schonbrunn, 1981) indicating a direct interaction between the GH and HPA axes. No published studies measuring GHRH concentration and GHRH-mRNA expression have been conducted in post-mortem tissue of depressed patients and matched controls, which, in light of the evidence presented here, is of interest. Similarly, CSF studies of GHRH are also lacking.

Table VII.4 GH axis changes in depression

↑ Circulating daily GH levels (uni- and bipolar depression)
↓ Noctural GH in depression
↓ Response of GH to clonidine

SUMMARY AND CONCLUSION

Basic clinical observations of psychiatric disorders associated with primary endocrine disorders such as Cushing's syndrome and

hypothyroidism have led to our broader understanding of the role of neuroendocrine disturbances in a variety of psychiatric disorders, including depression and bipolar disorders. These studies have led to major advances in biological psychiatry by helping to understand the brain circuits involved in the pathophysiology of mood and anxiety disorders. Foremost among these is the CRF theory of depression, which is supported by studies from a variety of disciplines, and which has led to the development of a novel therapeutic approach, namely CRF receptor antagonists. Further, this work has provided a mechanism to explain the increase in depression seen in patients exposed to trauma early in life (first postulated by Freud in the early part of the twentieth century). If CRF truly is the 'black bile' of depression, then CRF antagonists may represent a novel class of antidepressants with a unique mechanism of action from other commonly used antidepressants. Indeed, a number of CRF-receptor antagonists are now in clinical development as novel anxiolytics and antidepressants.

In addition to the HPA axis and CRF alterations observed in depression, HPT axis abnormalities are also very common; the majority of depressed patients, in fact, exhibit alterations in one of these two axes. Furthermore, there is widely replicated blunting of GH response to clonidine and the blunted prolactin response to serotonergic stimuli in depressed patients. Although these studies have not added much understanding to the prevailing monoamine theory of depression, the mechanistic studies that have followed have been remarkably fruitful. It is obvious that the vast majority of studies have been focused on patients with mood disorders, particularly unipolar depression. Clearly, other disorders, including eating disorders, anxiety disorders, schizophrenia and axis II diagnoses, should also be evaluated with similar scrutiny.

The availability of selective ligands that can be utilized with PET will mark the next major leap in our understanding of the neuroendocrine axes in psychiatric disorders. The ability to determine peptide-receptor alterations in the brains and pituitaries of patients with psychiatric disorders will contribute immensely to our understanding of the neurobiological underpinnings of such disorders.

Finally, a growing number of studies have demonstrated that depression is a systemic disease that increases vulnerability to other disorders. Depressed patients demonstrate increased incidence of coronary artery disease and stroke, osteoporosis and, perhaps, cancer. These observations may be attributed, at least partly, to the endocrine alterations observed in depression.

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VIII

Psychoneuroimmunology: Basic Principles

Ziad Kronfol and Madhavan M.P.N. Nair

Until recently, a chapter on psychoneuroimmunology, particularly in the basic principles part of a book entitled *Biological Psychiatry*, would have appeared as an anomaly. The brain, for a long period of time, has been considered an immunologically privileged organ, with immune responses different in quantity and quality from the rest of the organism. A series of discoveries over the last decade or two has provided us with startling information regarding the immune functions of the brain. The brain, we now know, plays an important role as a regulator of immune responses. We also know that this relationship is bidirectional, with the immune system also serving as a sensory organ, relaying to the brain important information about the internal milieu, particularly the presence or prevalence of invading micro-organisms and changes in antigenic properties. Furthermore, the brain influences immune responses not only directly but also through the endocrine system and the autonomic nervous system. In fact, interactions between cells and the products of nervous, immune or endocrine origins, and their clinical implications in health and disease, constitute a major focus of the field of psychoneuroimmunology today.

The goal of this chapter is to provide the reader with an overview of major advances in psychoneuroimmunology that may be of relevance to the general psychiatrist/clinical neuroscientist. Because most psychiatrists are not very familiar with the field of immunology, we provide a very brief introduction to basic immunological principles, including a distinction between innate and adaptive immune responses, as well as a summary of major principles in immune physiology. This section will also include a brief description of common laboratory tests, particularly those that are used frequently to assess immune functions in clinical psychoneuroimmunology. The following section deals with neural-immune interactions, stressing the bidirectional nature of these interactions. First, we summarize the neural effects on immune regulation, focusing on autonomic innervation of immune tissues and neurochemical, neuroendocrine and behavioural effects on different immune functions. Then we review the effects of changes in immune activity on central nervous system (CNS) function, again emphasizing neurochemical, neuroendocrine and behavioural effects. Both animal studies and human investigations are covered. We then turn our attention to the mediators of these neural-immune interactions, particularly neurotransmitters, neurohormones and cytokines. The chapter ends with a review of important clinical implications, particularly the role of stress in immune-related disorders on the one hand and the possible role of immune factors in the aetiology of psychiatric disorders on the other.

As with most other chapters in the first part of this book, this chapter presents an overview of basic principles, in this case the basic principles of psychoneuroimmunology. For some readers, the information may be rudimentary; for others, it may appear too advanced. Because of space limitation, we have refrained from detailed discussion of specific topics, suggesting to the reader specific references instead. Our hope is that the chapter will provide enough background information to allow the reader better appreciate the clinical syndromes that are discussed in detail in the second part of the book.

Neuroimmunological processes may play a role in the aetiology of various psychiatric disorders. They may also play a role in the pathophysiology of specific psychiatric symptoms. An understanding of basic principles in psychoneuroimmunology and an appreciation of the constant dialogue between the brain and the immune system are therefore warranted.

OVERVIEW OF IMMUNE PRINCIPLES

The word 'immunity' is derived from Latin, meaning protection from foreign substances, mainly against infectious and non-infectious agents and cancer. The organized system that consists of cells and molecules specialized in mounting defence against infection or insult is called the immune system, and the collective and coordinated response induced by the immune system against any infection or insult is called the immune response. The immune system operates in two fundamentally different ways: the natural or innate immune response, and the acquired or adaptive immune response. The extent of natural or innate immune response occurs essentially at the same degree or level every time an infection or insult to the body occurs, whereas the acquired or adaptive immune response increases in magnitude with repeated episodes of infection or insult.

The Innate Immune Response

The innate immune responses provide the first line of defence against infection or insult in the host. The main components of this system include both molecular and cellular elements. The cellular components include epithelial cells, basophils, mast cells, eosinophils and phagocytic cells such as neutrophils, monocytes, macrophages and natural killer lymphocytes. A key member of the cellular innate immunity is represented by the interdigitating dendritic cells, such as Langerhans' cells, which are located in the epithelia of the skin and the gastrointestinal and respiratory systems, and are heavily involved in the endocytosis of microbes (Bell *et al.*, 1999). Dendritic cells are functionally immature; however, as they migrate to the lymph nodes, they mature and become extremely efficient at presenting antigen to the T-cells to initiate an adaptive immune response.

Another prominent member of the cellular innate immune system is the natural killer (NK) cell (Biron *et al.*, 1999). The term 'natural killer' originates from the fact that these cells can kill certain target cells naturally or spontaneously without any previous activation. These cells derive from bone marrow precursors, and

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appear as large lymphocytes with numerous killer granules in their cytoplasm. NK cells can essentially be activated by three types of targets, namely antibody-coated target cells, cells infected with virus or intracellular bacteria, and cells lacking class 1 major histocompatibility complex (MHC) molecules. One of the most unusual specificities of NK cells is their inability to kill normal cells by virtue of the fact that most normal cells express class 1 MHC molecules. It is not known what activating receptors are involved in NK-mediated cytotoxicity. However, it is believed that NK cell activation is controlled by signals that are being generated between killer inhibitory and killer activating receptors that are characteristic of NK cells (Lanier, 1998).

The molecular basis of the innate immune response involves the complement system, acute-phase proteins and cytokines. The complement system consists of several blood proteins that help to develop inflammation and link effector cells to the site of microbial invasion. The complement usually operates through one of three different pathways: the classical pathway is activated by antigen–antibody complex, the alternative pathway is triggered by microbial antigen, and the lectin pathway is triggered by the interaction between mannose-binding lectin in the plasma and microbial carbohydrates. Irrespective of the pathway of complement activation, the final result is the formation of lysis complex on the membrane of the target, which leads to the death of the target cell.

The acute-phase proteins are plasma proteins. Their levels change rapidly in response to trauma, injury or inflammation. These proteins help to magnify the resistance to infection and promote the repair of tissue damage. Acute-phase proteins include complement components, haptoglobin, C-reactive proteins, serum amyloid A protein, protease inhibitors and coagulation proteins. These plasma proteins can recognize certain unique receptor structures present on invading microbes and thus afford protection to the host from these invading microbes. Further, mediator molecules of the innate immune response also stimulate and regulate the adaptive immune response. The acute-phase proteins appear in early stages of infection, and the hepatic synthesis of these molecules is upregulated by inflammatory cytokines, especially interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor-α (TNFα).

Cytokines represent another group of soluble immune mediators produced by both innate and adaptive immune cells in response to microbes and other antigen (Mire-Sluis and Thorpe, 1998). They act as an integrated network within and between humoral and cellular immune systems. They function at two main phases of the immune response, namely at the activation phase to stimulate the growth and differentiation of lymphocytes, and at the effector phase to eliminate infectious agents and foreign antigens. Because most of the cytokines are produced by leukocytes and act mainly on leukocytes, they are also called interleukins (incorrectly, because cytokines are also produced by non-leukocytes and act on cells other than leukocytes, as will be discussed later in this chapter). There are at least 21 different types of cytokines that have been cloned and characterized. They include interleukins 1–18 (IL-1–IL-18) in addition to other cytokines, such as TNFα tumour necrosis factor β (TNFβ), transforming growth factor β (TGFβ), granulocyte-macrophage colony-stimulating factor (GCSF), and interferons α, β and γ (IFN α, β and γ). In addition, another family of structurally homologous cytokines, called chemotactic cytokines, which stimulate or regulate leukocyte movements from blood to tissue, also participate in the innate immune response. The roles of cytokines in innate immunity and inflammation vary depending on the nature of microbes, such as extracellular or intracellular bacteria or lipopolysaccharide (LPS)-producing bacteria or viruses. Table VIII.1 describes the major cytokines and their sources, along with major biological functions in innate and adaptive immune responses.

Table VIII.1 Major cytokines with cellular sources and major biological activities

Cytokine	Cellular sources	Major biological functions
Innate immunity		
IL-1	Macrophages, endothelial cells	Inflammatory responses
IL-6	Th_2 helper cells, endothelial cells	Induces acute-phase proteins, B-cell differentiation
IL-8	Leukocytes, endothelial cells, epithelial cells, fibroblasts	Promotes leukocyte recruitment and inflammation
IL-10	Th_2 helper cells, macrophages	Inhibits IL-12 production Induces class 11 MHC molecules Stimulates B-cells
IL-11	Bone marrow stromal cells	Induces acute-phase proteins
IL-12	Macrophages, B-cells, dendritic cells	Induces IFNγ/cytotoxic reactions Th_1 differentiation
IL-15	Macrophages and other cells	Stimulates NK cells and T-cells
IL-18	Macrophages	Stimulates IFNγ production
TNFα	T-cells, B-cells, NK cells, macrophages, mast cells	Induces inflammation, apoptosis
IFNα	Macrophages	Viral resistance, MHC-1 molecules
IFNβ	Fibroblasts	Viral resistance, activates NK cells
Adaptive immunity		
IL-2	Th_1 helper cells	Induces T-/B-cell proliferation/clonal expansion Activates NK/macrophages Induces apoptosis
IL-4	Th_2 cells, mast cells, basophils, eosinophils	Th_2 cells differentiation, IgE class switching Activates monocytes
IL-5	Th_2 cells, mast cells, eosinophils	Eosinophil differentiation, B-cell proliferation Produces IgA
IL-13	Th_2 cells, epithelial cells	Suppresses macrophage activation
IFNγ	Th_1 cells, CD8 and NK cells	Inhibits Th_2 helper Activates macrophages, epithelial and endothelial cells Stimulates MHC-11 molecules Increases antigen presentation
TGFβ	T-/B-cells, macrophages, mast cells	Promotes immunosuppression/macrophage inhibition

The Adaptive Immune Response

The acquired or adaptive immune response traditionally has been divided into humoral immunity and cell-mediated immunity. The humoral immune response is mediated primarily by antibodies that can be transferred from an immunized donor to a naive host in the absence of cells. The effector phase of the humoral immune response involves the neutralization of extracellular microbes or toxins that are accessible to antibodies. The cell-mediated immune response (CMI) can be transferred by viable T-lymphocytes. The effector phase of the CMI involves the recognition of the antigen present on the surface of the cells or invading organism in the presence of self MHC molecules. CMI also eliminates foreign cells such as tumour or neoplastic cells or foreign MHC antigen such as allograft. T-cells also stimulate macrophages to become cytotoxic cells through cytokine production; these activated macrophages thus eliminate microbes that reside within the phagosomes.

The main cellular components of the adaptive immune system are B-lymphocytes and T-lymphocytes. B-cells recognize extracellular and cellular surface antigens and differentiate into antibody-secreting plasma cells. B-lymphocytes are the only cells capable of producing antibodies. The T-lymphocytes are divided further into the functionally distinct classes of helper T-cells and cytotoxic T-cells (Figure VIII.1). Helper T-cells secrete cytokines, whose function is to stimulate T-cells and other cells to eliminate microbes and other foreign antigens. Based on the profile of cytokines secreted, helper T-cells have also been divided into Th_1 and Th_2 cells. Th_1 cells secrete cytokines such as IL-2, IL-12 and IFN, which promote mostly cellular immune responses. Th_2 cells secrete cytokines such as IL-4, IL-6 and IL-10, which promote mostly humoral immune responses. The clinical value of such a distinction, however, has been questioned by some investigators. Another class of T-cells has been identified as suppressor T-cells, whose main function is to inhibit immune responses.

The adaptive phases of the immune response may be divided into different events, such as recognition of antigen, activation of lymphocytes, and finally elimination of the antigen. In the recognition stage, each foreign antigen is being recognized by specific lymphocytes, which proliferate and differentiate into memory cells that show enhanced immune response on subsequent exposure to antigen. In the effector phase, the antibodies and T-lymphocytes participate to eliminate the microbes or antigens. In addition to T-cells and antibodies, mediators of the innate immune system such as non-lymphoid cells, complement, phagocytes and other soluble mediators take part in the effector phase of the adaptive immune response.

The cardinal features of both the cell-mediated and humoral immune responses are specificity, diversity, memory and tolerance.

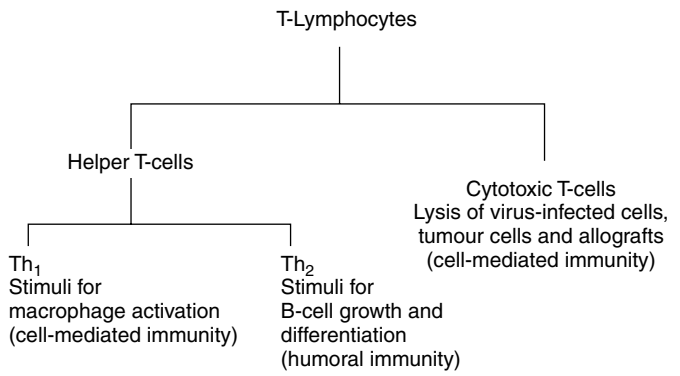

Figure VIII.1 T-lymphocyte subsets

The specificity refers to the distinct immune response for each antigen or each structural component of a complex protein, or any subtle differences in antigenic profile of a given macromolecule. The diversity enables the immune system to react to an array of microbes or invading stimuli simultaneously. Immunologic memory refers to the ability of the host's immune system to 'recall' a prior exposure to a particular antigen or microbe, and to react to it in an enhanced fashion. Both T- and B-cells play a significant role in this process. Tolerance refers to the remarkable feature of the normal immune system to recognize invading microbes or antigens and mount an efficient immune response against them, while at the same time not reacting harmfully to the self-antigenic system. Disorders of tolerance often lead to autoimmune diseases.

Methods Commonly Used in the Laboratory to Measure Immune Functions

Since the immune system can be divided mainly into humoral and cellular components, several assays are currently employed to measure these two immune functions (Lowell, 1990; Abbas *et al.*, 2000). Assays to measure the B-cell functions or humoral immune functions are separate from T-cell and other cellular immune functions. However, since both immune components and their soluble mediators, such as cytokines, chemokines and other immunoregulatory factors, are closely interlinked, the quantitative assessment of different immune functions is often quite interdependent. The widespread use of monoclonal antibodies has improved dramatically the method of antigen detection that forms the basis of antigen–antibody binding reactions. All modern antigen and antibody reactions are quantitated by an indicator molecule that is labelled with either a radioisotope (radioimmunoassay [RIA]) or an enzyme (enzyme-linked immunosorbent assay [ELISA]). Antibodies can also be used to purify proteins; the commonly used methods are immunoprecipitation and affinity chromatography. Western blot is commonly used to determine the size and the presence of proteins in biological fluids. The common methods to identify antigens expressed on given populations of cells, or to sort out a population of cells bearing a particular antigen (such as a specific cluster of differentiation (CD) antigen), utilize enzyme-linked or fluorescent-labelled antibodies by flow cytometry, fluorescence-activated cell sorting and immunofluorescence or immunohistochemistry techniques. Flow cytometry is one of the most commonly used assays in the clinical immunology laboratory; it allows for the sorting out and enumeration of T-cells (CD_3+), T-helper cells (CD_4+), T-suppressor cells (CD_8+), NK cells ($CD_{56}+$), and others.

The most commonly used clinical laboratory methods to detect cellular immunity are leukocyte phenotyping, delayed hypersensitivity skin testing, lymphocyte activation assays, cytokine production assays, and neutrophil function assays. Leukocyte phenotyping allows the quantification of cells carrying a specific antigen, as described above. This is usually done today using flow-cytometry technology. The delayed-type hypersensitivity skin testing is an *in vivo* test used primarily to assess immune competence and to test the memory T-cells that recognize a prior exposure of an antigen or infection. Lymphocyte activation assays are designed to determine the changes on the cell-surface markers and the ability of lymphocytes to undergo stimulation or proliferation in response to activation by a specific antigen, a mitogen such as phytohaemagglutinin (PHA) or concanavalin-A (Con A), or an endotoxin such as LPS.

Activation of lymphocytes also leads to the synthesis of cytokines and generation of cytotoxic cells. The production of cytokines in cells can be measured by ELISA or enzyme-linked immunospot (ELISPOT). Cytotoxicity can be measured by cytotoxic assays, such as cytotoxic T-cell (CTL), NK and antibody-dependent cellular cytotoxicity (ADCC) assays. Quantification of monocytes and macrophages, which are the essential components

of the innate immune system, can be made by the flow-cytometry method and histochemical staining for nonspecific esterase. Polymorphonuclear neutrophils (PMNs), which are the primary effector cells of the innate immune system, can be measured by neutrophil adhesion, chemotaxis, phagocytosis, and determination of respiratory burst and degranulation assays.

In recent years, advances in biomedical technology have permitted the characterization, expression, synthesis and modulation of various genes of interest in cells using molecular techniques. Southern blot hybridization is used to study the DNA profile, while Northern blot has been used to analyse the mRNA of the genes. The latest method of copying and amplifying specific DNA sequences is called polymerase chain reaction (PCR); this is used widely in molecular immunology. The most widely used *in vivo* methods for studying the functional effects of specific genes in immunology involve transgenic and gene-knockout animal models. In the case of transgenic models, a particular gene of interest can be overexpressed in a defined tissue, whereas in knockout models the function of a particular gene is knocked out by targeted mutation or disruption of the gene. The resultant changes in biological and/or clinical properties associated with the gene can then be assessed.

NEURAL-IMMUNE INTERACTIONS

Neural Effects on Immune Regulation

Autonomic Innervation of Immune Tissues/Organs

The immune system, while complex and intricately regulated, is not completely autonomous. Recent studies show that immunological processes can be influenced by signals from the central and/or peripheral nervous system. This is evidenced by the autonomic innervation of lymphoid tissue and organs, including thymus, bone marrow, spleen and lymph nodes. Work regarding the innervation of lymphoid tissue has been reviewed extensively by Bellinger *et al.* (2001). For most of these organs, there is evidence of autonomic innervation, with sympathetic, parasympathetic and/or peptidergic supply, as well as sensory innervation. The thymus, for instance, gets its sympathetic innervation from postganglionic cell bodies in the upper paravertebral ganglia of the sympathetic chain, mostly the upper superior cervical and stellate ganglia (Tollefson and Bulloch, 1990). Other innervation includes fibres from the vagus nerve, the phrenic nerve, and the recurrent laryngeal nerve (Bulloch and Pomerantz, 1984). The sympathetic innervation of the thymus is predominantly noradrenergic. Noradrenergic fibres enter the thymus mostly along blood vessels. They can form a dense plexus around the vessels or branch into the parenchyma, and can be found adjacent to thymocytes. Experimental studies suggest that noradrenaline affects the maturation of thymocytes. Through its action in β-adrenoreceptors, this catecholamine can inhibit proliferation and enhance differentiation (Singh, 1985a; Singh, 1985b). Cholinergic supply presumably provided through the vagus nerve appears weak and of doubtful clinical significance.

Nerve supply to the bone marrow is provided through the appropriate branch of the spinal nerve supplying the same region (Tokunaga, 1967). Most, if not all, of the nerve supply to the bone marrow appears to be noradrenergic (DePace and Webber, 1975). Sympathetic nerve fibres in bone marrow are usually associated with blood vessels. They are thought to be mostly vasomotor, controlling blood flow to the bone marrow.

The innervation of the spleen has been studied more extensively than other lymphoid organs. For a long time, the splenic nerve has been known to be rich in sympathetic nerve fibres (Elfvin, 1961). There is extensive noradrenergic innervation of the spleen, particularly in association with spleen vasculature and the smooth muscle of the capsule and trabeculae (Reilly, 1985). The functional aspects of this dense noradrenergic supply are not understood completely. In addition to the vasomotor component, there are probably complex neural-immune interactions that need to be defined more clearly; data remain rather slim regarding cholinergic innervation of the spleen.

There is also scattered evidence of innervation to lymph nodes and other lymphoid tissue (Felten *et al.*, 1984). As with other immune organs, the innervation could be noradrenergic, cholinergic or peptidergic in origin. There is also evidence of possible sensory innervation of different immune organs (Baron and Janig, 1988). More recently, efforts have been under way to describe lymphoid innervation not so much in anatomical terms, but more with regard to neurochemical pathways and their functional significance. More research is needed for a complete mapping of these networks.

Neuroendocrine Effects on Immune Function

The immune system and the endocrine system are tightly interconnected. There are several lines of evidence for these close interactions: (1) Immune cells express receptors for several hormones, such as cortisol, corticotrophin-releasing hormone (CRH), enkephalins, endorphins and neuropeptide Y (Schafer *et al.*, 1997; Malmstrom, 2001). (2) Both hormones and neurotransmitters, such as adrenaline, noradrenaline, dopamine and acetylcholine, affect immune function both *in vitro* and *in vivo* (McEwen *et al.*, 1997; Madden, 2001). (3) Surgical manipulations involving endocrine organs, such as hypophysectomy, thyroidectomy, adrenal removal or gonadal ablation, produce specific and predictable changes in the immune response (Goujon *et al.*, 1996). (4) Several immune parameters have intrinsic biological rhythms, usually circadian, that seem to be connected with the circadian rhythm of cortisol, albeit with a time lag of varying magnitude (Kronfol *et al.*, 1997). (5) Many immune cells are capable, under specific conditions, of secreting neurohormones that can influence directly or indirectly immune function through special feedback loops (Goetzl *et al.*, 1991). (6) Products of immune activation, such as cytokines, have well-documented neuroendocrine properties, such as hypothalamic-pituitary-adrenal axis (HPA) activation, which ultimately downregulates the immune response and prevents an excessive or exaggerated response from spiralling out of control (Sapolsky *et al.*, 1987; McEwen *et al.*, 1997). (7) Glucocortisoids have often been used successfully to treat immune-related disorders, such as rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus (LSE) (Kirwan, 1995). A detailed discussion of the immunological effects of various hormones is beyond the scope of this chapter; however, Table VIII.2 presents a summary of the *in vivo* and *in vitro* effects of glucocorticoids.

Effects of Brain Lesions on Immune Function

The production of specific lesions using stereotaxis procedures was among the earliest strategies to study central sites underlying

Table VIII.2 Effects of glucocorticoids on immune function

Affect leukocyte trafficking
Inhibit leukocyte migration and activation
Produce lymphopoenia
Inhibit lymphocyte proliferative responses
Inhibit NK and ADCC cytotoxic activities
Inhibit proinflammatory cytokine production
Produce a shift in Th_1/Th_2 balance
Inhibit polyclonal B-cell activation and immunoglobulin synthesis
Inhibit delayed hypersensitivity
Activate oncogenes

brain–immune system communications. Although somewhat primitive, this strategy provided a first insight into the potential brain circuitry underlying CNS–immune system communications. For instance, lesions in the anterior hypothalamus have been associated with a decrease in nucleated spleen cells and thymocytes (Brooks *et al.*, 1982), a decrease in proliferative T-cell responses to mitogen stimulation (Brooks *et al.*, 1982), a decrease in NK cell activity (Cross *et al.*, 1984), a decrease in antibody production (Tyrey and Nalbandov, 1972), and an inhibition of a lethal anaphylactic response (Stein *et al.*, 1981). These studies suggest that the anterior hypothalamus is implicated, either directly or indirectly, in the stimulation of both humoral and cell-mediated immune responses. Lesions of the medial or posterior hypothalamus have provided mixed results. Lesions in the limbic forebrain structures (e.g. dorsal hippocampus or amygdala) resulted in transient increases in splenocytes and thymocytes, as well as T-cell proliferative response to mitogen (Cross *et al.*, 1982). Some of these results could be reversed by hypophysectomy, suggesting that neuroendocrine pathways play an important role. Furthermore, work by Renoux and colleagues suggests that lesions in the cerebral cortex can affect immune regulation, and that there seems to be a lateralization effect, since lesions in the left cerebral hemisphere of mice were associated with decreased T-cell responses, whereas lesions in the right cerebral hemisphere produced increased T-cell responses (Renoux *et al.*, 1983; Renoux *et al.*, 1987). The notion that brain lesions can affect immune responses leads to the next question: can psychological stress have a similar effect? The behavioural effects on immune function are discussed next.

Behavioural Effects on Immune Regulation

It has been known for many years that stressed animals are more susceptible than non-stressed animals to developing infections following exposure to an infectious organism. The reasons for this increased susceptibility have never been very clear. With the influx of data showing extensive interactions between the brain and the immune system, the effects of stress on immune regulation became an important and fertile area for research investigation. Keller *et al.* (1981) showed that both lymphocyte numbers and function (response to mitogenic stimulation) were decreased in animal models of stress (foot shock). Further, they showed that intensity of the stressor, timing, duration, and control over the stressor were all important variables that could influence the overall effect of stress on immune function (Keller *et al.*, 1984). Another source of variation in these studies was the origin of the cells being investigated, since studies have also shown that the stress effects on lymphoid tissues derived from different compartments often differ substantially. Other experiments using restraint/immobilization stress, forced swim, maternal deprivation, and other stressors yielded mixed results. An intriguing and still somewhat unresolved issue is the notion that stress can also enhance certain immunological parameters (Lysle *et al.*, 1990; Shurin *et al.*, 1994). Dhabbar and McEwen (1997) proposed an interesting paradigm whereby acute stress stimulates certain components of the immune system, while chronic stress suppresses those same parameters. They argued that from an evolutionary perspective, an acute or fresh attack by a predator requires a strong immunological response to prevent the spread of infection, but if the stress becomes chronic, then a weakened immune response may be an advantage to the organism since it shifts limited resources away from the immune system and into more important organs, such as the muscles and the heart (Dhabbar and McEwen, 2001).

Human studies exploring the effects of stress on immune function have also been conducted (for a comprehensive review, see Biondi, 2001). The types of stressors used in these studies are either real-life stressors (e.g. bereavement, natural disaster such as earthquake or hurricane, academic examination, divorce, unemployment) or experimental stressors (e.g. mental challenge, sleep deprivation, acute overexertion). Many of the immune parameters were essentially the same as with the animal studies, notably enumeration of leukocyte and lymphocyte subsets, *in vitro* lymphocyte responses to mitogen stimulation, NK cell activity and cytokine production; again, results vary. Kiecolt-Glaser and colleagues, in a series of experiments, found that medical students had lower immune function indices the morning of a medical school examination compared with other less stressful mornings (Kiecolt-Glaser *et al.*, 1986). They also found that stress can modulate the immune response to vaccine (Glaser *et al.*, 1998). The same investigators have also found that wound healing was delayed (Kiecolt-Glaser *et al.*, 1995), and cytokine production within the wound decreased (Glaser *et al.*, 1999), in chronic caregivers to patients with Alzheimer's disease compared with non-stressed controls. Other investigators, however, have found the same immune parameters to be either increased or unchanged in association with similar or other stressors (Biondi, 2001). Therefore, it has been suggested that other factors besides the nature of the stressor, such as personality characteristics of the individual, amount of social support, and coping skills, all play a role in the immunological response to a specific stressor. As in the case of animal studies, more research is needed to elucidate the mechanisms of stress-induced immunomodulation. Another unresolved issue is the clinical significance of such changes. Any association between stress-induced immune changes and the risk of developing an immune-related disorder, such as infection or cancer, remains very tentative at best.

Immunological Influences on Central Nervous System Activities

Neural-immune interactions are bidirectional in nature. Not only does the CNS affect immune regulation, but recent evidence also indicates that the immune system influences different CNS functions. In the following sections we will briefly review the neurochemical, neuroendocrine and behavioural effects of immunological stimulation.

Neurochemical Effects

It is well known that viral infections produce specific changes in animal brain chemistry. The mechanisms of such changes, however, have been elucidated only recently. Controlled administration of Newcastle disease virus to mice was shown to alter the levels of several neurotransmitters and their metabolites in specific brain regions. More specifically, 3-methoxy-4-hydroxyphenylglycol (MHPG) and MHPG : noradrenaline ratios, an index of activation of noradrenergic neurons, dihydroxyphenylacetic acid (DOPAC) and DOPAC : dopamine ratios, an index of activation of dopaminergic neurons, and 5-hydroxyindoleacetic acid (5-HIAA) and 5-HIAA : 5-hydroxytryptamine (5-HT) ratios, an index of activation of 5-HT neurons, were all increased in a number of brain regions (Dunn *et al.*, 1987; Dunn and Vickers, 1994). Dunn and colleagues also showed a great similarity between the neurochemical responses to infections and other stressors, such as foot shock and physical restraint (Dunn *et al.*, 1999). Similar results were also obtained with the endotoxin LPS, particularly in relation to noradrenaline. *In vivo* microdialysis studies indicated increased extracellular concentrations of dopamine, DOPAC, noradrenaline, 3,4-dihydroxyphenylethyleneglycos (DHPG), MHPG and 5-HIAA in the medial prefrontal cortex and hypothalamus following LPS administration in rats (Lavicky and Dunn, 1995). Linthorst and colleagues, using similar techniques, found an increase in 5-HT in the hippocampus (Linthorst *et al.*, 1996) and increases in noradrenaline, MHPG, 5-HT and 5-HIAA in the preoptic area (Linthorst *et al.*, 1995). The effects of peripherally administered cytokines on

the turnover of specific brain neurotransmitters have been reviewed (Dunn *et al.*, 1999). IL-1, for instance, seems to be associated with an increased turnover of noradrenaline and 5-HT, IL-2 increases the turnover of noradrenaline and dopamine, IL-6 increases the turnover of 5-HT but not noradrenaline or dopamine, and IFN seems to produce no significant changes in any of these neurotransmitters.

Neuroendocrine Effects

The notion that infection is accompanied by an increase in glucocorticoid secretion is not new (Wexler *et al.*, 1957). However, this has recently been reinforced by experiments such as those described above with the Newcastle virus and showing stimulation of the HAP axis along with specific neurochemical changes (Dunn and Vickers, 1994). Here, again, the mechanism of these interactions has been elucidated only recently. The involvement of immune cells was first illustrated by the early experiments of Besedovsky *et al.* (1975), showing that the injection of sheep red blood cells into rats produced an increase in the level of glucocorticoid. The authors subsequently reported that supernatants from ConA-stimulated rat spleen cells administered into rats also produced an increase in the plasma levels of corticosterone (Besedovsky *et al.*, 1981). This was a clear demonstration that the activation of the neuroendocrine system was due to an immunogenic rather than a pathogenic mechanism. The bioactive product responsible for stimulating the HPA axis was identified later as the cytokine IL-1 (Berkenbosch *et al.*, 1987; Bernton *et al.*, 1987; Sapolsky *et al.*, 1987). It is now believed that IL-1 acts directly in the CNS to stimulate the HPA system. In addition to stimulating the release of CRH, there is also evidence that IL-1 acts directly on corticotrophs to induce the secretion of adrenocorticotropic hormone (ACTH) (Woloski *et al.*, 1985) and indirectly to produce excess glucocorticoids. The action of excess glucocorticoids on immune cells can result in downregulation of the immune response, thus providing a negative feedback to sometimes exaggerated and possibly damaging immune reactions (Table VIII.2) However, it is important to keep in mind that the effects of glucocorticoids on immune function are not always inhibitory, and that immune stimulation can occur under certain conditions (Dhabbar and McEwen, 2001). Immune responses can also affect other neuroendocrine pathways, such as the hypothalamic-pituitary-thyroid (HPT) axis (Rivier, 1993) and the hypothalamic-pituitary-gonadal (HPG) axis (Rivest and Rivier, 1993). There are also reports of interactions between growth hormone and the immune system, leaving some investigators to wonder whether growth hormone and insulin-like growth factor can be considered as cytokines (Venters *et al.*, 2001).

Behavioural Effects

As any patient knows, sickness is often accompanied by a number of behavioural attributes, such as apathy, fatigue, lack of motivation, and lack of concentration. Other attributes may include disturbed sleep, decreased appetite, or decreased sex drive. These symptoms have traditionally been considered a 'psychological reaction' to being ill. Research, however, indicates that many of these symptoms can be reproduced through the action of proinflammatory cytokines. Animal studies indicate that central or peripheral administration of IL-Iα, IL-1β or TNFα to healthy laboratory animals produces fever, activation of the HPA axis, and behavioural symptoms, such as a decrease in social exploration (Kent *et al.*, 1992), a decrease in night-time feeding (Plata-Salaman *et al.*, 1996), and an increase in delta-sleep (Krueger and Majde, 1994). For many of these cytokines, the association with the particular behaviour is dose-dependent, and tends to be abolished if the cytokine is heat-inactivated or given with its receptor antagonist, suggesting an important role for the specific cytokine in the pathophysiology of the specific behaviour.

Human studies addressing the behavioural effects of cytokines have been somewhat limited in scope because of ethical and safety considerations. However, because cytokine therapy, particularly IL-2 and IFN, has been used for a variety of medical conditions, investigators have been able to assess the behavioural effects of cytokines in these patients. Because of their antiviral as well as antiproliferative and immunomodulatory properties, interferons are now being used effectively in the treatment of several medical conditions, including malignant melanoma, hepatitis C and multiple sclerosis. The most common side effects include flu-like symptoms, such as fever, malaise and arthralgia. These side effects occur early in treatment and tend to decrease in intensity as treatment continues. Other side effects affecting roughly one-third of the patients on interferon are neuropsychiatric or behavioural in nature, and include mood changes (depression, anxiety, manic-like symptoms, irritability, and even suicidal behaviour), cognitive changes (decreased concentration, memory disturbance, delirium) and, rarely, psychosis. IL-2 and lymphokine-activated killer (LAK) cells have been used in the treatment of metastatic malignancies. In one longitudinal study, up to 50% of patients experienced severe cognitive and/or behavioural adverse effects (Denicoff *et al.*, 1987) that were dose-related but usually reversible. While the exact mechanism for these neuropsychiatric manifestations is not well understood, it is believed that cytokines interact with neurotransmitters and neurohormones to produce these adverse effects. Furthermore, in human experiments aimed at assessing directly the behavioural effects of cytokines, healthy volunteers were given LPS injections under controlled conditions. The associated rise in IL-1β and TNFα was accompanied by a significant increase in depressed mood and a significant decline in cognitive performance. There were also significant correlations between the change in the levels of these cytokines and the change in the associated behavioural measures (Reichenberg *et al.*, 2001).

MEDIATORS OF NEURAL-IMMUNE INTERACTIONS

Now that bidirectional communication between the brain and the immune system has been well established, the next important question relates to the mediators of these interactions. This section will summarize the role that neurotransmitters/neurohormones and cytokines play in this communication network.

Neurotransmitters and Neurohormones

As mentioned earlier, immune tissues and organs are innervated by the autonomic nervous system. Neurotransmitters such as adrenaline/noradrenaline, dopamine, serotonin (5-HT) and acetylcholine play a role in immunomodulation. Neurotransmitter receptors are found in most lymphoid cells. The functional evidence is based on both *in vivo* and *in vitro* findings using pharmacological agonists and antagonists selective for specific neurotransmitter subtypes. In addition, data have been presented to confirm the presence of the receptor at the cellular level using radioligand binding analysis and at the molecular level using reverse-transcription PCR and Northern analysis (for a comprehensive review, see Sanders *et al.*, 2001). Adrenergic receptors, for instance, can be found on thymic cells, bone marrow cells, T-cells, B-cells, NK cells and macrophages. Although results vary, β-adrenergic stimulation seems to increase bone marrow cell proliferation *in vivo* (Lipski, 1980), decrease *in vitro* thymic cell proliferation (Cook-Mills *et al.*, 1995), decrease T-cell proliferation by mitogen (Johnson *et al.*, 1981), and decrease IL-2 production (Selliah *et al.*, 1995). LPS-induced TNF production by macrophages is also decreased (Szabo *et al.*, 1997), as is lytic activity by NK cells (Takamoto *et al.*, 1991).

The results are mixed with T-cell-dependent antibody production (Besedovsky *et al.*, 1979; Shreurs *et al.*, 1982). Results with the α-adrenergic receptors differ. Studies have also shown that thymocytes and mature circulating T-lymphocytes express the muscarinic receptor, and that stimulation of this receptor increases cell viability and the proliferative capacity of these cells *in vivo* (Rinner *et al.*, 1995; Fujino *et al.*, 1997). As for serotonergic receptors, studies suggest that predominantly the 5-HT_1A receptor is expressed on T-cells, B-cells and macrophages, and that stimulation of this receptor can either suppress (Ferriere *et al.*, 1996) or enhance (Iken *et al.*, 1995) different immune functions. Results concerning the effects of dopaminergic stimulation on immune function have been mixed, so no definite conclusion can be reached at the present time (Panajotova, 1997).

In addition to the biogenic amine neurotransmitters, mediators of CNS-immune communication include specific neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P. These neuropeptides, which are synthesized in the cell body of the neuron and transported to the nerve ending where they are released when the neuron is depolarized, can diffuse out of the tissue and into the local microenvironment. Their functions range from control over local vasculature (Brain, 1997) to various immunological effects. For instance, substance P can regulate cytokine production by immune cells, immunoglobulin production by B-lymphocytes, and the migration of cells into sites of infection (Rameshwar *et al.*, 1994). CGRP on the other hand seems to exert inhibitory effects on mitogen-induced cell proliferation and cytokine production (Boudard and Bastide, 1991). It is now widely believed that these and other neuropeptides are involved in the regulation of immune and inflammatory responses in the local microenvironment. More research is needed to delineate their varied and adaptive roles further.

Cytokines

We have already addressed the immunological effects of cytokines. In addition to their immune and inflammatory effects, cytokines have been shown to be secreted by, and to affect the functions of, several organs, including the brain. The brain's effects of cytokines have been the subject of numerous reviews (Kronfol and Remick, 2000). However, before these effects are discussed, it is important to keep in mind that cytokine-to-brain signalling can occur in several ways: (1) passive transport of cytokines into the brain at circumventricular sites lacking a blood–brain barrier, such as the organum vasculosum of lamina terminalis; (2) binding of cytokines to cerebral vascular endothelium, thereby inducing the generation of secondary messengers, such as prostaglandins and nitric oxide; (3) carrier-mediated transport of cytokines across the blood–brain barrier and into the brain; and (4) activation by cytokines of peripheral afferent nerve terminals at the sites where cytokines are released. These mechanisms are not mutually exclusive. Furthermore, regardless of the mechanism(s) of brain signalling, it is now evident that cytokine genes and their receptors are expressed in the CNS and participate in the response to specific stimuli, such as infection or injury. The precise mapping of various cytokine pathways and their receptors in the brain, however, remains incomplete (Licinio and Wong, 1997).

A detailed discussion of the various cytokine actions in the brain is beyond the scope of this chapter. We will, however, offer examples of the various immunological/inflammatory, neurochemical, neuroendocrine and behavioural effects as an illustration (Figure VIII.2). The immunological/inflammatory effects of brain cytokines include gliosis, neovascularization and induction of other cytokines (Giulian *et al.*, 1988). The neurochemical effects were discussed earlier; they include changes in the brain turnover of the neurotransmitters noradrenaline, dopamine, serotonin and acetylcholine. The neuroendocrine effects include actions on the HPA (Sapolsky *et al.*, 1987), HPT (Rivier, 1993) and HPG (Rivest and Rivier, 1993) axes. The behavioural effects include changes in sleep regulation (Krueger and Majde, 1994), appetite regulation (Plata-Salaman *et al.*, 1996), sexual behaviour (Avitsur *et al.*, 1999) and cognition (Reichenberg *et al.*, 2001). There is also growing evidence that the behavioural effects of cytokines, including lethargy, increased sleep and decreased appetite, which are often referred to as 'sickness behaviour', are part of a well-organized central motivational state that, along with metabolic and physiological changes, represents the systemic response to local infection. From an evolutionary perspective, it has been suggested that these adaptive measures are meant to mobilize all the necessary resources to combat the infection/insult and return the organism to its previous state of homeostasis and health (Dantzer *et al.*, 2001).

CLINICAL IMPLICATIONS

Medical Illness

In spite of the dramatic advances in the field of psychoneuroimmunology at the basic science level, the advances at the clinical level have been somewhat timid in comparison. The immune system plays a major role in various medical illnesses, particularly infectious diseases, autoimmune/inflammatory disorders, and cancer. The notion that the CNS and the immune system are in constant dialogue raises the possibility that emotional factors and/or stressful life events may play a role in the aetiology and/or progression of these disorders through interference with immune regulation. Although to date there have been numerous studies addressing the issue of the connection between stress-induced immunomodulation and specific immune-related disorders, the results in general have been mixed and inconclusive. We will, however, provide some examples as an illustration.

Infection

The outcome of an infectious process depends on the interplay of complex phenomena related to both the infectious agent (e.g. specific strain, virulence, dose) and the host (e.g. genetic make-up, prior exposure, nutritional status). The importance of lifestyle factors in this equation has been recognized only recently. This may be obvious in cases such as the association between smoking and upper respiratory tract infection, and the association between sexual promiscuity and sexually transmitted diseases. The relation of infection to issues such as stressful life events, depression or exercise, however, has been addressed only recently. In one such study, Cohen *et al.* (1991) experimentally exposed a number of healthy volunteer subjects to five different respiratory viruses following a thorough psychological assessment that included life events, perceived stress and negative affect. The subjects were followed up for 7 days for signs of the common cold (illness) and for 28 days for rise in viral-specific antibody titre (infection). Results indicate that higher levels of stress were associated with significantly higher incidences of both infection and illness (common cold). The same pattern was seen for all five viruses. In a subsequent study, the same authors examined the effects of stress characteristics and social support on susceptibility to the common cold (Cohen *et al.*, 1998). They found a significant positive association between the duration of life stressors and the risk of having a cold. They also found that social support network diversity tended to decrease the frequency of the cold. There is also recent evidence that stress-induced increases in IL-6 concentrations may play a role as

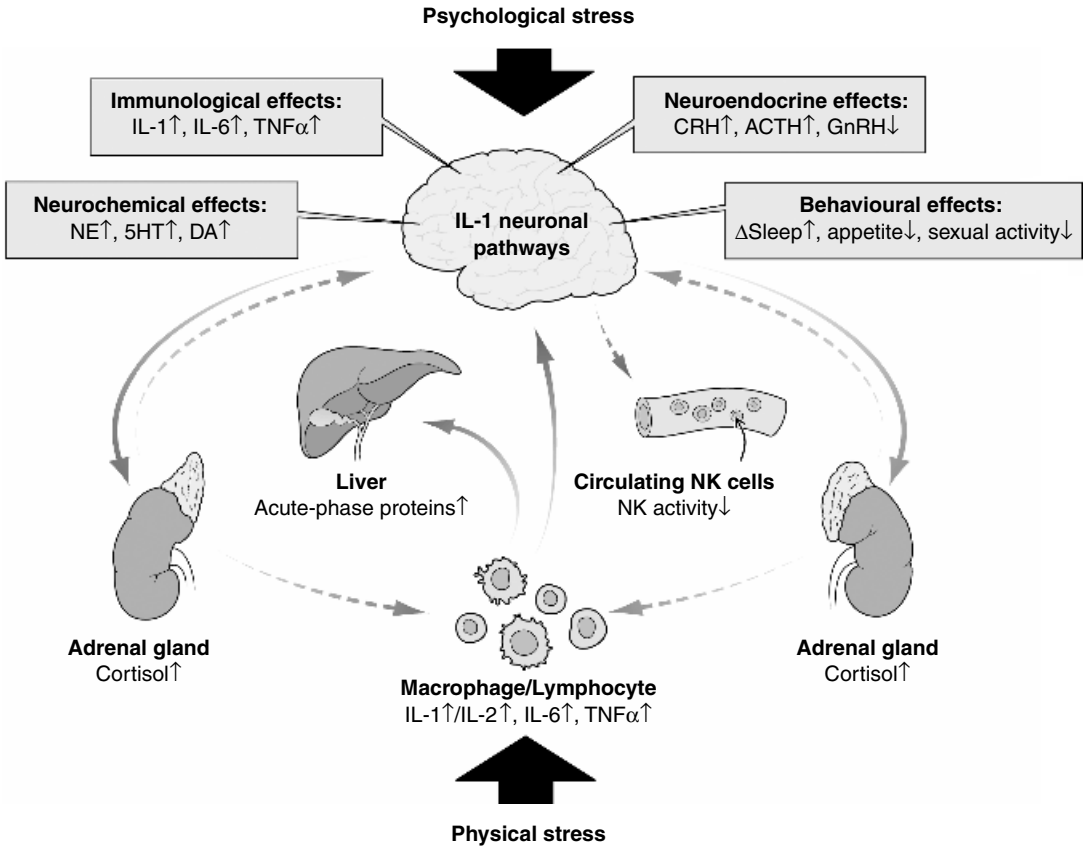

Figure VIII.2 Stress and IL-1 secretion. Both psychological stress (e.g. academic stress, depression) and physical stress (e.g. infection, trauma) can activate IL-1. IL-1 activation is associated with various phenomena, both peripherally (e.g. cascade activation of other cytokines, induction of acute-phase proteins) and centrally (e.g. various immunological, neurochemical, neuroendocrine and behavioural effects). Feedback mechanisms occur at several levels; they include negative feedback exerted by cortisol. Only selected effects are shown as an example. *Reproduced by permission from The American Journal of Psychiatry*, 157, 683–694, 2000. Copyright 2000, The American Psychiatric Association. GnRH, gonadotropin-releasing hormone; NE, noradrenaline

mediators between stress and upper respiratory infection (Cohen *et al.*, 1999).

Cancer

The relation between psychosocial stress and cancer is complex and multifaceted. Several questions have been addressed in the literature: Does psychosocial stress and/or depression increase the risk for the development of cancer? Does psychosocial stress and/or depression play any role in cancer progression? Can psychosocial intervention improve the outcome? Are there specific neuroimmunological pathways that explain these interactions? Whereas animal studies have established an association between stress-induced immunosuppression and both tumour development (Ben Eliyahu *et al.*, 1999) and tumour metastasis (Ben Eliyahu *et al.*, 1991), the association in humans between psychosocial factors and cancer development and/or progression has not been well documented. In a meta-analysis of studies addressing the relation between depression and the development of cancer, there was a small but statistically significant association between depressive symptoms and the occurrence of cancer (McGee *et al.*, 1994). The effects of stress and depression on cancer progression have also been controversial, with some studies reporting an association between stressful life events and cancer recurrence (Ramirez *et al.*, 1989), and others showing no evidence for such an association (Cassileth *et al.*, 1985). One reason for the variation in findings may be related to coping styles, with studies showing that a 'fighting spirit' is associated with a more positive prognosis (Greer and Watson, 1985), and other studies showing a fatalistic attitude or helplessness to be associated with a negative outcome (Temoshok *et al.*, 1985). Regarding the effects of psychosocial intervention, the evidence remains inconclusive. Spiegel and co-workers, in a 10-year follow-up study of women with metastatic breast cancer, found that women who participated in a group psychotherapy intervention lived for up to 18 months longer than those in the non-intervention group (Spiegel *et al.*, 1989). Similar effects were also reported by Fawzy *et al.* (1990b) in malignant melanoma patients. Unfortunately, not all studies agree (Gellert *et al.*, 1993; Van der Pompe *et al.*, 1997). Furthermore, in the few studies where immunological measures were obtained (e.g. relative percentage of CD_4+ and/or NK cells), results regarding these measures in the intervention and non-intervention groups were also mixed (Van der Pompe *et al.*, 1997; Fawzy *et al.*, 1990b).

Acquired Immune Deficiency Syndrome

The course of infection with the human immunodeficiency virus (HIV) is highly variable, with some patients remaining asymptomatic for many years but others progressing quickly to full-blown acquired immune deficiency syndrome (AIDS). While many factors relating to both the virus (such as the specific strain) and the host (such as concurrent infection) have been recognized to explain this discrepancy, psychosocial influences have also been suggested. A

summary of the studies addressing these factors has been provided by Cole and Kemeny (2001). Again, the psychosocial factors have included stressful life events, such as awareness of a serious health problem (a positive HIV test), bereavement (particularly death of a loved one), and financial difficulties, and also depression, coping skills and social support. As in the case of other infectious diseases and cancer, the results have varied. In a follow-up study of 330 gay men, Burack *et al.* (1993) found a significant negative association between CD_4+ cell levels and depressive symptoms. This association, however, did not affect disease progression or mortality. In a larger study of 1809 gay men, Lyketsos *et al.* (1996) were unable to find any relationship between CD_4+ cells, morbidity or mortality. In a different line of work, Cole *et al.* (1996) showed that gay men who concealed their homosexual identity had lower CD_4+ levels and faster HIV progression than those who did not conceal their homosexual identity. These and other findings illustrate the extent and the complexity of the relationship between psychosocial factors, immune parameters, and HIV progression.

Psychiatric Illness

Because major psychiatric disorders such as schizophrenia and bipolar affective disorder are thought to be brain disorders associated with abnormal brain chemistry and/or disrupted neuronal circuitry, and because these same neurochemicals and/or neuronal pathways have been shown to affect immune regulation, it has been postulated that major psychiatric disorders will also be accompanied by dysregulation of immune function. In fact, a good deal of research over the last few years has addressed the characterization and clinical significance of immune system abnormalities in patients with schizophrenia, major depression, Alzheimer's disease and other diseases. These issues are addressed in the clinical chapters later in this book. In addition to examining the immunological characteristics of the disorder (Kronfol and House, 1989), researchers have also investigated the relation of any demonstrated immunopathology to the clinical manifestation of the disorder (Ganguli *et al.*, 1995), as well as the possible association between neurochemical/neuroendocrine characteristics of the disorder and the demonstrated immunological anomalies (Maes *et al.*, 1993). Furthermore, in view of the frequent comorbidity between medical and psychiatric illness, and the poor prognosis associated with such events, researchers have studied a possible role for specific immune measures in such interactions (Burack *et al.*, 1993).

CONCLUSION

As this chapter demonstrates, the CNS and the immune system are in constant dialogue. In addition to its traditional role in defending the organism against invading micro-organisms and other insults, the immune system can also act as a sensory organ. In that capacity, components of the immune system collect information about the local microenvironment and transmit it to the brain (afferent loop), which in turn analyses the information and accordingly initiates a series of steps to either enhance or suppress the immune response (efferent loop). The purpose of this dialogue, therefore, is to coordinate between the local microenvironment and the rest of the organism for the purpose of mobilizing the necessary resources to best deal with a stressful event such as infection, inflammation or trauma.

As some of the reasons for this dialogue between the CNS and the immune system are getting clearer, the language used is also unfolding. Neurotransmitters, neuropeptides, hormones and cytokines are all involved. It is this constant dialogue that keeps the organism healthy by facilitating activities such as controlling and eliminating an infection and healing a wound. However, if for whatever reason the dialogue is compromised or interrupted, then the risk for medical and/or psychiatric symptoms increases significantly. Relations between the immune system and specific psychiatric disorders are presented later in this book.

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IX

Psychophysiology

Gary G. Berntson, John T. Cacioppo and Martin Sarter

Psychophysiology is an interdisciplinary science that seeks to elucidate the relations between the mind and the body. Historically, psychophysiologists have been interested in the impact of psychological states and processes on physiological (especially autonomic) functions, and hence have often focused on psychosomatic or psychophysiological disorders. Psychophysiology is considerably broader than this, however, and psychophysiological perspectives have expanded and matured considerably over the past decades. Currently, many psychophysiologists are equally interested in the impact of neural and physiological factors on psychological processes. In fact, it is difficult to draw clear distinctions between psychophysiology and disciplines such as psychobiology, behavioural neuroscience, cognitive neuroscience and neuropsychology. There are a few general perspectives, however, that characterize the contemporary field of psychophysiology. These include an emphasis on reciprocal relations between psychological and physiological domains, a focus on interactions among systems (e.g. behavioural, endocrine, autonomic, immune) and levels of neurobehavioural organization (e.g. reflexive, affective, cognitive), and an interest in explicating higher-level psychological processes.

HISTORICAL TRENDS IN PSYCHOPHYSIOLOGY

Although the roots of psychophysiology may be traced back thousands of years, its establishment as an independent, formal discipline is generally pinpointed to the 1960s with the formation of the Society for Psychophysiological Research in 1960 and the publication of its official journal *Psychophysiology* in 1964 (Cacioppo *et al.*, 2000b; Sternbach, 1966). Systematic research with a predominant psychophysiological perspective, however, has been pursued since the late nineteenth century. This includes investigations of electrodermal responses and their sensitivity to psychological processes (Fere, 1888; Tarchanoff, 1890), studies of emotion and autonomic control (Cannon, 1928), and work on the conditioning of autonomic and visceral responses (Pavlov, 1927). From these early beginnings, the field of psychophysiology has developed and matured considerably. Of note are several general conceptual trends in this historical development that are relevant to applications of psychophysiology to contemporary psychology and psychiatry. This is an especially important consideration, as the prevailing conceptual landscape serves to frame empirical findings and theoretical perspectives, and thus can powerfully shape clinical concepts, research and applications.

These closely related historical trends include (1) a general shift in perspective from a peripheral to a central psychophysiology; (2) a progressive trend from an emphasis on lower reflex mechanisms to the influence of higher and more complex rostral neural systems, together with a related shift from simplistic regulatory models to more complex, multidetermined conceptions of psychophysiological regulation; (3) a corresponding shift from a predominant focus on efferent processes to the recognition of reciprocal afferent–efferent interactions; and (4) a parallel transition from constructs of global influences on mind and body to more specific patterns of determinants.

From Peripheral to Central Psychophysiology

Much of the early history of psychophysiology revolved around the autonomic nervous system (ANS) and measures of visceral function. Nineteenth-century concepts of the involuntary or vegetative nervous system generally focused on peripheral components; Langley (1921) originally proposed the phrase 'autonomic nervous system' to refer to the visceral nerves of the thoracic, cranial and sacral outflows. By the beginning of the twentieth century, the impact of this peripheral system on visceral function was recognized, and opposing effects of the vagal and sympathetic branches on heart rate had been articulated. Both Langley (1921) and Cannon (1928) recognized central nervous system (CNS) components of the ANS, including the sympathetic motor neurons of the thoracic and lumbar divisions of the cord, and Cannon specifically elucidated the impact of psychological factors such as fear and rage on autonomic function. Even behavioural influences, however, were considered as manifestations of relatively simple, reflex-like reactions that mediate peripheral adjustments to adaptive challenge and support the requisite energy mobilization for emergency reactions of fight or flight (Cannon, 1939, p. 227).

The baroreceptor–heart rate reflex is a prototypic brainstem reflex that exerts powerful control over the autonomic outflows in the service of blood pressure regulation. As depicted in Figure IX.1, baroreceptor reflexes are controlled by afferents from pressor receptors in the heart and great arteries. An increase in blood pressure results in enhanced baroreceptor afferent activity to the nucleus of the tractus solitarius (NTS), which in turn issues a relatively direct excitatory projection to the parasympathetic source nuclei in the nucleus ambiguous and dorsal motor nucleus of the vagus. The resulting increase in parasympathetic outflow slows the beat of the heart and reduces cardiac output, thereby opposing the pressor increase. This is accompanied by a reciprocal inhibition of sympathetic motor neurons in the intermediolateral cell column of the cord. The resulting decrease in sympathetic cardiac outflow tends to further diminish the heart rate and to reduce ventricular contractility, which, together with reductions in adrenergic vasoconstrictor tone, synergistically serve to compensate for the disturbance and to restore blood pressure. The opposite pattern of autonomic control (i.e. sympathetic activation and reciprocal parasympathetic withdrawal) is triggered by the unloading of arterial baroreceptors (e.g. during assumption of an upright posture), which, provides important cardiovascular support to maintain adequate blood pressure and circulation in the face of this

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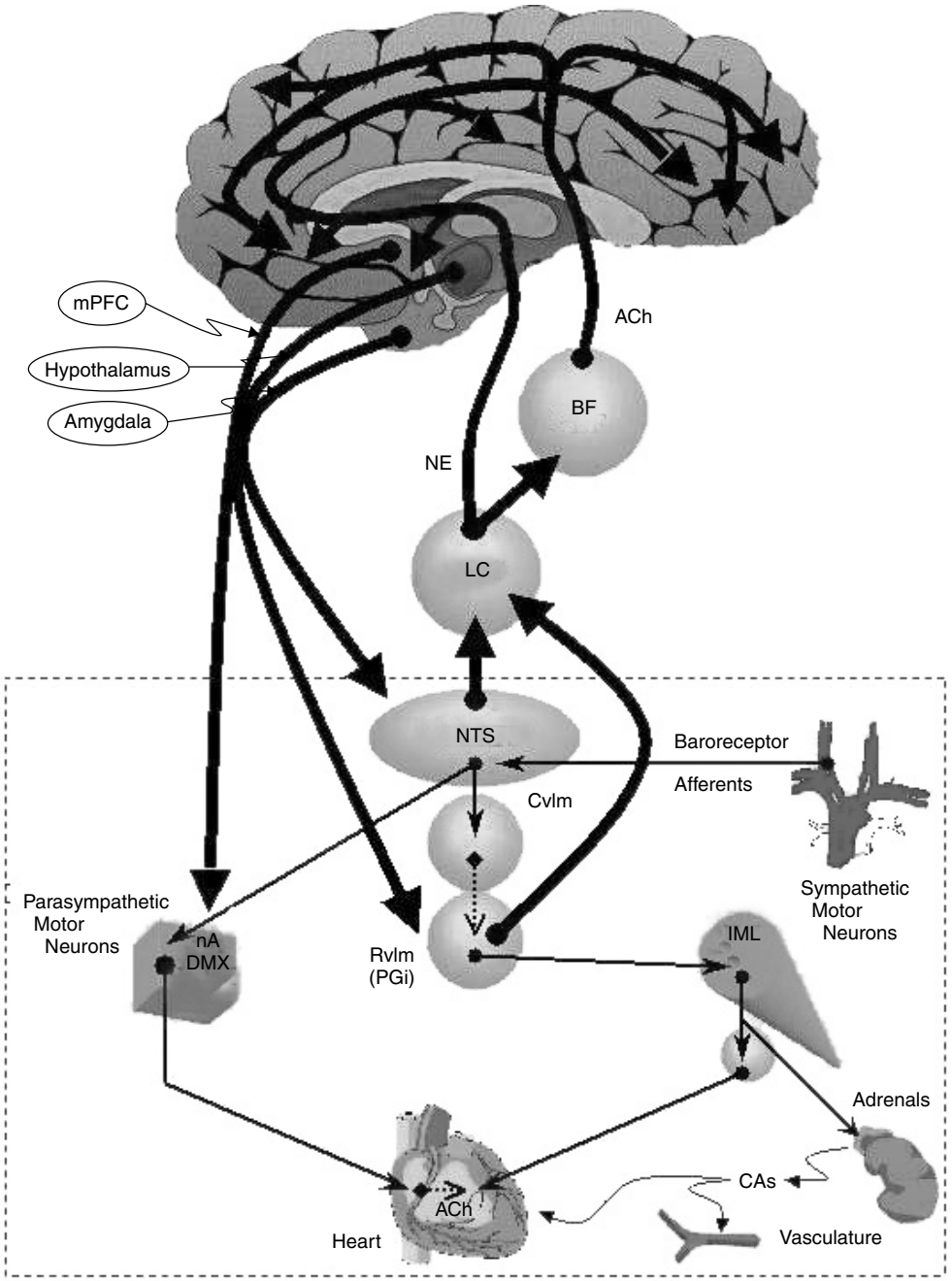

Figure IX.1 Baroreflex circuits. Dashed box: classical brainstem systems underlying baroreceptor cardiac reflex. Baroreceptor afferents project to nucleus tractus solitarius (NTS), which in turn leads to activation of parasympathetic motor neurons in the nucleus ambiguous (nA) and dorsal motor nucleus of the vagus (DMX). The NTS also activates the caudal ventrolateral medulla (Cvlm), which in turn inhibits the rostral ventrolateral medulla (Rvlm), leading to a withdrawal of excitatory drive on the sympathetic motor neurons in the intermediolateral cell column of the cord (IML). Upper section: expansion of the baroreflex circuit to illustrate the ascending and descending pathways to and from rostral neural areas, such as the medial prefrontal cortex (mPFC), hypothalamus and amygdala. Ascending systems include routes from the rostral ventrolateral medulla (Rvlm) and the nucleus of the tractus solitarius (NTS) to the locus coeruleus (LC) noradrenergic system, and indirectly to the basal forebrain (BF) cortical cholinergic system. Adapted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 466, with permission from Cambridge University Press. ACh, acetylcholine; CAs, catecholamines; NE, noradrenaline; PGi, paragigantocellular nucleus (See Colour Plate IX.1)

orthostatic challenge. Although mediated by the CNS, baroreceptor reflexes are rather rigid in organization and expression, being regulated by specific baroreceptor afferent activity, and characterized by a reciprocal excitatory/inhibitory control of the two autonomic branches.

The early focus on peripheral components and lower reflexes was natural given the existing state of knowledge, the relative accessibility of the peripheral autonomic nerves and visceral organs, and the limited methods for central physiological measurement and analysis. Consequently, the empirical database was most conducive

to the development of a peripherally focused psychophysiology, and this focus impacted both research and theory.

An example of this focus comes from the early work of Eppinger and Hess (1915) on individual differences in autonomic reactivity, which were considered to reflect distinct constitutional dispositions. Some individuals were reported to show relatively greater parasympathetic reactivity to pharmacological or physiological challenges (*vagotonia*), whereas others displayed greater sympathetic reactivity to the same challenges (*sympaticotonia*). Based on these and other findings, Eppinger and Hess formulated an early individual differences model of psychosomatic disorders, in which these basic constitutional factors were seen to differentially dispose the individual toward particular types of pathology (e.g. asthma in vagotonics, Reynaud's disease in sympathicotonics). This work spearheaded an extensive literature on individual differences and the situational and constitutional determinants of autonomic responses. The subsequent work of Wenger (1941) confirmed individual differences in autonomic dispositions, but suggested that autonomic balance was distributed normally rather than categorically across individuals (i.e. vagotonics and sympathicotonics). Additional research suggested a further differentiation of complex but stable patterns of reactivity across multiple measures that cannot be characterized along a single sympathetic–parasympathetic dimension (Lacey and Lacey, 1958). Other early work suggested that the pattern of autonomic response was influenced by the specific evocative stimulus or psychological state (e.g. fear *v*. anger, Ax, 1953; anxiety *v*. resentment, Wolf and Wolff, 1947).

Research on these issues continues, and it is now clear that patterns of autonomic response are determined by multiple factors, including individual differences in response disposition (*individual response stereotypy*) as well as stimulus or situational variables (*stimulus-response specificity*). Of particular importance is the contribution of psychological states, and interactions with response dispositions and situational factors, in the determination of psychophysiological relations. This is an important area, because experimental interest often concerns the central psychological state whereas classical measures have been primarily peripheral. Because the complexity of psychophysiological mappings is likely to increase with the number of intervening mediational steps or processes (Cacioppo *et al.*, 2000a), there has been increasing recognition of the need for more proximal (central) as well as distal (peripheral) measures.

The importance of this is apparent, even at the most peripheral levels. Heart rate is a common psychophysiological measure with documented relations to psychological states and processes, and heart rate measures have been reported to predict atherosclerosis (Manuck *et al.*, 1997), survival after myocardial infarction (La Rovere, 2001), and immune responses (Cacioppo *et al.*, 1995; Cohen, 2000). The heart, however, is innervated dually by both branches of the ANS, and an increase in heart rate, for example, could arise from sympathetic activation, vagal withdrawal, or both. This is significant, because the central processes underlying these patterns of autonomic response may be fundamentally different and may have distinct psychophysiological implications. Thus, it appears to be the sympathetic component of heart rate response, and not the heart rate response *per se*, that is most predictive of the neuroendocrinological and immunological response to stress (Cacioppo *et al.*, 1995). Moreover, it is the relative sympathetic and parasympathetic control of the heart that is most predictive of recovery after myocardial infarction (Altschuld and Billman, 2000). These and other findings reveal that the understanding of peripheral psychophysiology can be informed by knowledge of the more central origins of these relations. This has led to the development and deployment of more specific measures of activities of the autonomic branches, including selective pharmacological blockades and non-invasive measures, such as pre-ejection period (PEP), which provides an index of sympathetic control, and respiratory sinus arrhythmia (RSA), which reflects parasympathetic control (see Berntson *et al.*, 1994a; Cacioppo *et al.*, 1994). Further elucidation of psychophysiological relations is emerging from more direct measures of higher neural activity, including electrophysiological recording and brain imaging methods, as well as from the consequences of natural (neuropsychological) or experimental manipulations (e.g. direct or magnetic stimulation, pharmacological manipulations, selective inactivations, etc.). We consider next the historical shift from a focus on lower to higher neural systems.

From Lower to Higher Levels, and from Simple to Complex Neural Regulation

In his treatise on Evolution and dissolution of the nervous system, John Hughlings Jackson in 1878 recognized that functions are represented at multiple levels of the neuraxis, as they become represented and elaborated by higher-level systems in the progress of neural evolution. Reactions to aversive stimuli, for example, can be seen in pain-withdrawal reflexes organized at the level of the spinal cord. Elaborated systems of the brainstem, however, can generate more complex and organized patterns of escape and aggressive reactions as evidenced by decerebrate organisms (Berntson *et al.*, 1993a). Higher limbic structures, such as the amygdala, have been shown to be important in affective processes and conditioned fear reactions, whereas cortical systems appear to be particularly important for contextually based fear and anxiety and for cognitively and strategically based reactions (Berntson *et al.*, 1998; LeDoux, 2000; Lang *et al.*, 2000). The multiple levels of organization and processing in neurobehavioural systems have substantive implications for psychophysiology. A comprehensive conceptualization of psychobiological and psychophysiological relations will require attention to the multiple levels of processing, as distinct levels may be differentially sensitive to specific aspects of the stimulus and associative context, and may vary in their response repertoires and access to output mechanisms. Of special relevance are interactions among levels of processing, and the conditions that foster expression of one of more processing levels. In view of these considerations, an understanding of a given functional level of processing may require an appreciation of the broader range of neuraxial levels of function, as well as a multilevel analysis of events in the psychological, neural, cellular and molecular domains.

The historical shift in focus across levels of neural organization represents an extension of the trend from a peripheral to a central emphasis in psychophysiology. At progressively higher levels of organization, one sees an expansion in the range and relational complexity of stimulus processing, and an increase in the variability and flexibility of responses. This is highly relevant for the present consideration because it implies that psychophysiological mappings for higher psychological processes, although lawful, may be highly complex. Moreover, as noted above, the complexity of this mapping may increase with the number of intervening processing levels or steps. Consequently, an understanding of the autonomic psychophysiology of anxiety, for example, may benefit from experimental methods that allow for more direct measurement or manipulation of the higher central systems that underlie these relationships.

Although simple fear conditioning may be mediated at the level of the amygdala, cortical systems appear to be especially important for contextual fear conditioning and the cognitive aspects of anxiety (Berntson *et al.*, 1998; LeDoux, 2000; Phillips and LeDoux, 1992). In addition to supporting the highest-level cognitive and behavioural strategies for adaptive response, these rostral processing substrates are capable of a top-down activation of lower-level systems involved in more basic behavioural reactions as well as autonomic and neuroendocrine responses. This top-down activation can manifest even in the absence of a relevant environmental fear stimulus. Mental imagery of aversive or anxiogenic contexts is

associated with cortical activation, especially in limbic, paralimbic and associated cortical areas, as evidenced by positron emission tomography (PET) studies (Kosslyn *et al.*, 1996; Rauch *et al.*, 1995; Rauch *et al.*, 1996; Rauch *et al.*, 1997; Shin *et al.*, 1999). Such imagery can evoke autonomic responses characteristic of anxiety (Witvliet and Vrana 1995), can potentiate startle responses in a fashion similar to a conditioned fear conditioned stimulus (Vrana 1995), and can trigger anxiety symptoms in patients with phobias or post-traumatic stress disorder (McNeil *et al.*, 1993; Rauch *et al.*, 1995; Rauch *et al.*, 1996).

On the other hand, particular contexts may not require, or may even preclude, higher-level processing, and the relative contributions of processing levels may vary across conditions. Conditioned fear stimuli have been shown to increase activity in both the amygdala and cerebral cortex, as revealed by PET and functional magnetic resonance imaging (fMRI) studies (Hugdahl *et al.*, 1995; LeBar *et al.*, 1998; Morris *et al.*, 1999). Backward visual masking of a fear stimulus, however, may preclude conscious awareness and higher-level processing of the stimulus, without eliminating lower-level processing and associated autonomic responses (Ohman and Soares, 1994; Ohman and Soares, 1998). In this regard, a recent fMRI study revealed that masked-fear stimuli, relative to non-masked stimuli, preferentially enhance activity in thalamic-amygdala circuits and decrease amygdala-cortical activity (Morris *et al.*, 1999). These findings are consistent with the view that processing of fear- and anxiety-related stimuli and associations can occur at multiple interrelated levels of the neuraxis, and illustrate the utility of recently developed psychophysiological methods that provide relatively direct measures of patterns of higher neural activity. These findings may help clarify the functional bases for some complexities in the autonomic features of anxiety states.

Levels of Organization and the Complexity of Psychophysiological Mappings

The levels of neurobiological function within the CNS are often considered to be organized in a hierarchical fashion, with higher levels receiving inputs from, and implementing actions via, immediately lower levels (Figure IX.2). Because of the afferent convergence and efferent divergence of higher levels, a hierarchical organization would allow for broadened and more elaborated stimulus processing and an increased range of outputs at progressively more rostral levels. Furthermore, this organization would ensure integration and coordination among levels because of the hierarchical processing of ascending and descending information.

The hierarchical model is compatible with the views of Walter Cannon, who significantly shaped early conceptions of autonomic control and autonomic psychophysiology (Cannon, 1928; Cannon, 1929). Cannon proposed that a primary role of the ANS was in maintaining the constancy of the internal mileau, a regulatory process he termed 'homeostasis' (Cannon, 1929; Cannon, 1939). Cannon viewed the sympathetic branch as the primary homeostatic regulator, with the parasympathetic branch serving to fine-tune reactions across organs. Historically, the two autonomic branches have been considered to be reciprocally regulated by central systems (Fulton, 1945), a view that continues to be espoused in the contemporary literature (Malliani *et al.*, 1997). There is ample evidence for such a reciprocal mode of autonomic control in simple reflex responses such as the baroreceptor–heart rate reflex, and this mode of control would have considerable regulatory advantage as reciprocal changes would synergistically amplify actions at the end organ and expand the dynamic range of control of the heart (see Berntson *et al.*, 1993b).

The homeostatic model of autonomic function dominated early conceptions of psychophysiology, and behavioural-autonomic relations were often viewed as hierarchical extensions of homeostatic processes. In a hierarchical system, rostral levels could access a wider range of response mechanisms, but the actions of these systems would be constrained by the more primitive organizations at lower levels. Hence, the basic reciprocal mode of control apparent in brainstem reflexes might be expected to manifest in behavioural contexts as well. However, it is now clear that while there are hierarchical aspects to neural organization, there also exist long ascending and descending projections that cross multiple levels of organization and allow far more flexibility of control by rostral neural systems, a pattern of organization that has been termed 'heterarchical' (Berntson and Cacioppo, 2000). Figure IX.2(b) illustrates long descending projections that extend from rostral brain regions such as the hypothalamus, amygdala and medial prefrontal cortex to lower reflex substrates of the brainstem and even directly to autonomic source nuclei. Thus, higher systems may be able to bypass or override lower substrates, yielding non-homeostatic and/or non-reciprocal modes of autonomic control. It has now been shown that autonomic responses in behavioural contexts can exceed metabolic demands (Obrist, 1981; Turner, 1994), that conditioned autonomic responses may be seen prior to homeostatic perturbations and their associated afferent feedback (Dworkin, 1993), and

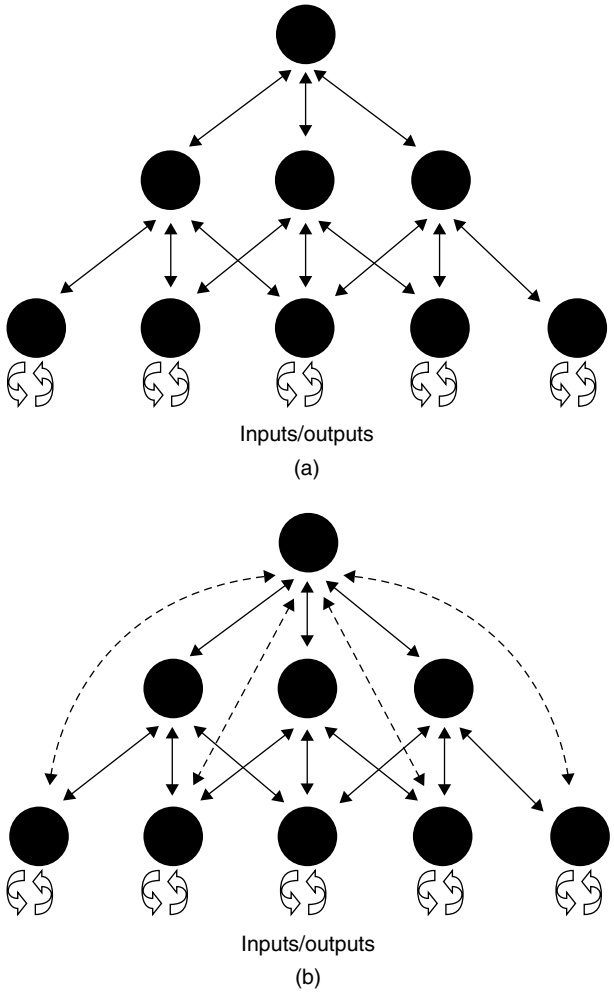

Figure IX.2 Organizational structure. (a) Classical hierarchical organization, with sensory inputs and motor outputs associated with lowest levels, and maximal convergence/divergence at the highest level. (b) Heterarchical organization, with long ascending and descending pathways (dashed arrows). Not illustrated are additional complexities associated with the presence of lateral interactions

that autonomic responses in behavioural contexts may entail non-reciprocal changes in activities of the autonomic branches (Berntson *et al.*, 1993b; Berntson *et al.*, 1994b). Moreover, under some conditions, psychological stressors may actively inhibit homeostatic baroreceptor reflexes (Steptoe *et al.*, 1996; Nosaka, 1996), permitting the concurrent increase in heart rate and blood pressure often observed under stress. These responses may well be adaptive, but they are certainly not homeostatic.

Whereas lower-level autonomic substrates may be characterized by relatively fixed patterns of response, higher-level systems can generate a broader and more flexible range of reactions. This increases the complexity of psychophysiological relations. Although baroreflex responses may entail tightly regulated reciprocal patterns of autonomic control, the autonomic branches may change reciprocally, independently or coactively in behavioural contexts (Berntson *et al.*, 1993b; Berntson *et al.*, 1994b). This has necessitated an expansion in the simple reciprocal bipolar model of autonomic control (Figure IX.3), in which autonomic states are considered to lie along a single continuum with maximal sympathetic (and minimal parasympathetic) activity at one end and maximal parasympathetic (and minimal sympathetic) activity at the other. Although this model may apply to simple brainstem reflexes, the greater output flexibility of higher neural systems in behavioural contexts necessitates a bivariate model of autonomic space with sympathetic activity along one axis and parasympathetic activity along the other. As illustrated in Figure IX.3, this has important implications for psychophysiological mapping. For the bipolar model, there would be a rather simple mapping between heart period and the underlying autonomic state, and hence measures of heart rate would offer an index of that underlying state. In contrast, for a bivariate mode of control, heart period is ambiguous with regard to its autonomic origins, as evidenced by the isofunctional contour lines on the autonomic plane. These lines illustrate different locations on the autonomic plane that translate into equivalent chronotropic states of the heart. Although knowledge of autonomic states could allow inferences concerning heart period, heart period measures do not permit ambiguous inferences concerning autonomic states.

In addition, there may be far greater individual differences in autonomic responses arising from the operations of rostral neural systems in psychological contexts. At a group level, the heart rate response of human subjects to orthostatic stress and to standard psychological stressors (mental arithmetic, speech stress, reaction time task) were found to be similar (Berntson *et al.*, 1994a; Cacioppo *et al.*, 1994). Analysis of the separate contributions of the two autonomic branches by the use of single and dual pharmacological blockades revealed that the orthostatic stress (transition from sitting to standing) yielded a rather consistent response across subjects, characterized by a highly correlated sympathetic activation and parasympathetic withdrawal. In contrast, psychological stressors yield a more varied pattern of response across subjects, with no overall correlation between the responses of the autonomic branches. Some subjects showed a predominant sympathetic activation, others a predominant parasympathetic withdrawal, and others varying combinations of these responses (Figure IX.4). Although there were considerable individual differences in the responses, individual response patterns were stable across the three psychological stressors (Berntson *et al.*, 1994a; see also Malarkey *et al.*, 1995). These

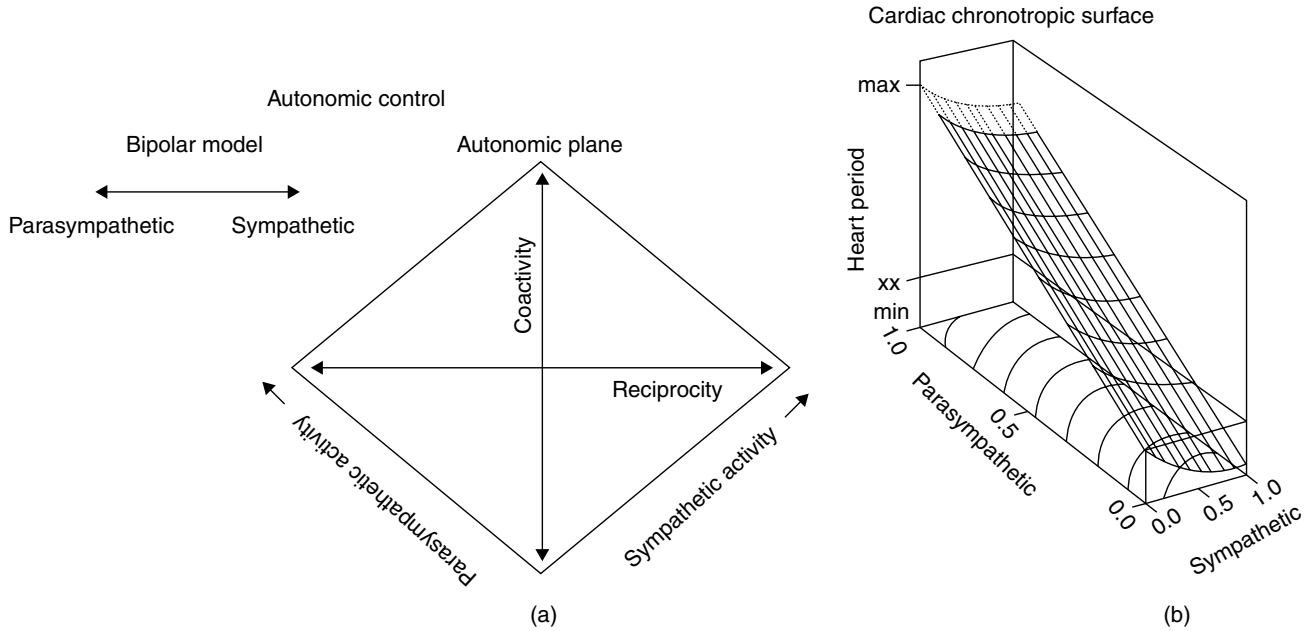

Figure IX.3 Autonomic space. (a) Bipolar versus bivariant representations of sympathetic and parasympathetic control. (b) Bivariate autonomic plane and associated chronotropic effector surface. The plane bounded by the parasympathetic and sympathetic axes represents all possible combinations of sympathetic and parasympathetic activities (on a relative scale from 0 to 1). The effector surface overlying the autonomic plane represents the chronotropic state of the target organ, expressed in heart period, for all loci within autonomic space (see Berntson *et al.*, 1993b for details of derivation). Parasympathetic activation exerts relatively linear effects on the heart period, whereas sympathetic activation yields somewhat nonlinear effects. The dotted surface at maximal levels of parasympathetic activation represents the ambiguity in effects at this extreme. For illustrative purposes, the lengths of the axes are scaled in proportion to relative dynamic ranges of control of the autonomic branches; Beta represents the 'intrinsic' heart period in the absence of autonomic input. The curved lines on the autonomic plane represent isoeffector contours projected from the effector surface on to the autonomic plane. These contour lines illustrate loci on the autonomic plane that yield equivalent chronotropic effects. Adapted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 467, with permission from Cambridge University Press

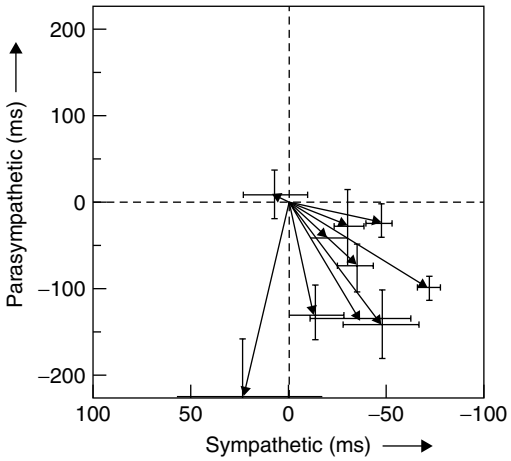

Figure IX.4 Individual patterns of response to psychological stress depicted on the autonomic plane. The intersection of the dotted lines in the centre of the graph represents the basal resting state, and the arrows depict the individual response vectors along the sympathetic and parasympathetic axes, with the peak response at the arrowheads. Vectors were derived from independent estimates of the contributions of the autonomic branches under selective pharmacological blockades. Note that some subjects responded primarily with parasympathetic withdrawal, some primarily with sympathetic activation, and some with reciprocal sympathetic activation and parasympathetic withdrawal. These individual response vectors were stable. Three stressors were used (mental arithmetic, reaction time task, speech stressor), and the error bars at the tip of each response vector illustrate the standard errors of the response across the three tasks. Units on the axes are in milliseconds of heart period. Adapted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 470, with permission from Cambridge University Press

considerations are important because they bear on the probable complexity of psychophysiological relations in behavioural contexts.

Fear, Anxiety and Autonomic Control

The importance of multilevel analysis in psychophysiology is illustrated by recent work on anxiety and autonomic control. Anxiogenic or fear-eliciting contexts are often associated with robust autonomic responses, and the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) recognizes abnormal visceral reactivity as a common feature of anxiety disorders (American Psychiatric Association, 1994). The empirical research on the specific autonomic correlates of anxiety is complex, however, and the behavioural significance of the autonomic correlates of fear and anxiety remain uncertain (Berntson *et al.*, 1998; Cacioppo *et al.*, 1992). This is a consequential issue, because anxiety disorders represent a clear risk factor for cardiovascular disease and sudden cardiac death (Hayward, 1995; Kawachi *et al.*, 1994; Kubzansky *et al.*, 1998), and autonomic states may underlie these relations in part.

As considered above, fear- and anxiety-related stimuli may be processed at multiple levels of the neuraxis, which may contribute to the complexity of psychophysiological relations in anxiety states. Studies on the pharmacology of anxiety support this view. The classical antianxiety agents, the benzodiazepine receptor (BZR) agonists such as chlordiazepoxide (CPZ, Librium®), are positive γ-aminobutyric acid (GABA) modulators (increase GABA-gated chloride flux) and selectively reduce anxiety reactions with minimal effects on pain sensitivity. Conversely, BZR inverse agonists or partial inverse agonists, such as the beta carbolines, exert opposite actions at the GABA/BZR receptor complex (negative GABA modulators that decrease GABA-gated chloride flux) and have been shown to have potent anxiogenic-like actions in a number of paradigms (see Berntson *et al.*, 1998). In uncontrolled human studies, for example, BZR partial inverse agonists have been reported to induce severe anxiety, fear, restlessness, and striking autonomic reactivity (Dorow *et al.*, 1983; Gentil *et al.*, 1989).

In a series of studies, we found that the BZR partial inverse agonist FG 7142 selectively enhances the behavioural and autonomic (cardioacceleratory) defensive-like responses to contextually conditioned stimuli but not to a simple conditioned fear stimulus (Berntson *et al.*, 1998; Stowell *et al.*, 2000). Because the effects of FG 7142 interact with the behavioural context, they cannot be considered to reflect a direct drug-induced state. Rather, this compound appears to selectively facilitate a specific class of processing. This selectivity may be due to differences in requisite levels of processing underlying contextually and explicitly conditioned stimuli. Simple fear conditioning entails a close temporal association between an explicit fear conditioned stimulus and an aversive unconditioned stimulus, and can be mediated at the level of the amygdala. In contrast, contextual conditioning entails an aversive reaction to more generalized and pervasive cues, more reminiscent of clinical anxiety, and has been shown to depend on cortical processing.

In this regard, FG 7142 has been shown to enhance cortical processing, and its anxiogenic effects appear to arise in part from this action. The effects of BZR agonists and (partial) inverse agonists on anxiety are mediated in part by modulations of the basal forebrain cortical cholinergic system, which constitutes the primary source of cholinergic innervation of the cortex (see Berntson *et al.*, 1998; Hart *et al.*, 1999; Stowell *et al.*, 2000). This system constitutes an important regulator of cortical activation and cortical/cognitive processing, and atrophy of this system underlies in large part the cognitive decline and dementia of Alzheimer's disease (Sarter, 1994). Because the processing of contextually conditioned stimuli is cortically dependent, it would be expected to be enhanced by FG 7142. In contrast, simple conditioned fear stimuli can be processed subcortically at the level of the amygdala, and consequently may be less subject to modulation by FG 7142. Thus, FG 7142 appears to selectively promote the cortical/cognitive processing of, and autonomic reactivity to, more anxiogenic stimuli (see Berntson *et al.*, 1998). This is consistent with emerging cognitive theories of anxiety (e.g. Eysenk, 1991; Gray and McNaughton, 2000).

Activity of the basal forebrain cholinergic system is normally regulated in part by inhibitory GABAergic inputs, which serve to dampen ongoing activity. Moreover, this GABAergic inhibition is enhanced by BZR agonists and reduced by BZR inverse agonists or partial inverse agonists. Indeed, it is likely that the dampening effects of BZR agonists on cortical cholinergic activity underlie the clinical anxiolytic actions of these agents, and account for the well-known impairments in cognitive function attendant on use of these compounds. This is supported by the finding that the bidirectional modulation of anxiety-associated behaviours and autonomic reactivity is abolished by selective immunotoxic lesions of the basal forebrain cholinergic neurons (Stowell *et al.*, 2000).

A model of selected components of central systems underlying fear, anxiety and autonomic control is illustrated in Figure IX.5(a). An important feature of this model is that autonomic responses are determined by multiple processes and at multiple levels of the neuraxis. Consequently, it is not surprising that psychophysiological relations are complex, especially when indexed by peripheral visceral responses that represent the final common pathway for autonomic reactions arising from varied levels of processing. A meaningful understanding of these relations will require attention to the multiple levels of neural processing and the physiological, behavioural and social factors that impact these processes.

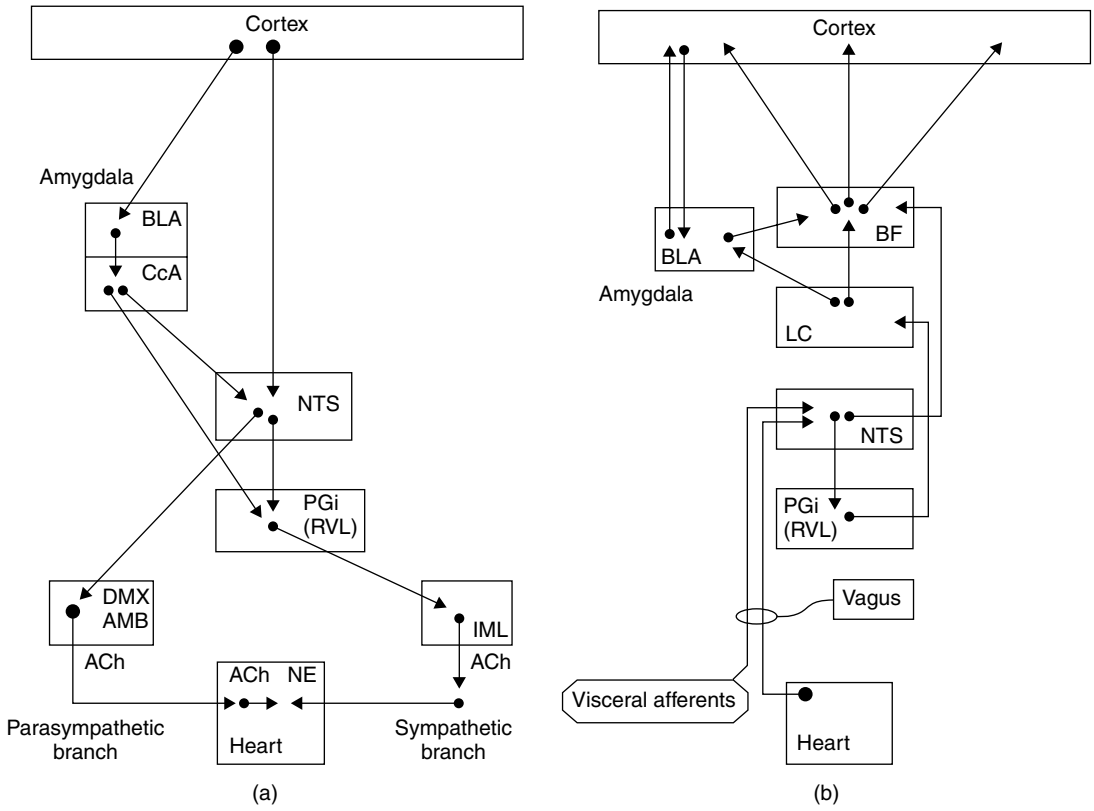

Figure IX.5 Anxiety and autonomic control. (a) Descending branch of an anatomical model of neuronal substrates by which cortical/cognitive processes may contribute to the development and expression of anxiety and its autonomic features. (b) Ascending branch of an anatomical model of neuronal substrates by which cortical/cognitive processes may contribute to the development and expression of anxiety and its autonomic features. Ascending pathways illustrate the potential routes by which sympathetic activity and visceral afference may modulate rostral systems. The model is not intended to present an anatomically complete description of relevant circuits and transmitters; rather, it is conceptually driven and focuses on hypotheses derived in part from experimental evidence (see Berntson *et al.*, 1998). Adapted from *Behavioural Brain Research*, Volume 94, Berntson, G.G., Sarter, M. and Cacioppo, J.T. Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link, 2000, p. 236–237, with permission from Elsevier Science. ACh, acetylcholine; AMB, nucleus ambiguous; BF, basal forebrain; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; DMX, dorsal motor nucleus of the vagus; IML, sympathetic preganglionic neurons of the intermediolateral cell column; LC, locus coeruleus; NE, noradrenaline; NTS, nucleus tractus solitarius; PGi, nucleus paragigantocellularis; RVL, rostral ventrolateral medullary pressor area

From Efferent Processes to Reciprocal Interactions

Much early work in psychophysiology focused on the efferent side, with a primary emphasis on peripheral effector systems and patterns of physiological reaction to psychological states and processes. Although limited in perspective, this prevailing focus on outputs fostered important theory and research on the determinants of autonomic response patterns. Early theoretical models ranged from generalized autonomic and neuroendocrine reactions to stress and arousal (Cannon, 1936; Selye, 1956) to a bipartite focus on stimulus or situational determinants on the one hand and individual response dispositions on the other (stimulus-response specificity versus individual response stereotypy; e.g. Lacey and Lacey, 1958).

However important they are, efferent processes represent only one aspect of psychophysiological relations, and there were early exceptions to the predominant efferent focus in psychophysiology. A notable example was the James and Lange conception of the role of visceral feedback in emotion. In contrast to Cannon's (1927) thalamic theory, in which separate thalamic output pathways were considered to underlie emotional behaviour (via descending projections) and emotional feeling (via cortical projections), James (1884) considered strong emotion to reflect the perceptual consequence of afferent feedback from somatic and visceral responses. Based in part on the effects of adrenaline priming on emotional reactions, Schachter and Singer (1962) later proposed an alternative view that specific emotions arise from cognitive appraisals initiated by the perception of a rather undifferentiated physiological arousal. Although these theories have been challenged on both empirical and conceptual grounds (e.g. Cacioppo *et al.*, 1992; Reisenzein, 1983), it does appear that somatovisceral afference can prime or bias affective reactions (for review, see Cacioppo *et al.*, 1992). Somatic flexion and extension responses have been shown to bias evaluative judgments (Cacioppo *et al.*, 1993), and adrenaline or other visceral priming has been shown to enhance emotional memories in humans and rats (Ferry *et al.*, 1999; McGaugh *et al.*, 1993).

There are ample routes by which visceral afference can modulate higher neural systems. The vagus nerve and its terminal site in the NTS has been shown to be a critical pathway for the visceral priming of emotional memories, as this priming is blocked by either vagotomy or NTS lesions (Ferry *et al.*, 1999; McGaugh *et al.*, 1993; Williams and McGaugh, 1993). Figure IX.5(b) illustrates a proposed ascending model of central systems involved in anxiety and autonomic control (Berntson *et al.*, 1998). A important component of this model is the paragigantocellular nucleus (PGi). Activity of the PGi is closely related to the state of sympathetic activity, as it receives direct afferent projections from the NTS and is partially coextensive with the rostral medullary pressor area that

contributes direct projections to the sympathetic motor neurons in the intermediolateral column of the cord (Aston-Jones *et al.*, 1996). The PGi also projects extensively to the noradrenergic neurons in the locus coeruleus (LC), which is known to be highly activated in emotional states (Aston-Jones *et al.*, 1996). The LC, in turn, issues ascending noradrenergic projections to the amygdala and cerebral cortex, the former being particularly important for visceral memory priming (Clayton and Williams, 2000). As illustrated in Figure IX.5, the LC also has an additional route of access to cortical systems via an excitatory projection to the basal forebrain cholinergic system (Berntson *et al.*, 1998). This may be an important route by which visceral feedback can activate cortical processing systems, including those of the medial prefrontal cortex that have been implicated previously in anxiety and autonomic control (see Berntson *et al.*, 1998; Hart *et al.*, 1999). Indeed, we have speculated that this may be a conduit by which visceral activity is able to trigger panic attacks via a bottom-up activation of rostral systems.

It is now clear that, in addition to traditional afferent sensory systems, there are important neural and chemical signals emanating from the periphery that can exert powerful control over the operations of central networks. Even the 'sickness response' (lethargy and behavioural depression, anorexia, fever, etc.) to the presence of foreign antigens now appears to be triggered in part by a lymphocyte-derived peripheral cytokine (interleukin 2), which triggers a critical, vagally mediated signal to the brain (Maier and Watkins, 1998). Contemporary psychophysiology is increasingly cognizant of the reciprocal interactions between central and peripheral processes.

From Global to Specific Influences on Mind and Body

An additional trend that characterizes the historical development of contemporary psychophysiology is the shift from conceptual guidance by nonspecific, unitary or global influences on mind and body to more realistic and useful models that include complex and often interacting sets of more specific determinants. An example derives from the discussion above of the homeostatic model of autonomic function and regulation. Although the ANS may play an important role in maintaining the internal environment within limits compatible with life, the feedback-regulated, homeostatic model of autonomic control is far too restrictive. Rather, as discussed above, autonomic adjustments are far more flexible, and may entail conditioned adjustments that anticipate and thereby precede perturbations (Dworkin, 1993), may exceed metabolic demands (Obrist, 1981; Turner, 1994), and may reflect an active suppression of homeostatic reactions such as stress-related inhibition of baroreflexes (Steptoe *et al.*, 1996; Nosaka, 1996), allowing the conjoint increase in both blood pressure and heart rate often observed during stress. In each of these cases, the autonomic adjustment may be highly adaptive, but they are not homeostatic. Rather than a generalized homeostatic pattern of response, the ANS is capable of a much wider range of context-specific adjustments. This is important because models of physiological regulation shape concepts of health and disease.

In keeping with early global models, Selye (1956) proposed a generalized pattern of autonomic and neuroendocrine response to stress, the *general adaptation syndrome* (GAS), which was considered to reflect a uniform pattern of reaction to stress. Although autonomic and adrenocortical responses were seen to have distinct temporal dynamics, even these differences were thought to be consistent across individuals and stress conditions. The global features of the GAS, in turn, fostered a relatively simplistic peripheral model of psychosomatic disease (diathesis stress model), in which distinct disorders to a common stress-reaction were considered to reflect specific end-organ vulnerabilities. Thus, although Selye acknowledged individual differences in stress-related disorders, these differences were considered to arise largely from peripheral end-organ dispositions.

Other early researchers considered individual differences not only in psychosomatic symptomatology but also in the individual pattern of autonomic response to a given stressor. Eppinger and Hess (1915) observed that some individuals tend to show larger and more pervasive sympathetic reactions (sympathicotonics) whereas others display more parasympathetic-like responses (vagotonics) to the same set of challenges. For Eppinger and Hess, it was the difference in autonomic response disposition, rather than end-organ vulnerabilities, that disposed toward distinct patterns of psychophysiological disorder. Accordingly, vagotonics were more likely to display asthma whereas Reynaud's disease was more frequent in sympathicotonics. Moreover, different evocative stimuli or psychological states were also found to yield differentiated patterns of autonomic response (Ax, 1953; Wolf and Wolff, 1947). These early studies spearheaded an extensive literature documenting multiple determinants of autonomic response, including complex patterns of individual response dispositions that do not fall along a simple sympathetic–parasympathetic dimension (individual response stereotypy), as well as stimulus or situational determinants (stimulus-response specificity) associated with orienting, defensive and other contextually-specific reactions (Graham and Clifton, 1966; Lacey and Lacey, 1958).

An additional example comes from the literature on activation and arousal. In the first half of the twentieth century, Cannon emphasized the activational role of generalized sympathetic discharge in mobilization for emergency reactions. Elizabeth Duffy (1951) focused attention on the role of activation as a determinant of motivation, emotion and performance, and suggested that this construct could be indexed by autonomic and electroencephalographic measures. This work spearheaded the widespread application of concepts of arousal and activation in psychology, and generalized arousal became a central organizing theme in psychophysiology. Although it was recognized that the relationship between activation and performance may not be simple or monotonic (e.g. the inverted U function; Hebb, 1955), it was thought that the unitary dimension of activation exerted a pervasive impact on cognition and behavior (Lindsley, 1957). Contributing to the notion of a generalized dimension of activation were rapidly emerging developments on the role of the brainstem reticular formation and the ascending reticular activating system (ARAS) in cortical and behavioural arousal (Lindsley, 1957; Magoun, 1960). The powerful influence that the construct of general arousal had on psychology and psychophysiology was due in part to the promise that the ARAS concept held as a fundamental link between physiology and behaviour. Partly because of this appeal, the concept of general arousal survived despite the fact that such a unitary dimension was belied by early recognition of distinct attentional processes and distinctions between phasic and tonic arousal (Pribram and McGuinness, 1975), as well as by lesion-induced dissociations of cortical and behavioural arousal (Feldman and Waller, 1962).

Neither psychophysiological nor behavioural measures of arousal correlate very highly across contexts, and it is now clear that arousal and attention are multidimensional phenomena (Cacioppo *et al.*, 1996; Neiss, 1988). This does not undermine the early hope of relating behavioural processes to specific neural systems. Indeed, the concept of a general ARAS is also no longer tenable from a neurobiological standpoint, as recent work has documented multiple and differentiated ascending systems (adrenergic, cholinergic, dopaminergic, serotonergic) underlying sleep, arousal and attention (Robbins *et al.*, 1998; Sarter and Bruno, 2000; Hobson *et al.*, 2000). Moreover, increasingly it has been possible to relate specific ascending neural systems to particular aspects of attention and performance (Robbins *et al.*, 1998; Sarter and Bruno, 2000). Of these multiple systems, perhaps the two related most closely to the original ARAS concept are the activational influences of the ascending noradrenergic systems and the 'amplifying' effects of the basal forebrain

cortical cholinergic system on cortical processing (Robbins *et al.*, 1998; Sarter and Bruno, 2000).

CONTEMPORARY PSYCHOPHYSIOLOGY

As a discipline, psychophysiology has advanced and matured considerably over the past several decades. Of particular importance in this development were the historical trends outlined above, which served to position psychophysiology as a critical hub discipline between psychology, psychiatry and the neurosciences. Also influential has been the expanding recognition of the complex interactions between psychological processes and a range of systems, such as the immune system, in addition to traditionally recognized links with the autonomic and hypothalamic-pituitary-adrenal (HPA) systems. Of further impact have been the dramatic development of sophisticated methods and measures, especially those related to the brain, that allow meaningful studies of these interactions. Especially consequential have been the growing interdisciplinary collaborations between psychophysiology and behavioural and cognitive neuroscience, neuropsychology, endocrinology, neurochemistry, immunology, molecular biology and behavioural genetics.

At present, it is difficult to distinguish clearly the work of many contemporary psychophysiologists from other neuroscientists. Psychophysiology is no longer limited to behavioural independent variables and peripheral physiological dependent measures. Psychophysiologists are as likely to measure central events and processes as peripheral physiological changes, and they increasingly employ psychological states and processes as dependent as well as independent variables. Moreover, with the increasing understanding of the integration among multiple behavioural and physiological systems, there has been a considerable expansion of the scope of psychophysiology into areas such as psychoneuroimmunology, neuropsychology and psychiatry. What continues to characterize psychophysiology is its abiding interest in psychological processes, and how the understanding of these processes can be informed through multilevel analysis of the interactions between psychological and physiological systems. This includes the manner in which psychological processes are implemented in, and in turn impact on, physiology.

In summary, what characterizes contemporary psychophysiology is its emphasis on reciprocal relations between psychological and physiological domains, a focus on interactions among systems (e.g. behavioural, endocrine, autonomic, immune) and levels of neurobehavioural organization (e.g. reflexive, affective, cognitive), and an interest in explicating higher-level psychological processes. As a critical bridge discipline, psychophysiology is particularly relevant for psychiatry.

SOME APPLICATIONS OF PSYCHOPHYSIOLOGY IN PSYCHIATRY

Clinical issues were often central in the early history of psychophysiology, and a clinical focus within the discipline continues in the contemporary literature. In this section, we consider three examples of psychophysiological approaches in clinical psychiatry, and see how these approaches have contributed to theory and research. The following discussions are not intended to be comprehensive or exhaustive, but rather to illustrate the utility of psychophysiological perspectives and multilevel analysis.

Anxiety

As discussed above, fear- and anxiety-related stimuli can be processed at multiple levels of the neuraxis, each with distinct access to autonomic source nuclei and regulatory systems of the brainstem and cord. An additional complexity is that simple fear may be primarily an emotional response, whereas anxiety is characterized by salient cognitive components. These considerations, together with the wide range of anxiety conditions, individual response stereotypy, and stimulus-response specificity in autonomic reactions, preclude a simple psychophysiological mapping of autonomic states and reactions in clinical anxiety. In anxiety conditions where there is a focal evocative stimulus or event, such as specific phobias or post-traumatic stress disorder (PTSD), a relatively selective enhancement of autonomic reactivity to the target stimulus is often observed (Davidson *et al.*, 2000; McNeil *et al.*, 1993; Pitman *et al.*, 1999). In some cases, this may reflect processing in subcortical systems. Backwardly masked visual fear stimuli can trigger exaggerated autonomic responses in phobic subjects, and can support conditioned fear responses, despite being unrecognized (Ohman and Soares, 1994; Ohman and Soares, 1998). In accord with animal research, PET studies have suggested that these reactions are mediated by a subcortical pathway entailing the superior colliculus, pulvinar and right amygdala (Morris *et al.*, 1999). In contrast, more cognitively mediated anxiety-like reactions appear to be dependent on cortical systems (Berntson *et al.*, 1998; Lang *et al.*, 2000; McNaughton and Gray, 2000; Morris *et al.*, 1999). As might be expected, psychophysiological mappings, like behavioural relations, become more complex with the involvement of higher-level processing systems. Anxiety patients may show either increases or decreases in basal and reactive autonomic states, or they may show more complex patterns of response among autonomic measures (see Berntson *et al.*, 1998). A decrease in high-frequency heart rate variability (respiratory sinus arrhythmia), an index of vagal tone and vagal reactivity, has been reported to be diminished in a number of anxiety conditions, including general anxiety disorder, panic disorder, and blood phobia (Thayer and Lane, 2000). This and other findings have led to the suggestion that anxiety may be associated with a diminished physiological variability and flexibility (Thayer and Lane, 2000; Hoehn-Saric and McLeod, 2000).

From a psychiatric perspective, however, a more relevant issue might be the functional consequences of autonomic and neuroendocrine reactions for psychological states. At the extreme, it has been suggested that frank neural degeneration resulting from excess glucocorticoids may underlie PTSD (Bremner, 2001; Pitman, 2001), although questions remain concerning this hypothesis. Prolonged stress has been shown to result in glucocorticoid-dependent hippocampal degeneration in animals, and several imaging studies have reported a reduced hippocampal volume, as well as memory impairments that might be expected from such damage, in PTSD (Bremner, 2001; Sapolsky, 2001). However, it remains unclear whether the smaller hippocampal volumes in PTSD are due to enhanced cortisol levels or even whether they are related causally to PTSD or its symptoms (McEwen, 2001; Pitman, 2001; Yehuda, 2001). This is an important area for further study, and the implications of the neurotoxicity hypothesis, if confirmed, would be substantial for our understanding of psychiatric conditions.

Regardless of the resolution of this issue, evidence for other feedback effects of autonomic or neuroendocrine responses is growing. The effects of glucocorticoids on multiple brain systems is well established, and reciprocal relations between the brain and adrenal cortical hormones has been studied extensively. Glucocorticoids, for example, have complex and dose-dependent effects on fear and anxiety via multiple actions at central glucocorticoid and mineralocorticoid receptors (Korte, 2001). In addition, brain corticotropic releasing hormone (CRH) systems appear to play a central role in the orchestration of behavioural, autonomic and neuroendocrine responses to stress, and glucorticoids modulate these systems by regulating a CRH-binding protein (Behan *et al.*, 1996; Heim and Nemeroff, 1999). Recent developments suggest an important role for central CRH systems in anxiety. CRH acts on two receptor

populations, Crhr1 and Crhr2, with the Crhr1 receptor type primarily responsible for the stress-like and anxiogenic actions of CRH (Heim and Nemeroff, 1999; Owens and Nemeroff, 1999). In view of these considerations, and evidence of CRH neuronal hyperactivity in affective disorders and anxiety, Crhr1 antagonists are currently undergoing clinical trials to determine their clinical efficacy in patients with affective and anxiety disorders (Owens and Nemeroff, 1999).

As illustrated in Figure IX.5, there are also ample ascending routes by which the peripheral autonomic states can influence higher neural systems. Although it is no longer tenable to maintain that emotional feeling is merely the perceptual consequence of somatovisceral feedback, there are a variety of ways in which visceral afference may impact emotional reactions. Even a rather generalized pattern of autonomic reaction may contribute to specific emotional states by priming or biasing neural processing, or by a 'visceral illusion' process akin to the distinct percepts that are possible with visual ambiguous figures (Cacioppo *et al.*, 1992). Empirically, it has been shown that post-training visceral activation by systemic adrenaline can enhance emotional memories in rats (Williams and McGaugh, 1993; Croiset *et al.*, 2000) and increase pain sensitivity in humans (Janssen *et al.*, 1998). Moreover, adrenaline administration has been reported to trigger panic attacks in human subjects (van Zijderveld *et al.*, 1999). As this effect was not related to anxiety sensitivity or the fear of autonomic reactions, it did not simply reflect a perceptual consequence of visceral activity (Veltman *et al.*, 1998). As illustrated in Figure IX.5, a final common pathway by which visceral afference may enhance anxiety is the basal forebrain cortical cholinergic system (see Berntson *et al.*, 1998). The importance of this system in anxiety is supported further by the report that anxiolytic actions of the BZR agonist, chlordiazepoxide, are mediated at least in part via an inhibition of this system (Stowell *et al.*, 2000). Moreover, Crhr1 receptors that underlie the anxiogenic actions of CRH have been identified on basal forebrain cholinergic neurons. Together, these findings support the view that basal forebrain cholinergic systems may enhance anxiety by promoting the cortical processing of threat- and anxiety-related stimuli.

In summary, psychophysiological approaches to the study of anxiety can contribute to our understanding of the nature of anxiety. Moreover, conceptual models derived from multilevel analysis and psychophysiological studies can help to elucidate the neurobiological bases of anxiety, and contribute to the development of more effective pharmacological and behavioural treatment strategies.

Schizophrenia Spectrum Disorders

There has been a substantial history of psychophysiological studies of schizophrenia. Although much remains to be learned about this spectrum of disorders, a broad albeit sketchy conceptualization of this disorder is emerging from the integration of multidisciplinary data and perspectives. It is now recognized that among the central cognitive disturbances in schizophrenia are disorders of attention (for reviews, see Braff, 1993; Gray, 1998; Keller *et al.*, 2000; Sarter, 1994; Sarter *et al.*, 2001). Deficits in sensory or attentional 'gating' have been reported regularly in pre-pulse inhibition paradigms with measures that include acoustic-event-related potentials (e.g. P50) and electromyographic startle responses (Dawson *et al.*, 2000; Keller *et al.*, 2000; Light and Braff, 1999), and neuroleptic medication may normalize gating deficits (Weike *et al.*, 2000). Additional studies document high basal levels of autonomic variables, low reactivity, and slow habituation of autonomic responses (Zahn, 1988; Zahn *et al.*, 1997).

Slowed habituation is consistent with a gating or filtering deficit, although the low level of autonomic reactivity is more consistent with additional findings suggesting deficient attentional processing in schizophrenia, as indicated by smaller evoked potentials, or by behavioural measures (Keller *et al.*, 2000; Knight, 1993). Other studies suggest a deficiency in working memory in schizophrenia (Keller *et al.*, 2000; Selemon and Goldman-Rakic, 1999; Perlstein *et al.*, 2001). The latter is in accord with indications of 'hypofrontality' or dysfunction in schizophrenia, as indicated by structural, functional brain imaging, and behavioural measures (Callicott *et al.*, 2000; Hazlett *et al.*, 2000; Perlstein *et al.*, 2001; Selemon and Goldman-Rakic, 1999; Staal *et al.*, 2001).

These and other psychophysiological approaches have been highly significant in defining the cognitive and behavioural features of schizophrenia. In view of the above findings, additional work will be needed to characterize more precisely the nature of the cognitive processing in schizophrenia spectrum disorders. Differences in the literature on deficits in gating, attentional failures and working memory may be more apparent than real. Inability to selectively filter or disattend may tax the processing capacity of working memory (as would a primary working memory deficit) and lead to attentional failures due to capacity limits, and any of these features may manifest in schizophrenia depending on the context (Sarter, 1994; Sarter *et al.*, 2001).

Psychophysiological studies have also been influential in highlighting potential neural systems underlying the cognitive and behavioural features of schizophrenia, and contribute importantly to the multilevel analyses that are essential in understanding the basic neurobiology and neuropsychology of schizophrenia. Indeed, multilevel interdisciplinary approaches are now beginning to clarify the links between underlying neuropathology and the cognitive features of schizophrenia. Because of the therapeutic efficacy of dopamine receptor (D2) antagonists, schizophrenia has often been considered to reflect a disturbance in dopamine systems. Dopamine releasers such as amphetamine or cocaine can trigger or exacerbate schizophrenic symptoms, and specialized single positron emission computerized tomography (SPECT) and PET brain imaging techniques have revealed sensitized mesolimbic dopaminergic systems and enhanced amphetamine-induced dopamine release in schizophrenics (Laruelle, 2000; Kugaya *et al.*, 2000). Phencyclidine (PCP) and other antagonists of *N*-methyl-D-aspartate (NMDA) glutaminergic receptors, however, may provide an even better model of schizophrenia than amphetamine, especially for both positive and negative symptoms (Jentsch and Roth, 1999). These and other findings lead to hypoglutaminergic models of schizophrenia. Additional results have suggested a hypercholinergic state in schizophrenia. Amphetamine and dopamine agonists can both enhance cortical acetylcholine release, schizophrenic patients have been reported to show abnormal acetylcholine receptor density in the prefrontal cortex, and potentiation of the basal forebrain cortical cholinergic system may provide the closest model of many of the cognitive features of schizophrenia (Crook *et al.*, 2000; Sarter, 1994; Sarter *et al.*, 2001). These seemingly disparate findings are beginning to be organized by more integrative, multilevel theories and models.

Although there is considerable evidence implicating altered dopamine function in schizophrenia, this may not be the primary disturbance. Based on extensive studies and integrative reviews of the literature, a primary cortical disturbance has been proposed in schizophrenia, one consequence of which is a disturbance in the (glutaminergic) cortical regulation of subcortical dopamine systems (Grace, 2000; Moore *et al.*, 1999). Reduced tonic glutaminergic driving of dopamine systems is hypothesized to result in dopamine upregulation and enhanced phasic dopamine driving, which in turn can enhance cholinergic activity by disinhibiting the basal forebrain cortical cholinergic system (Sarter, 1994; Sarter *et al.*, 2001). While much work remains to be done in this area, the emerging schema organizes a wide range of otherwise disparate findings extending from the anatomy (e.g. hypofrontality), to the neuropharmacology (dopaminergic, glutaminergic, cholinergic), to the psychological

features (attentional and working memory impairments and frontal lobe symptoms) of schizophrenia.

What has been important to the progress in this area is the integration of information derived from multiple levels of analysis, which provides the empirical and conceptual depth to allow meaningful hypotheses and constructive theorizing. Thus, although atypical antipsychotic compounds may not have the dopamine-blocking effects of the conventional antipsychotics, they do share a common effect of attenuating cholinergic activity, which may account for their therapeutic benefit on cognitive and attentional processing in schizophrenia (Sarter, 1994; Sarter *et al.*, 2001). We can look forward to additional genetic and molecular biological contributions to our understanding of schizophrenia, but molecular biology alone can never yield a comprehensive account of schizophrenia or any other psychiatric condition. For this, multilevel analyses are required, and psychophysiological approaches can play an important role in these analyses.

Summary

The examples above illustrate some applications of psychophysiology in psychiatry, and how such approaches may inform psychiatric issues. Psychophysiological methods can provide a unique perspective, although as with any approach there are limits to the inferences that can be derived. Consequently, psychophysiological approaches are most useful in multilevel analyses, where they can provide information that complements and converges with that derived from other methods and levels of analysis. Below, we consider some of the features of psychophysiological methods and designs, and the logic of psychophysiological inference.

PSYCHOPHYSIOLOGICAL INFERENCE

Psychophysiology seeks to elucidate the reciprocal relations between mind and body, including the highest-level processes of language, symbolic representation and problem solving. In psychophysiology, 'the emphasis is on integrating data from multiple levels of analysis to illuminate psychological functions and mechanisms rather that physiological structures *per se*' (Cacioppo *et al.*, 2000b). Methodologically, this may entail measures of behavioural, physiological or neural activity in psychological contexts. Important advances continue to be made in measurement systems, and current devices for neural and physiological recording are often highly sophisticated and precise. Regardless of measurement precision, the question arises as to how signals are to be interpreted and inferences drawn. Generally, physiological measures are of interest primarily to the extent to which they allow one to index a psychological process.

One common approach in the literature is the subtractive method, in which experimental and control or contrast conditions differ by one element, stage or process (Cacioppo *et al.*, 2000b). Outcome differences between the experimental and control conditions are then considered to reflect the impact of that single component. In this fashion, physiological indices of particular psychological processes can be identified, and within a hypothetico-deductive framework, concepts and theories can be developed and tested. This approach, for example, allowed Helmholtz to determine the propagation velocity in nerves by the difference in reaction times to a somatic stimulus delivered at a proximal and a more distal site on the arm. The assumption was that all aspects of the task and neural processing were identical, except for the extra component associated with the longer distance the neural signal must travel when the stimulus was presented distally. Knowing the difference in reaction times and the distance between the stimulus sites, we can derive the propagation velocity (dx/dt).

A contemporary example comes from brain imaging studies, in which the difference in the neural maps under experimental and control conditions are interpreted to reflect the activity uniquely associated with the experimental condition. If, under a sufficient set of conditions, a consistent difference is apparent that comports with a model or hypothesis (e.g. amygdala activation mediates aversive reactions to pain), then the results provide some support for the hypothesis. This approach does not allow a strong inference, however. The confidence in such an interpretation would be enhanced if the particular pattern (amygdaloid activation) was shown to arise consistently under a wide range of pain conditions and could not be attributable to some other aspect of the contexts (i.e. well-designed control conditions). Even then, amygdaloid activation could not be used to infer the presence of pain or aversion, as other emotional states may yield the same result. Moreover, even if amygdala activation was shown to be highly specific to pain conditions, its causal role in that psychological state would need to be confirmed by converging evidence (e.g. amygdaloid stimulation or inactivation, anatomical connections, etc.). In fact, converging evidence is always important because the subtractive method assumes that components or processes can be inserted or deleted without altering other components or processes, and that may not always be true. We will return to this example below.

More generally, the question arises as to how we are to understand psychophysiological relations. Below, we present a framework for thinking about such relationships (for further development, see Cacioppo and Tassinary 1990; Cacioppo *et al.*, 2000b). We further outline the rules of evidence for, and limitations to, psychophysiological inference.

Psychophysiological Relations

Psychophysiological relationships are relations between two general domains: the psychological domain, which can be represented as comprised of a set of elements ψ, and the physiological domain, which we can represent as the set ϕ. Figure IX.6 illustrates the logical relations between elements in these two domains. All psychological elements in the set ψ are assumed to have some physiological referent or physical substrate.

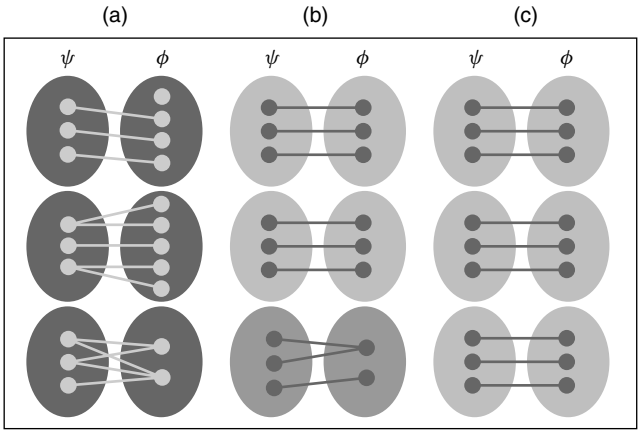

Figure IX.6 Logical relations among elements in the psychological (ψ) and physiological (ϕ) domains. (a) Relations between ψ elements and individual physiological responses. (b) Relations between ψ and physiological response patterns. (c) Relations between ψ elements and profiles of physiological response across time. Reprinted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 13, with permission from Cambridge University Press

Figure IX.6 illustrates several types of relationships. First, there are elements within the physical domain that have no psychological referent (Figure IX.6a, top row). This represents psychologically irrelevant sources of variance in physiological measures (e.g. temperature effects on electrodermal activity or postural effects on heart rate). These are important because they constitute a source of variance or artefact in physiological measures that needs to be controlled or accounted for. This is accomplished by careful recording methods, good research designs, and rigorous experimental controls. In the ideal case, this effectively eliminates the impact of these elements on the results (Figure IX.6b, top row).

As illustrated in Figure IX.7, there are five general relations that could characterize the mapping between elements in the sets ψ and ϕ:

- *One-to-one*, in which an element in ψ is associated with one, and only one, element in ϕ.
- *One-to-many*, in which elements in ψ are related to more than one element in ϕ.
- *Many-to-one*, in which two or more ψ elements are associated with a single element in ϕ.
- *Many-to-many*, where two or more ψ elements are associated with overlapping ϕ elements
- *Null*, in which there is no association between elements in ψ and ϕ.

Of these relations, only the first and third allow logical inferences concerning psychological states or processes (ψ) from physiological measures (ϕ). This is important, because psychophysiological research often entails manipulation or blocking of elements in the psychological domain and measurement of elements within the physiological domain. Consequently, the basis for theoretical interpretations in psychophysiology can be enhanced if relationships between ψ and ϕ could be specified in terms of one-to-one or even many-to-one.

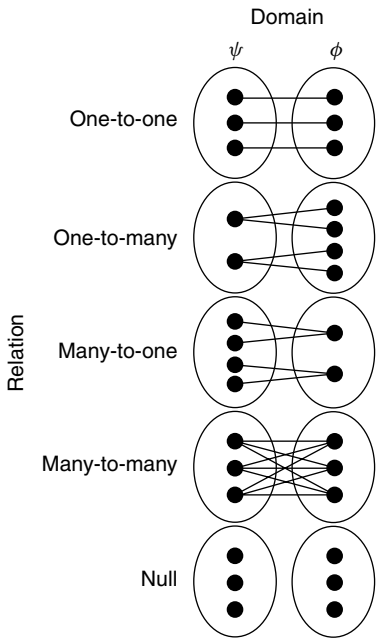

Figure IX.7 Possible relationships between elements in the psychological (ψ) and physiological (ϕ) domains. Reprinted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 13, with permission from Cambridge University Press

Unfortunately, one-to-one relationships that allow strong inference are not common in psychophysiology. One approach to this problem is to recast the ϕ elements of a one-to-many relationship into a higher-order syndrome (ϕ') that is comprised of a combination or pattern of ϕ elements (Figure IX.6b). In this fashion, a one-to-one relationship may be identified between a psychological element and a pattern of physiological responses.

Similarly, the many-to-many relations may be simplified by reconceptualizing the physiological elements into a syndrome or profile of physiological responses (Figure IX.6b). This serves to simplify a many-to-many relation to a many-to-one (or even a one-to-one) relation. Adding additional dimensions (ϕ'') to the definitions of the physiological elements or profile (e.g. amplitudes, temporal dynamics, etc.) may allow further simplification to a one-to-one relationship (Figure IX.6c). Complex psychophysiological relationships, however, may not always be reducible to a one-to-one or even a many-to-one relation.

Psychophysiological Inference

When a psychological factor serves as the independent or blocking variable and a physiological factor the dependent variable, the experimental design can be conceptualized as examining the $P(\phi/\psi)$, or the probability of ϕ given ψ. In contrast, when a physiological factor serves as the independent or blocking variable and a psychological factor the dependent variable, the experimental can be conceptualized as investigating the $P(\psi/\phi)$, or the probability of ψ given ϕ.

For illustration, we return to our brain imaging study of pain and the amygdala. If differences in amygdala activation ϕ (assuming that the imaging method is actually measuring activation) are found during aversive reactions (ψ) to pain compared with control conditions, the data are often interpreted prematurely to indicate that ϕ (the amygdala activation) is associated with ψ (aversive reactions). These types of results are also sometimes treated as if ϕ were manipulated directly (amygdala activation as the independent variable) and ψ was measured (aversive reaction as the dependent variable). This form of interpretation, however, assumes an isomorphism between ψ and ϕ that may not be true (Sarter *et al.*, 1996). Causal hypotheses regarding the involvement of a brain structure (ϕ) in a psychological process (ψ) are of the form $\psi = f(\phi)$. This implies that the occurrence of ϕ (e.g. amygdala activation) is a sufficient condition for ψ (aversive reaction), but it does not imply that ψ is necessarily preceded by ϕ. Moreover, brain activity is often of interest as an index of a psychological state or process, and the desired inference is of the form $\psi = f(\phi)$ rather than not-$\psi = f$(not-ϕ). Consequently, the goal of psychophysiology may be characterized by the conditional probability of ψ given ϕ:

$$P(\psi|\phi) = 1$$

But imaging studies are generally of the form:

$$P(\phi|\psi) = ?$$

Psychophysiological measures provide information on ϕ as a function of ψ, but the conditional probabilities are equivalent $[P(\psi|\phi) = P(\phi|\psi)]$ only if there is a one-to-one relationship between the physiological element ϕ (e.g. amygdala activation) and the psychological element ψ (e.g. aversive reaction) (Cacioppo and Tassinary, 1990). This cannot be assumed but must be tested. In the present example of aversive reactions and the amygdala, the isomorphism does not exist, as aversive reactions do not depend on amygdala activation (although data discussed earlier suggest that conditioned aversive reactions may, and that redefining the element ψ

may be in order). In interpreting psychophysiological data, isomorphism between a psychological variable and a physiological variable (one-to-one relationship) must be tested, and deviations from isomorphism must be considered. In this regard, interdisciplinary, multilevel approaches are particularly useful as they can also provide complementary and converging evidence from direct manipulations of the physiological variables (ϕ as independent variable).

Categories of Psychophysiological Relationships

In addition to the mappings illustrated in Figure IX.7, psychophysiological relations can vary in terms of their generality, yielding four general classes of relations: outcomes, markers, concomitants and invariants.

A psychophysiological *outcome* is a many-to-one, situation-specific relation. If a physiological element (ϕ) is shown to vary as a function of a psychological element (ψ), then there is at least an outcome relationship among the variables. This is often the first level of findings in psychophysiology. If that relation can also be shown to be isomorphic (one-to-one), then it becomes at least a marker, whereas if it is shown to be independent of context, it is at least a concomitant. If it proves to be both isomorphic and context independent, then it is an invariant relationship.

The inferential power of an invariant is far greater than an outcome, but even an outcome relation can provide the basis for strong inference. For example, if two psychological theories predict different psychophysiological outcomes, then the outcome alone can serve as a test of those theories, based on hypothetico-deductive logic. An outcome relationship, however, does not allow one to draw inferences concerning the psychological state from the physiological measure. As an example, an increase in heart rate may have an outcome relation to the state of arousal, but an increase in heart rate alone cannot be interpreted as a reflection of an increase in arousal. As the probability of ψ given $\phi[P(\psi, \phi)]$ approaches 1, and the $P(\text{not-}\psi, \phi)$ approaches 0, then the element in the physiological domain becomes an ideal marker of the element in the psychological domain.

A psychophysiological *marker* is a context-specific, isomorphic relation between ψ and ϕ. Consequently, markers provide a valid indication of one element (often ψ) on the occasion of the other (often ϕ) within an assessment context. Markers may merely signal the occasion of an event in the other domain, or they may inform as to the amplitude or temporal features of the other event. To be established as a marker, an element in one domain must be shown to predict reliably an element in the other domain, and the boundary conditions for this relation must be identified. A heart rate decrease might be considered a concomitant of the orienting response within an experimental context that sufficiently controls other physical (e.g. temperature) and behavioural (e.g. activity, arousal) variables that can also alter heart rate.

A psychophysiological *concomitant* is a context-independent, many-to-one relationship between ψ and ϕ. It entails a cross-situational covariation between events in the two domains. It is less restrictive than an invariant, as it does not require a one-to-one relationship. Consequently, although a psychological event (e.g. aversive reaction to pain) may predict reliably the occurrence of a physiological event (amygdala activation), the presence of the amygdala activation event cannot be interpreted as a reflection of a pain reaction, as other psychological events may also produce activation of the amygdala.

Finally, a psychophysioloigcal *invariant* is a context-independent, isomorphic (one-to-one) relation between ψ and ϕ. This requires that the element ψ is present if, and only if, the element ϕ is present, and vice versa, across contexts. It is the only relation in which $P(\psi|\phi) = P(\phi|\psi)$ and $P(\text{not-}\psi, \phi) = P(\text{not-}\phi, \psi) = 0$. Invariant relations allow for strong inferences in psychophysiology. Unfortunately, however, invariant relations are sometimes assumed, rather than established, which can lead to specious interpretations and obfuscations. Although invariant relations afford the most powerful basis of inference, as considered above, some psychophysiological inferences can be derived with other categories of relation. It is crucial, however, to be cognizant of the differences.

CONCLUSION

Psychophysiology is the study of the relations between the mind and the body, or between psychology and physiology, hence it is extremely relevant to psychiatry. Its emphasis on multilevel analysis and the explication of psychological processes offers an important approach to the understanding and treatment of psychiatric disorders. The multilevel, interdisciplinary nature of psychophysiology is particularly viable in view of increasing documentation of extensive links and reciprocal interactions between psychological processes and autonomic, neuroendocrine and immune systems. The utility of psychophysiological approaches, however, is dependent on the recognition and application of the logic and principles underlying psychophysiological inference.

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X

Neuropsychology

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INTRODUCTION

Neuropsychology is the study of how the neurological organization of the brain influences the way people think, feel and act. Knowledge about the relationship between the brain and the mind is obtained from a broad array of techniques and from a range of different populations. Traditionally, neuropsychology weds a fine-grained behavioural assessment of individuals who have suffered brain damage with data that localize the anatomical location of such damage. With the advent of new brain-mapping methods, neuropsychological inquiry has expanded to include investigations that provide information on the location and/or time course of brain activity in either brain-damaged individuals, psychiatric patients or neurologically intact people. In the first part of this chapter, we provide an overview of these methods and how they can be fruitfully applied to a deeper understanding of psychiatric disorders. In the second part of the chapter, we give an overview of the organization of the brain for different mental functions, with an eye towards delineating those regions that are most likely to be affected in psychiatric disorders.

METHODS

Examining Patients with Delineated Brain Damage: The Lesion Method

The most venerable method of linking activity of the brain with mental functioning is that of the lesion method. The seeds for this method date back some 2000 years. During Roman times, Galen, a physician who ministered to the wounds of gladiators, noticed that contestants sustaining injury to the arm, leg or torso retained their powers of thought, whereas those who sustained injury to the head or the brain did not. From these observations, he inferred that the brain was linked to the mind, becoming one of the first scientists to use individuals with brain damage to understand brain–behaviour relationships.

The logic of the lesion method is to assume that if damage to a particular region of the brain results in an inability to perform a specific function, then the lost function must be supported by that brain region. Because damage to different regions of the human brain has distinct functional consequences, we now assume some degree of localization of function, i.e. that a particular brain region supports a specific mental function. This was not always accepted fact, as it had been proposed that the brain worked via mass action, meaning that all pieces of brain contributed to all functions. According to this theory, the nature of cognitive deficits observed after brain damage hinged not on which brain regions were destroyed but on the extent of the damage.

Even though specific functions may appear to be localized, it is important to remember that the brain is comprised of about 50 billion interconnected neurons. Thus, almost all functions rely on the smooth and integrated functioning of many of these areas. In addition, there is some redundancy, so that lesions in different brain regions can sometimes have similar effects. Therefore, we must remember that the brain relies on both localization of function and diffuse processing to carry out mental function in a seamless fashion.

The major strength of the lesion method is that it allows a direct linkage between a specific region of brain tissue and a particular aspect of mental processing. When using the lesion method, a researcher can take one of two conceptual approaches. One approach emphasizes knowledge about neural substrates; the other emphasizes knowledge about mental function. For the purposes of expanding our understanding of the neuropsychological organization of the brain, researchers often ask, what functions are supported by a particular piece of brain tissue? Under such conditions, a researcher assembles a group of individuals in whom the site, cause and extent of damage are as comparable as possible. An example of the power of this approach is the work of Brenda Milner and colleagues at the Montreal Neurological Institute. By examining patients who underwent removal of portions of the temporal lobe (because it was the source of epileptiform activity), they were able to demonstrate that removal of the hippocampus and nearby structures are critical for the production of new long-term memories about factual information (e.g. Milner, 1978).

The other conceptual approach to the lesion method emphasizes mental functioning. When taking this approach, researchers select a group of individuals that have the same diagnosis or exhibit the same behavioural symptoms and then determine the neural concomitants of those behavioural characteristics. One example of this approach is to differentiate schizophrenics who predominately exhibit negative symptoms (e.g. anhedonia, alogia, avolitional behaviour, etc.) from those that exhibit positive symptoms (e.g. hallucinations, delusions) and examine the integrity of frontal regions in each. A greater degree of frontal atrophy is associated with negative symptoms compared with positive symptoms (Baaré *et al.*, 1999). Such findings suggest that degeneration of neural tissue, especially in prefrontal regions, may in fact be predictive of the poor outcome associated with negative symptoms (Sanfilipo *et al.*, 2000). Another variation of this approach is to make an even more fine-grained selection with regard to a specific behavioural symptom (e.g. alogia), rather than grouping individuals according to a diagnostic category that encompasses a variety of behavioural manifestations (e.g. negative symptoms). Such an approach assumes that a particular syndrome may result from dysfunction with regard to a variety of brain regions, and that associations may best be revealed by examining the relationship between a specific symptom and a specific brain region (e.g. Stolar *et al.*, 1994).

The patterns of mental dysfunction among groups of individuals with lesions in different locations can provide important insights into the architecture of the mind. First, comparisons across groups

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can help to distinguish whether a brain region is *involved* in a function, or whether it is *critical* to that function. Taking our example above, Milner was able to demonstrate that the hippocampus was critical in this long-term memory function because individuals with damage to these other regions, such as the frontal lobes, did not exhibit problems in forming new long-term memories.

Second, comparisons across groups, via a double dissociation, can provide information as to whether two mental functions are independent (e.g. Shallice, 1988; Teuber, 1955). A double dissociation occurs when lesions have converse effects on two distinct cognitive functions: one brain lesion causes a disruption in cognitive function A but not cognitive function B, whereas a different lesion causes a disruption in cognitive function B but not cognitive function A. We infer that the functions are independent because the viability of one cognitive function does not depend on the viability of the other. For example, one might assume, a priori, that all auditory language processing is localized to the same region of the brain. If that were the case, then losing the ability to understand auditory language would be accompanied by a loss of speech output as well. However, the dissociation between Broca's and Wernicke's aphasia illustrates that the ability to produce and the ability to comprehend spoken language are distinct mental faculties. In Broca's aphasia, comprehension of auditory language is, for the most part, intact, but speech production is halting, slow and effortful. In contrast, in Wernicke's aphasia, individuals cannot understand what is said to them but nonetheless fluently produce grammatically correct sentences (even though these sentences are usually nonsensical). Hence, disruptions in speech output are independent of whether a disruption in speech comprehension occurs, and vice versa.

The importance of the lesion method in expanding our knowledge in neuropsychology cannot be underestimated. It has led to classic conceptualizations about the neural underpinnings of language, memory and perception, to mention just a few areas (see Damasio and Damasio, 1989 for further discussion). Nonetheless, the lesion method has two major limitations. First, variability in characteristics of the participant population and inconsistency in the location and the extent of the lesion can make straightforward inferences difficult. Prior to brain damage or a psychiatric illness, individuals have often had diverse life experiences, typically varying widely in socioeconomic status, educational background, and age. Afterwards, their life experiences also vary, depending on the type of treatment they receive, their compliance with treatment regimens, their social support network, and time since onset or insult, to name only a few factors. Differences in these characteristics between populations can produce seemingly contradictory results across studies, and the variable(s) responsible for such dissimilarities may remain obscure or difficult to tease out.

The second major limitation of the lesion method is that it does not allow direct observation of the function performed by the damaged portion of the brain. Rather, all we can do is observe how the rest of the brain performs without that particular area, and from these observations infer the previous role of the damaged region. Although such inferences are usually sound, they may have certain limitations and liabilities. One is that the lesion method can identify only the regions of the brain that are absolutely necessary to perform a given mental function; it cannot identify the entire set of brain regions that may participate in that function. Another limitation is that a brain region's contribution to a particular cognitive function can go undetected if a task can be performed via multiple strategies. If an individual utilizes an effective alternative strategy, performance may appear intact when indeed some functions have been lost. Finally, behavioural impairment may result after damage to a region not because that region is critical to the task, but because that region connects two or more brain regions that must interact for correct performance of the function, a situation known as a disconnection syndrome.

In some cases, generalizations from the results obtained with the lesion method have been applied fruitfully to psychiatric disorders; in other cases, they have clear limitations. One case in which generalizations have been relatively straightforward is that of major depression. Lesions to left frontal lesions result in major depression in half of all individuals who sustain them, and an extensive body of research indicates that underactivity of left frontal regions is associated with major depression (We discuss this issue in more detail later.) However, other psychiatric disorders, such as schizophrenia, are much less likely to result from dysfunction of a specific region. Although much research indicates dysfunction of the frontal region in schizophrenia (e.g. Goldstein *et al.*, 1999), there are numerous other brain regions, including the basal ganglia (e.g. Menon *et al.*, 2001), hippocampus, temporal regions (e.g. Sigmundsson *et al.*, 2001), and thalamus (e.g. Blennow *et al.*, 2000), that also appear to be dysfunctional. Furthermore, recent research has suggested that disconnections between different brain regions, either between prefrontal and temporal regions (Halliday, 2001; Fuster, 1999) or within temporal regions themselves, such as between the hippocampus and entorhinal regions (e.g. Young *et al.*, 1998), may characterize schizophrenia. Clearly, such findings indicate that brain dysfunction in this disorder involves a diffuse and broad network, rather than being well characterized as resulting from a circumscribed lesion.

Techniques for Determining Brain Anatomy

As should be obvious from the discussion above, the lesion method relies critically on determining where in the brain damage was sustained. Our ability to make such linkages has been undergoing a revolution since the mid-1970s due to the advent of different brain-imaging techniques that provide information on the location of damage in living individuals. The first of the modern brain-imaging techniques, computerized axial tomography (CAT), uses X-rays to provide information on the density of brain structures. Although CAT scanning was a breakthrough, in many ways it has been superseded by magnetic resonance imaging (MRI). This technique relies on the use of magnetic fields to distort the behaviour of atoms; the information gained on how long the atoms take to recover from this distortion can then be used to create an image of the anatomy of the brain (for a more advanced, but readable, discussion of this method and some of its applications, see Andreasen, 1989).

MRI has two main advantages over CAT. First, MRI does not require X-rays and hence does not involve transmitting high-energy radiation through the body. Second, the spatial resolution of the image is far superior with MRI than with CAT. However, MRI cannot be performed on individuals with pacemakers or people with metal in the body that is not connected to hard tissue (e.g. a clip on an artery or a metal shaving in the eye from welding). A new, MRI-derived method, diffusion tensor imaging, has the potential to provide much information about the anatomical connectivity of different brain regions. This method looks at the axes along which water diffuses, and hence provides information on the main directional orientation of white matter tracts as well as their structural integrity (Conturo *et al.*, 1999). Since white matter tracts connect distant brain regions, this method can be utilized to detect disorders that arise from a partial or complete disconnection between brain regions, or to detect demyelinating disorders.

Techniques for Determining Physiological Activity

Following quickly on the heels of the revolution of brain-imaging techniques that provide a structural description of the brain's anatomy has been a revolution in the ability to measure the

physiological functioning of the brain. These methods divide into two major classes: those that mainly provide information about *where* activity is occurring, and those that mainly provide information about *when* activity is occurring. These two classes of methods can be viewed as complementary rather than rivals, since each provides a different type of information (i.e. where *v*. when). As such, researchers are attempting to gain a more complete picture of brain systems supporting mental thought by performing identical experiments with each type of method and cross-comparing the results (e.g. Martinez *et al.*, 1999). Although these functional brain-imaging techniques are often used with individuals with brain insult or psychiatric disorders, these techniques can be used with neurologically intact individuals to great advantage. They allow the direct observation of the activity of a brain region during performance of a specific task, and they can identify the entire network of brain structures that participate in performing a particular mental function, neither of which is provided by the lesion method.

Methods that Provide Information about the Location of Brain Activity

The two main techniques for localizing where activity is occurring are positron emission tomography (PET), which uses a radioactive agent to determine the brain's metabolic activity, and functional magnetic resonance imaging (fMRI), which uses a variant of the MRI techniques discussed above. In PET, the radioactive tags utilized to measure brain activity typically are either 2-deoxy-D-glucose, a physiologically inert sugar similar to the one that supplies the brain with energy, which is altered by the introduction of a radioactive fluorine atom (^{18}F) into 2-deoxy-2-fluoro-D-glucose, or ^{15}O delivered in water. To reach a more stable state, the radioactive isotope emits a positively charged electron (positron). After emission, the positron collides with an electron, causing annihilation of their mass and a release of energy in the form of two photons of light that travel from the site of annihilation exactly 180 degrees opposite each other. A ring of photocells surrounding the patient's head detects the coincident arrival of two photons of light from opposite directions. Extrapolating backwards, region(s) of the brain from which the photons came can be determined, providing information on brain activity. The time required to obtain a picture of brain activity is dictated by how quickly a given isotope goes from a radioactive state to a non-radioactive state (known as its half-life), because a critical number of photons must be detected before an image is interpretable. A related technique, single photon emission tomography (SPECT), is a much scaled-down version of PET using a small set of sensors and isotopes that have a longer half-life. Hence, the spatial resolution of the image and the temporal precision of SPECT images are reduced relative to PET.

PET has a distinct advantage over other functional brain-imaging methods in that it allows investigation of the metabolic activity with regard to a large number of molecules. As long as a (positron-emitting) version of the molecule can be created, metabolic action of that molecule can be observed. This advantage makes PET a particularly important method for understanding psychiatric disorders because it allows for the investigation of neurotransmitter function (e.g. Wong *et al.*, 1986). There are many linkages between psychiatric illnesses and dysregulation of neurotransmitter systems, including (1) the dopaminergic system with regard to schizophrenia (e.g. Lidlow *et al.*, 1998), certain forms of substance abuse (e.g. Spanagel and Weiss, 1999), and attention deficit disorder (e.g. La Hoste *et al.*, 1996); (2) the serotonergic system with regard to depression (e.g. Blier and de Montigny, 1999) and obsessive–compulsive disorder (Baumgarten and Grozdanovic, 1998; Delgado and Moreno, 1998); and (3) GABAergic transmission in alcoholism (Hoffman and Tabakoff, 1996) and the anxiety disorders (Leonard, 1999). As an example of the utility of PET in exploring such issues, this method has demonstrated that the binding of dopamine D1 receptors in prefrontal regions of the brains of schizophrenics predicts the severity of negative symptoms as well as dysfunction on a cognitive tasks relying on frontal regions (Okubo *et al.*, 1997).

Despite this major advantage, PET has some drawbacks. First, it involves radiation, which limits an individual to about three to four scans a year. This precludes certain studies, such as those designed to monitor changes in brain activity during the course of a treatment regimen. Second, the time periods over which brain activity is recorded are exceedingly long (on the order of minutes or hours), making precision with regard to specific mental processes difficult.

With fMRI, changes in neuronal activity are accompanied by local changes in cerebral blood flow and blood oxygenation (e.g. Fox *et al.*, 1988), which in turn can be used to infer the activity levels of different brain regions. Since the mid-1990s, there has been an explosion of research using the blood oxygenation level-dependent (BOLD) method, which measures the relative proportion of oxygenated blood to deoxygenated blood. Active brain regions exhibit an increase in oxygen-rich blood, which is infused to these regions faster than it can be extracted by brain tissue. This increase can be picked up as an amplification of the magnetic resonance signal because the binding of iron to haemoglobin varies between oxygenated and deoxygenated blood (e.g. Kwong *et al.*, 1992).

For a number of reasons, fMRI has characteristics that make it advantageous compared with PET. First, scans can be obtained with standard clinical MRI machines routinely located in hospitals (rather than relying on a facility for creating a radioactive isotope, as is the case with PET). Second, it does not involve ionizing radiation. Third, multiple scans can be run on a single subject, which allows examination of changes in brain function over time, such as might occur during treatment or time from insult.

The main drawback of this method, however, is that it is measuring the haemodynamic response of the brain, rather than a derivative of neuronal activity. This haemodynamic response has a characteristic waveform, peaking about 4–6 seconds after stimulation and coming back to baseline after around 12–16 seconds. Although certain experimental designs make the temporal precision much greater than 16 seconds (Aguirre and D'Esposito, 1999), the time course of the haemodynamic response does impose limitations.

Methods that Provide Information on the Time Course of Brain Activity

There are other electromagnetic methods that provide precise information about the time course of brain activity, although they do not provide as much precision with regard to the location of such activity. The main methods for examining the time course of brain activity are event-related potentials (ERPs) and magnetoencephalography (MEG). ERPs are recordings of the brain's electrical activity that are linked to the occurrence of an event (e.g. the presentation of a stimulus) rather than a continuous measure of ongoing activity, as is the case with electroencephalography (EEG). For ERPs, metal electrodes positioned on the scalp record the electrical signals produced by the brain, which are then amplified. The electrical waveform recorded on the scalp is the summed or superimposed signal of electrical activity of fields of postsynaptic potentials of neuronal dendrites. The electrical potential recorded in response to a stimulus can be divided into components, which are characteristic portions of the waveform that have been linked to certain psychological processes, such as sensory processing, attention and memory. ERP components are labelled with a letter and a subscript number (e.g. P_{300}). The letter, *P* or *N*, denotes whether the deflection of the electrical signal is positive or negative, while the number represents, on average, how many milliseconds after stimulus presentation the

component appears. Whereas the early components (up to first 150 ms) tend to be driven by physical features of the presented stimulus (e.g. loudness, physical similarity to the last item), the later components tend to be linked to internal cognitive states, such as the degree to which memory must be updated in the current context (P_{300}) (Donchin and Coles, 1998) or the degree to which a stimulus is semantically anomalous given the context (N_{400}) (Kutas and Hillyard, 1984). A related method, MEG, examines the magnetic fields at the brain's surface derived around dipoles located in the brain. This method has the advantage of providing some information about the depth within the brain from which activity is emanating (as derived from the characteristics of the magnetic field recorded at the surface). Development of methods for interpreting MEG data as well as high-density ERP recordings (e.g. 128 electrodes) are allowing more precise localization of the source of electrical activity (see Davidson *et al.*, 2000 for a discussion of some of these issues). One common approach is to utilize information derived from PET or fMRI that serve to constrain modelling the source of electrical activity (see Mangun *et al.*, 1998 for a longer discussion of this issue).

A number of different components, ranging from those that reflect perceptual process to those that reflect more central processes, have been linked to specific psychiatric disorders. We will cite a couple of examples here. Schizophrenic individuals show an abnormal P_{50}, which has been suggested to index the early gating of sensory information. Furthermore, the degree of abnormality of this component has been found to predict negative (but not positive) symptoms (e.g. Erwin *et al.*, 1998) and appears to result from nicotinic receptor desensitization (for a longer discussion of this association, see Adler *et al.*, 1998). An asymmetry between the hemispheres in the N_{100} recorded via MEG has been found in paranoid schizophrenics (for a review of the utility of MEG in psychiatry research, see Reite *et al.*, 1999). The latency of the P_{300}, which is though to reflect the attentional resources that can be brought to bear to a task, has been found to be elongated in depressed individuals and to predict performance on executive tasks (e.g. Kindermann *et al.*, 2000). Furthermore, the latency of the P_{300} in depression is linked to serotonergic activity, implicating this neurotransmitter system in the generation of this component (Hansenne and Ansseau, 1999).

A newly developed method, event-related optical signal (EROS), has the exciting potential to provide simultaneously information about the location and time course of brain activity. This method utilizes the beaming of near-infrared light through the skull into the cortex. The deformation of the path of the light back out of the head provides two types of information. The first, known as the slow signal (which tracks changes on the order of seconds) appears to detect changes in the brain's haemodynamic response similar to that measured by fMRI. The second, known as the fast signal (which tracks changes on the order of milliseconds), appears to detect changes in the shape of neurons associated with neuronal firing. Because the position of input of the light source, and the site of the detector, can be controlled, it is possible to localize the source of the signal. In addition, the data can be recorded in a millisecond-by-millisecond manner, providing temporal information (see Gratton and Fabiani, 1998 for a longer description of the technique, validation of the method, and applications).

This new method has large potential. It is less costly than MRI systems, can provide both temporal and spatial information, and is non-invasive. Its limitations are that it cannot reveal activation of deep structures in the brain (because light will not penetrate that deeply) and that it is relatively untested at present.

Methods that Modulate Brain Activity

Recently, methods have emerged that temporarily modulate the activation of the brain in humans. These methods have been used both with regard to understanding brain–behaviour relationships and in a therapeutic manner. The most notable of these is transcranial magnetic stimulation (TMS). In this method, a magnetic coil is placed over a particular portion of the brain and a pulse of magnetic stimulation is applied. This magnetic field disrupts the electrical activity of the neurons in the vicinity. Although this method has been referred to as causing a 'reversible' lesion, which would suggest that it blocks neuronal activity, it is better understood as influencing activity by causing neurons to fire in a random pattern rather than in a coherent manner. The result is that at low frequencies TMS disrupts mental functioning, whereas at higher frequencies it can enhance activity (see Fitzpatrick and Rothman, 2000 and Walsh and Cowey, 2000 for a description of these techniques).

This method has numerous theoretical advantages. First, it can be used to confirm findings from the lesion method that implicate a brain region as playing a critical role in a specific mental function. If a brain area is critical for a particular mental function, then applying TMS to the region should disrupt that function. Second, it has been useful in demonstrating that some clinical syndromes result from an imbalance of activity between two brain regions, rather than dysfunction of a specific region. For example, hemi-neglect (a syndrome we will discuss in more detail later on) causes an individual to ignore information on one side of the world (e.g. information on the left). There are various theories as to the mechanism underlying this disorder, which typically occurs after unilateral damage to parietal regions of the right hemisphere. Recently, it has been demonstrated that TMS to the intact left hemisphere can eradicate hemi-neglect (Oliveri *et al.*, 1999), restoring normal function. Such a finding indicates that it is an imbalance between the left and right hemispheres, rather than damage to the right hemisphere, that causes hemi-neglect. Third, TMS can be used clinically to restore imbalances in activation between brain regions. Much work suggests that underactivation of left as compared with right frontal regions is associated with depression (Heller and Nitscke, 1998). Repetitive trains of TMS (rTMS) over left prefrontal areas have been used successfully to treat depression (e.g. Eschweiler *et al.*, 2000). Finally, TMS can be utilized along with another brain-mapping method, such as PET, to determine the functional connectivity between brain regions. In a series of clever experiments, Paus (1999) applied TMS in a focal manner while simultaneously obtaining PET data on activation across the entire brain. He reasoned that remote areas of the brain influenced by the region disrupted by TMS should exhibit decreased activity. In this way, a map of the functional connectivity of the brain could be obtained.

Assessment of Behaviour

Careful and thoughtful behavioural testing is one of the most powerful tools we have for analysing how the brain constrains and influences the way we think. Just as we need precise tools to measure brain anatomy and brain physiology, we also need precise tools to examine behaviour. Although a variety of quick screening devices, such as the mini-mental status exam (MMSE), are available and used widely in the medical profession, they provide a very poor metric of mental abilities. They are lacking as measures on which to base treatment decisions or mental competency (Guilmette and Krupp, 1999). Rather, a standard neuropsychological assessment is a far superior method for evaluating the degree to which damage to the central nervous system may have compromised a person's cognitive, behavioural and emotional functioning. The goals and uses of this assessment are numerous: to provide a profile of mental capacity exhibited by an individual including both strengths and weaknesses, as a tool for providing prognosis, and as a means to evaluate the person's progress during rehabilitation or treatment.

Table X.1 Components of the Halstead–Reitan neuropsychological test battery

Test	Measures	How the ability is measured
MMPI	Psychiatric symptomatology, e.g. depression and schizophrenia	Individual answers a large number of yes/no questions to provide a profile relative to individuals who have been diagnosed with specific psychiatric disorders.
Categories test	Abstract reasoning	Individual views four items on the screen and pushes one of four buttons, with different sets of items requiring different responses (e.g. push the button corresponding to the atypical item, push the button corresponding to the Roman numeral on the screen). The only feedback provided is a bell for correct answers and a buzzer for incorrect answers.
Rhythm test	Auditory perception and timing	Individual decides whether two patterns of sounds are similar.
Speech sounds perception test	Verbal abilities Attentional abilities	On each trial, the individual chooses a previously heard sound from among a number of choices. The sounds are nonsense syllables that begin and end with different consonants.
Finger tapping test	Motor function	Tapping rate of each index finger is determined.
Grip strength test	Motor function	Strength with which a dynamometer can be squeezed by each hand is assessed.
Trail making test	Visual search Attention	*Part A*: examines whether the individual can draw a line connecting consecutively numbered circles. *Part B*: examines whether the individual can connect, in an alternating manner, numbered and lettered circles (e.g. A1B2C3).
Aphasia screening test	Language	Examines the ability to use and perceive language, to pantomime simple actions, and to reproduce simple geometric forms.
Tactile perception test	Tactile ability	Determines whether the individual can identify objects by touch (each hand separately), identify letters traced on the fingertips (when the eyes are closed), and perceive being touched on different fingers of both hands.
Tactual performance test	Tactual memory Spatial localization	Without any visual input (blindfolded or eyes closed), a set of felt shapes must be placed into a single board from which they are cut out. Afterwards, with eyes open and the board obscured from view, each shape must be drawn at its correct location in the board.
Sensory-perceptual exam	Sensory loss Hemi-neglect	Assesses the perception of simple information in the visual, tactile and auditory modality. To determine whether neglect is present, stimuli are presented to just one side of the body or to both simultaneously.
WAIS-III	General intellectual abilities	11 separate subtests that assess a variety of intellectual functions.

MMPI, Minnesota multiphasic personality inventory-2; WAIS-III, Wechsler Adult Intelligence Scale–Third edition.

There is a tension in neuropsychological assessment between the desire to provide a tool that will detect the presence of brain damage regardless of its source and the desire to provide a fine-grained componential analysis of behaviour that delineates precisely the specific cognitive or emotional functions that have been disrupted. To cast a wide net to detect brain dysfunction of either neurological or psychiatric origin, neuropsychologists often utilize a test-battery approach. These batteries typically assess a variety of mental functions and have at least one test designed to measure overall intelligence. Probably the most widely used neuropsychological test battery is the Halstead–Reitan battery, which consists of a number of tests that generally require about 6–8 hours to administer. The abilities examined in this battery range from simple tests of sensory function to complex tests of reasoning, from tests of verbal function to tests of spatial function, and from tests of immediate recognition to tests of memory. In addition, the battery is used to assess functioning in different sensory modalities. A complete Halstead–Reitan battery for adults (Boll, 1981) includes the tests described briefly in Table X.1.

Other test batteries, such as the Luria–Nebraska test, take a similar approach. The tasks on the Luria–Nebraska test are divided into 12 content scales: motor functions, rhythm and pitch, tactile and kinaesthetic functions, visual functions, receptive language, expressive language, reading, arithmetic, writing, memory, intermediate memory, and intelligence. These batteries were designed to determine whether an individual suffered brain damage, and they are effective at discriminating patients with brain damage from neurologically intact individuals (e.g. Golden *et al.*, 1978; Vega and Parsons, 1967). However, these batteries may not be as effective at discriminating between individuals with brain damage and those with psychiatric disorders (e.g. Adams, 1980).

Another popular battery is the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized battery. This has been designed to emphasize assessment of abilities that rely on frontal regions and associated subcortical areas, but also assesses parietal and temporal lobe function. All tests in this battery are non-verbal (Sahakian and Owen, 1992). One set of tasks act as control tasks designed to assess psychomotor speed, the ability to follow instructions, and gross spatial perceptual skills. Another set is designed to be sensitive to frontal lobe damage; this set consists of tasks that test the ability to utilize a search strategy to find a target, the ability to switch sets (e.g. from one dimension of a stimulus to another), the ability to plan towards a goal, and the ability to remember the spatial location of a visual stimulus. The tests sensitive to functioning of the temporal and parietal lobe assess the ability to recognize complex visual displays after different time delays, the ability to recognize abstract patterns, the ability to associate visual patterns with specific locations, and the ability to remember the order in which visual stimuli are presented. This battery has been utilized with a variety of populations, ranging from people with dementia and Parkinson's disease (e.g. Sahakian, 1990) to those with bipolar and unipolar mood disorders (e.g. Sweeney *et al.*, 2000). Unfortunately, the test–retest reliability of certain portions of the battery is not as high as would be ideal (e.g. Lowe and Rabbitt, 1998).

An alternative strategy to the test-battery approach is customized neuropsychological assessment. In such an assessment, a small set of standard tests is used, such as the Wechsler Adult Intelligence Scale–III (Wechsler, 1997) to survey general intellectual

abilities, and the Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 1983) to ascertain language functioning. The examiner uses information from the initial set of tests to generate hypotheses about the set of particular abilities that were compromised by the brain damage. Each hypothesis is evaluated with a specific neuropsychological test, and, depending on the individual's performance, the hypothesis is either pursued further with another test or abandoned (e.g. Lezak, 1995). Myriad tests can be used for assessing specific functions, such as those measuring memory, attention, spatial ability and abstract reasoning. Compared with a standardized battery, the individualized approach requires a more skillful examiner. In this case, the neuropsychologist's expertise may be critical to elucidating the nature of the disorder, especially in atypical or unusual cases. The interested reader is referred to Lezak (1995), which is the definitive reference with regard to clinical assessment of neuropsychological dysfunction.

Probably the set of tests used most widely to assess intellectual abilities is the Wechsler family of intelligence tests: the Wechsler Pre-school and Primary Scale of Intelligence–revised (WPPSI-R; Wechsler, 1989), for children aged 3 years and 7 months to 7 years and 3 months; the Wechsler Intelligence Scale for Children–third edition (WISC-III; Wechsler, 1991), for children aged 6 years to 16 years and 11 months; the Wechsler Adult Intelligence Scale III (WAIS-III; Wechsler, 1997); and the Wechsler Adult Intelligence Scale–revised as a neuropsychological instrument (Kaplan *et al.*, 1991). These tests generally take about 3 hours to administer. They all provide an overall or full-scale estimate of IQ (FSIQ). In addition, they provide scores on two major subscale scores, a verbal IQ (VIQ) and a performance IQ (PIQ), which, generally speaking, break down into verbal tests and non-verbal tests, respectively. The WAIS family of tests are useful because (1) they provide a profile of abilities rather than a single score; (2) they are well-normed, based on a large sample of individuals who were chosen to match the demographics of the US population with regard to gender, region of the country in which they reside, and type of environment (i.e. rural or urban); and (3) they provide different norms for different age ranges. The WAIS-NI is tailored to neuropsychological assessment because it allows for an evaluation of an individual's abilities, even if he or she has difficulty in motor output, and it provides the examiner with information on the strategies that a patient uses in solving problems.

Even though test batteries and tests of general intellectual abilities provide information on how well a person is functioning, it is often important to have some estimate of premorbid functioning before an injury or onset of psychiatric illness. Sometimes, a person's educational and occupational history can serve as such a standard, but in other cases this may be inadequate. The vocabulary subtest of the WAIS-III has been used to estimate premorbid IQ, because the abilities it measures seem relatively resistant to brain damage, even damage that affects many different arenas of intellectual functioning, such as Alzheimer's disease. Another test used to estimate premorbid functioning is the National Adult Reading Test (Nelson, 1982), which is an oral reading test consisting of 50 words, most of which are short and do not follow normal rules of pronunciation (e.g. 'ache'). Because the words cannot be sounded out, an ability to read them indicates some previous familiarity and hence provides an estimate of premorbid abilities (Crawford, 1992). When estimates of premorbid intelligence are much higher than present test scores, the results suggest that psychiatric illness or brain damage has affected adversely the individual's intellectual abilities.

Although many researchers who are trying to understand fundamental aspects of brain–behaviour relationships often adopt tests utilized in neuropsychological assessment (e.g. the Wisconsin card sorting test) in brain-mapping studies, such an approach may not be optimal. These tests serve as good assessment tools because they are sensitive to dysfunction due to a variety of aetiologies involving a number of brain regions (e.g. Anderson *et al.*, 1991). However, that makes them inherently poor at pinpointing a specific cognitive operation. For example, numerous brain-imaging studies have utilized the Stroop task, which is known as the gold standard of attentional functioning (MacLeod, 1992). In this task, individuals attend to and identify the colour in which a word is printed while ignoring the word. Attentional demands are increased in the incongruent condition, in which it is hard to ignore the word because it names a colour distinct from the word's ink colour (e.g. the word 'red' printed in blue ink), than in the neutral condition, in which the word contains no colour information (e.g. the word 'lot' printed in blue ink). Although many researchers have identified a series of neural structures that aid in attentional selection of this task (Pardo *et al.*, 1990; Bench *et al.*, 1993; Carter *et al.*, 1995; Carter *et al.*, 1997; Bush *et al.*, 1998; Brown *et al.*, 1999; Petersen *et al.*, 1999), the specific components of attentional control have not been well specified.

An alternative approach is to understand more precisely the different component processes and the neural regions that support those processes by performing specific comparisons, while considering neurocognitive theories of how these processes might dissociate. We took such an approach with the Stroop task. Our guiding heuristic was a neurocognitive theory of attentional control that conceives it as being divided into an anterior system that provides top-down control of attention and a posterior system that selects information on the basis of sensory characteristics (e.g. Posner and Dehaene, 1994). We reasoned that any top-down system that serves as a general mechanism for selecting task-relevant information should be engaged, regardless of the specific attribute to which an individual must attend. In contrast, we hypothesized that posterior brain regions should be sensitive to the type of information that is being selected, and that the pattern of activity in posterior regions should differ between these two regions. To test these predictions, we compared brain activation for the standard colour-word Stroop task described above to that of a spatial-word Stroop task. In this latter task, an individual identifies the position of a word relative to a box (an incongruent trial would be one in which the word 'below' is positioned above the box). Notice in this comparison that we have held the task-irrelevant information constant — in both cases the information to be ignored is a word. Hence, similarities between patterns of activation between the two tasks must indicate a common mechanism for attentional selection, regardless of the task-relevant attributes, whereas differences in patterns would indicate sensitivity to the nature (e.g. colour, spatial) of task-relevant information. We found the expected dissociation between anterior and posterior regions. Both tasks activated similar regions of the middle frontal gyrus, whereas a differentiation in the pattern of brain regions activated was observed for posterior regions (Banich *et al.*, 2000).

PRINCIPLES OF HUMAN BRAIN ORGANIZATION FOR MENTAL FUNCTION

Having reviewed the ways of obtaining information about the brain and behaviour, we will now spend the rest of the chapter providing an overview of how the brain is organized for different cognitive and emotional functions. On a gross level, the brain has a functional organization front/back and left/right. Frontal regions are involved in motor programming and planning, whereas posterior regions are responsible for sensory processing. The two hemispheres of the brain also play distinct roles in mental operations. We will discuss the organization for the receipt of sensory information and the control of motor output. This is followed by a discussion of the differences between the cerebral hemispheres, and the role of

the four major lobes of the brain in higher-order mental functions: frontal, parietal, occipital and temporal.

Primary Sensory and Motor Cortices

In general, the frontal regions are involved in motor processing, while sensory processing occurs in the posterior regions. The organization of primary sensory and motor areas share some general characteristics of organization. First, all these brain areas are organized so that specific aspects of the physical world are 'mapped' on to brain tissue (e.g. a particular region of visual space is perceived by a specific region of primary visual cortex). Second, these maps are distorted relative to the physical world, so that more brain tissue is devoted to those regions for which we have a higher density of receptors (e.g. more of the primary visual cortex is devoted to processing visual information from the fovea). Third, for vision, touch and motor control, the mapping of the world on to brain tissue occurs in an upside-down, backward manner (e.g. information from the upper right-hand portion of the visual world is processed by the ventral region of the primary visual cortex of the left hemisphere).

The primary motor cortex resides directly in front of the central fissure in a long, narrow band that begins deep within the longitudinal fissure and continues down until the Sylvian fissure. The amount of brain tissue devoted to a body part is proportional to the degree of fine motor control exhibited by that body part. Neurons in the motor cortex control the amount of force to be applied by muscles, so that damage to primary motor cortex leads to muscle weakness on the opposite side of the body, especially those abilities required for fine motor control, such as are needed to grasp something between the thumb and forefinger.

The primary somatosensory cortex is located directly posterior to the central fissure and receives information about tactile stimulation, proprioception, and pressure and pain sensations from internal organs and muscles. The distortion is similar but not identical to that observed for the motor strip, because the parts of our body for which we have fine motor control, such as our hands, are the same areas for which we need a fine sense of touch. Damage to the somatosensory strip compromises fine discriminations of touch (e.g. differentiating velvet from burlap, or knowing how many times one is touched in succession) on the side of the body contralateral to the damaged primary somatosensory cortex.

The primary visual cortex is located within the calcarine fissure in the occipital cortex. Neurons in this region code the contrast between light and dark, and destruction of the visual cortex leaves an individual functionally blind, as no visual information can be perceived. The mapping of the visual world on to the brain occurs such that information to the right of fixation (i.e. the right visual field) projects to the left hemisphere, whereas information to the left of fixation (i.e. the left visual field) projects to the right hemisphere. Almost the entire occipital cortex is devoted to processing visual information. After being processed by the primary sensory cortex, information is relayed to the secondary sensory cortex, which, like primary sensory regions, processes information from only one sensory modality. Although there are secondary sensory regions for all modalities, those for visual processing are vast, comprising more than 30 distinct regions of secondary visual cortex, each of which varies in its sensitivity to important visual attributes, such as colour, orientation and motion.

The primary auditory cortex is located in Heschl's gyrus, tucked inside the Sylvian fissure. Unlike other sensory systems in which information from one side of the body projects solely to the contralateral hemisphere, the organization of the auditory system is such that there are both ipsilateral and contralateral projections from the ear to the brain; hence, auditory information received at the right ear projects to the left and right hemispheres. The organization of the primary auditory cortex is tonotopic, meaning that it is organized with regard to the frequency of a tone. The mapping of the auditory cortex is such that the lowest tones are processed caudally and laterally, and tones of increasing frequency are processed as one moves rostrally and medially through the cortex. Unilateral damage to the primary auditory cortex does not impair the ability to perceive all sound because of the redundancy provided by both crossed and uncrossed connections in the auditory system. Rather, the softest intensity that can be perceived, i.e. the sound threshold, becomes higher contralateral to the damaged hemisphere, and the ability to perceive the location of a sound becomes poorer for the contralateral side of space.

Lateralization of Function

The idea that the hemispheres had different functions first caught the attention of the scientific community in the 1860s when Paul Broca, a French neurologist and anthropologist, provided evidence from numerous case studies that the left hemisphere, but not the right, was critical in language processing. Broca's discovery was sparked by an encounter with one of his patients, who exhibited an unusual syndrome. The man could utter only the syllable 'tan', yet he could understand language as evidenced by his ability to follow simple verbal commands. Furthermore, his problem was specific to speech output, in that he did not exhibit paralysis of the vocal musculature or the vocal tract. At autopsy, Broca noticed that a specific region of the left hemisphere was damaged. He then proceeded to accumulate a small series of brains from individuals who had the same type of language problem, and noticed that in each case the damage was restricted to the same brain region; more importantly, he noticed that the damage was always localized to the left hemisphere. Furthermore, Broca found that individuals with damage to the analogous area of the right hemisphere displayed no difficulties in language. The importance of the left hemisphere for language processing was confirmed by other neurologists, such as Karl Wernicke. He found that individuals with lesions to the temporal-parietal region of the left hemisphere exhibited the converse syndrome of that discovered by Broca — difficulties in language comprehension along with intact production. By the end of the 1800s, the scientific community had accepted the idea of cerebral dominance, the notion that one hemisphere dominates or leads for a given mental function. At that time, it was believed that the left hemisphere dominated almost all mental thought because language was considered synonymous with thought.

Research in the late 1950s and early 1960s dramatically changed scientists' conceptions of the functioning of the hemispheres, revealing each to have its own specialization. Effects of the surgical removal of regions of the right hemisphere, or disruption in right hemisphere performance after brain lesions, were noted by researchers across the globe, including those in Canada (e.g. Milner, 1968), France (e.g. Hecaen, 1962) and the USA (e.g. Benton, 1969). Probably the most dramatic demonstration of differences in function between the hemispheres, however, came from the research of Roger Sperry and associates (e.g. Sperry, 1974) at the California Institute of Technology, who tested a unique set of patients known as split-brain patients. As treatment for severe intractable epilepsy, the corpus callosum, the major fibre tract connecting the cerebral hemispheres, was cut in these individuals. This split-brain surgery provided a fascinating and unique opportunity — the ability to test the individual competency of each hemisphere separate from that of its partner.

Because the observations of Broca and subsequent neurologists suggested that the left hemisphere was dominant for speech output, Sperry and colleagues wished to determine which hemisphere controlled speech output in these split-brain patients. To do so, these researchers asked the patients to feel objects hidden from view

with just one hand (limiting information to the tactile modality). Because of the contralateral organization of the somatosensory system, objects felt by a given hand are perceived exclusively by the contralateral hemisphere. The patients were able to name the objects placed in the right hand but not those placed in the left hand. To determine whether the right hemisphere was indeed without much mental competency, or whether it just could not express itself, the researchers changed the task so that the patient had to demonstrate the correct use of familiar objects, such as pencils, cigarettes and drinking glasses. Under these conditions, the objects placed in the left hand could be identified correctly, indicating that the right hemisphere does have knowledge about the world, even though it has no control over speech output (Gazzaniga *et al.*, 1962).

Subsequent findings indicated that although mute, the right hemisphere has some albeit limited linguistic capacity. It can read most concrete words (i.e. words that represent real objects in the world), and it can make simple grammatical comparisons (e.g. differentiate between 'The boy went to the store' and 'The boy did not go to the store') (e.g. Zaidel, 1990). However, it cannot process more complicated grammatical constructions in which word order is important (e.g. 'The dog that the cat chased ran under the table behind the garage that had been condemned by the sheriff last week') (Zaidel, 1978), and it cannot perform phonological processing (e.g. Levy and Trevarthen, 1977), which is the ability to break down words into their constituent sounds.

In contrast, the right hemisphere excels at tasks that are spatial and non-verbal in nature. For example, when given blocks that must be arranged to form a pattern, the right hand (left hemisphere) performs in a hapless and disorganized manner, whereas the left hand (right hemisphere) performs the task rapidly and accurately. In addition, the left hand can depict three-dimensional structures in a two-dimensional plane, such as is required when a cube must be drawn on a piece of paper, but the right hand can not (e.g. Gazzaniga, 1970). The right hemisphere also excels at processes such as recognizing faces, non-algebraic aspects of geometry, and so forth. Findings of differences between the hemispheres in mental functioning have been confirmed not only in patients with unilateral lesions but also in neurologically intact individuals (see Banich, 2003, Chapter 4 for a longer discussion of these results).

Whereas earlier models suggested that the hemispheres differed in the kinds of information they each process (e.g. spatial *v*. verbal), more recent research suggests that the hemispheres differ in *how* they process information (see Banich and Heller, 1998). These studies suggest that the hemispheres have different modes of processing (e.g. holistic *v*. piecemeal), and that certain materials or tasks are handled better by one mode than the other. Although many dichotomies have been suggested to describe the differences between the hemispheres, they are summed up well by saying that the left hemisphere processes information in a piecemeal and analytic fashion, with a special emphasis on temporal relationships, whereas the right hemisphere processes information in a gestalt and holistic fashion, with a special emphasis on the spatial relationships. From this viewpoint, language is processed better by the left hemisphere because it relies on distinctions of fine temporal ordering in the comprehension and production of speech sounds, and the gestalt processing of the right is well suited for tasks such as face recognition.

Some researchers posit that the hemispheres selectively filter different aspects of basic sensory features, and that this selective filtering of sensory information underlies hemispheric differences for more complicated cognitive tasks. This viewpoint posits that although both hemispheres are equivalent in their receipt of sensory information, the left hemisphere filters information so that it preferentially processes information of relatively higher frequency, whereas the right hemisphere filters information of relatively lower frequency. (In the visual modality, frequency refers to how quickly information shifts from dark to light, so that information of higher spatial frequency provides the detail, and that of lower spatial frequency provides gross form.) This asymmetry is relative, so that information processed preferentially by the left hemisphere in one context (i.e. when it constitutes the higher-frequency information) may be processed by the right hemisphere in another context (i.e. when it constitutes the lower-frequency information). (For a book-length description of this theory, see Ivry and Robertson, 1998.)

The concept that the hemispheres are specialized for different modes of processing implies that both hemispheres can contribute simultaneously to performance. For example, in face processing, the right hemisphere is involved in processing the overall configuration or gestalt of the face, while the left hemisphere is engaged in processing the details, such as the shape of mouth. This parallel processing brings an important issue to the fore — that of how the hemispheres end up coordinating processing to provide the seamless mental experience that we all have. Recent work suggests that interaction between the hemispheres not only merges together these two modes of processing but also serves an important role in attentional regulation. When attentional or task demands are low, the hemispheres work independently, as coordinating their activity imposes a communication 'overhead'. But as demands increase, their processing becomes increasingly coupled. In this manner, more brain 'power' can be brought to bear on a task, and the cost of communication is relatively small in comparison to the additional computational power provided by an additional hemisphere. Interestingly, a number of clinical disorders, including schizophrenia (e.g. Mohr *et al.*, 2000), multiple sclerosis (Pelletier *et al.*, 1993), and closed head injury (Vuilleumier and Assal, 1995), are characterized by atypical or disrupted interhemispheric interaction and are also associated with attentional difficulties (For a review of the role of interhemispheric interaction in cognitive processing, especially attention, see Banich (2002).

Association Areas

Association areas, where information from multiple sensory modalities converges, are responsible for those abilities that we tend to think of as distinctly human, such as language, compassion and foresight. Because it is involved mainly in processing visual information, the occipital lobe does not serve as large an associative function as the other three major lobes of the brain (frontal, parietal and temporal).

The Frontal Lobe

In discussing the frontal lobes, researchers and clinicians generally describe it as having three distinct regions: the primary motor region (discussed previously), the premotor region, and the prefrontal region. Prefrontal regions are often divided further into dorsolateral, orbital and medial regions. The distinction among these regions is based on major cytoarchitectonic subdivisions. Recent evidence suggests that these regions may play very different roles in mental functioning. Although we will discuss these subdivisions in more detail later, as an overview, dorsolateral regions have been implicated in memory and attentional processing, orbital regions have been implicated in reward processes and decision making, and medial regions have been implicated in judgement and the detection of errors.

Frontal regions are often thought of as the source of some of the most uniquely human abilities. A good generalization about the role of frontal regions is that they are associated with the planning, guidance and evaluation of behaviour. Just as the head of a corporation oversees its day-to-day operations and plans its long-term goals, the frontal lobes are considered to be the 'executive' of the brain. Not only are the frontal regions important for organizing behaviour coherently, but research also suggests that they may

allow us to extrapolate forward in time, enabling us to realize the future consequences of our current behaviour.

The abilities that are destroyed by frontal lobe damage are not categorized easily under a simple rubric, but the loss of these abilities can nonetheless be profound. For example, individuals with frontal lobe dysfunction may exhibit little decrement in performance when given standardized IQ tests that assess fact knowledge and academic skills, such as knowing the definitions of words, knowing how to solve arithmetic problems, knowing how to put together jigsaw puzzles, and so forth. Impairments in functioning may nonetheless be severe. Disruptions may occur in any or all of the following areas: the ability to organize and sequence behaviour; the ability to modulate behaviour, especially its initiation or its cessation; the ability to generate an appropriate emotional response; and the ability to use strategies and tags for retrieving memories.

People with frontal lobe damage generally have difficulty knowing how to organize behaviour to reach a goal. Although they may know the components or steps in a process, they are unable to put them together in a coherent manner to reach a goal. For example, if they go to change a light bulb that has burned out, they will go to the cabinet where they typically store light bulbs. But if a bulb is not there, they may have difficulty organizing themselves to find the keys to the car, drive to the store, purchase new light bulbs, and then come back and replace the burned out bulb, although under other circumstances they are perfectly capable of finding their keys, driving to the store and back, and making a purchase.

Another problem observed in individuals with frontal lobe damage is an increase in 'psychological inertia', the force that must be overcome to either initiate or stop a process. Individuals with frontal lobe damage have an inability to overcome the inertia required to initiate a new behaviour or to cease the task in which they are engaged. This handicap may take many forms. For example, people with frontal lobe damage may not bathe and change their clothes each day on their own volition, but they will do so if directed by another individual; or an individual with frontal lobe damage may sit on a couch for hours without initiating any behaviour. Conversely, once involved in a behaviour, an individual with frontal lobe damage may find it impossible to stop acting in a particular manner. Such patients are likely to perseverate, i.e. perform the same behaviour repeatedly. There is now evidence that obsessive–compulsive disorders, which are characterized by people who engage in the same stereotyped motor behaviour to ward off some danger (e.g. constantly washing hands to prevent infection by germs), result from dysfunction of the frontal lobe, specifically the orbitomedial areas and subcortical regions to which they connect (e.g. Saxena *et al.*, 1998; Tallis, 1997).

Because the frontal lobes are relatively immature in young children, they tend to exhibit perseverative tendencies. If you hide a child's favourite toy behind one of many pillows, the young child will reach out and move the pillow behind which he or she believes the toy to be, but then sees that the toy is not there. Rather than moving another pillow, the child moves the same pillow, which still does not conceal the toy. The child is likely to repeat this behaviour many times. Although the child appears to forget where the toy is hidden, research suggests that very young babies (2–2.5 months old) can remember the location of an object, even when it is hidden behind another (e.g. Baillargeon, 1994). The problem is not so much one of memory but rather that the child seems to be locked into a perseverative behavioural loop in which the only way to look for the toy is to reach out and move that one particular pillow. This sort of behavioural loop is exactly the type of perseverative behaviour that may be exhibited after frontal lobe damage (Diamond, 1990).

Individuals with frontal lobe damage also have more general difficulties in modulating behaviour. For example, patients with frontal lobe damage are often socially uninhibited and inappropriate. They may make unwanted sexual advances to medical personnel, tell jokes at inappropriate times, be insensitive to the social context in which they find themselves, and so forth. This inability to modulate behaviour leads to the paradoxical effect that at certain times individuals with frontal lobe damage are insensitive to their social surroundings, whereas at other times they are unduly swayed by them. For example, by talking about depressing topics, physicians and other medical personnel may find an individual with frontal lobe damage moved to tears, but they can reverse the effect and put him or her in an ecstatic mood within minutes by talking about the person's favourite possessions or activities.

Frontal regions of the brain are also involved in 'metamemory functions', which can be thought of as the abilities that allow for the strategic use, deployment and retrieval of memories. These functions are clearly related to memory, but they do not involve actually remembering a particular item, individual or fact. In general, the difficulties in metamemory observed after frontal lobe damage are problems with the temporal sequencing or tagging of memories. For example, individuals with frontal damage are unable to determine which of two items in a sequence occurred more recently (Milner *et al.*, 1991), even though they can clearly distinguish between items that appeared in a sequence and those that did not (Milner and Petrides, 1984). Moreover, if we show individuals with frontal lobe damage a series of items in which some items appear randomly on multiple occasions (e.g. three times, five times, seven times), these people have difficulty in estimating how often an item occurred although they have no difficulty in discerning whether an item was viewed previously (Smith and Milner, 1988). Finally, individuals with frontal lobe damage have trouble knowing the source of a memory (e.g. remembering that they learned in a travel guide that Rome had a million inhabitants in AD 100) (Janowsky *et al.*, 1989). These types of deficits are often observed in the dementias associated with neurological disorders such as Parkinson's disease and Huntington's disease, because they compromise the integrity of basal ganglia–frontal connections (e.g. Cummings and Benson, 1988). In such cases, recognition memory (e.g. 'Was Churchill the leader of England during the Second World War II?') tends to be intact because the individual need not organize an effortful search through memory. In contrast, effortful retrieval of information (e.g. recall) or metamemory functioning is compromised (e.g. Ivory *et al.*, 1999).

Frontal regions, especially prefrontal areas, are also considered important for keeping memory information online to be used in giving a response, also known as working memory. Working memory is memory that has a limited capacity (7 ± 2 items) and that can be retained without rehearsal for only a short period of time (e.g. 10 s). The role of the prefrontal region in this function is well established, coming from various sources, including work with monkeys (e.g. Goldman-Rakic, 1990) young infants (Diamond, 1990) and adults (e.g. Belger *et al.*, 1998). Some researchers have suggested that ventrolateral regions are important for the storage of information in the working memory buffer, whereas more dorsolateral regions are important for manipulating the contents of working memory (e.g. Petrides, 1998; Wagner, 1999) or for selecting the contents of working memory (see Smith and Jonides, 1999 for a short review of this work). In addition, the prefrontal region seems especially important for creating an attentional 'set', i.e. holding online what it is that one must attend to (Banich *et al.*, 2000b). Dysfunction of the dorsolateral prefrontal region with concomitant disruptions in working memory has been implicated specifically in schizophrenia (e.g. Callicott *et al.*, 2000). If one cannot hold information online or select what is relevant for task performance, then serious disruption in mental processing is likely to ensue.

Damage to frontal regions can have profound effects on personality. Often, the family and other loved ones of an individual who has sustained frontal lobe damage will comment that the individual does not seem to like him- or herself any more. A formerly quiet and peaceful person may be described as argumentative and prone

to outbursts, a previously conscientious and hard-working person may be characterized as irresponsible and lazy, and a previously kind and considerate person may appear to be selfish and uncaring. One of the most famous early cases in which such changes were documented was that of Phineas Gage, a railroad worker. While clearing a way for the railroad in 1848, an explosion blew a steel rod through his skull, damaging sections of his frontal cortex. Family and friends complained that he 'was no longer Gage', and that although his body was the same, the man they knew seemed to have vanished.

More recent research suggests that frontal lobe functioning not only influences personality, a characteristic considered to be constant over time, but that such functioning can also influence a person's internal emotional state or mood, which can vary over time from happy to sad, from calm to frustrated, from peaceful to agitated. Certain aspects of mood are related to the difference in activation between the right and left frontal regions. In particular, relatively higher activation of the left frontal region compared with that of the right is associated with positive mood. In contrast, relatively higher activation of the right frontal region compared with that of the left is generally associated with negative or dysphoric mood (e.g. Davidson, 1992). For example, after damage to left frontal regions, activation of such regions is clearly lower than that of right frontal regions — an imbalance associated with depressed mood. In fact, more than half of individuals with damage to left frontal regions due to stroke fit psychiatric definitions of either major or minor depression (Starkstein and Robinson, 1999). It is notable that the extent of the depression does not depend on the severity of the person's cognitive deficits, but rather is related to the location of the lesion within the left frontal lobe. Because it is an imbalance of frontal regions, rTMS over the left frontal regions, which has the effect of stimulating these regions by increasing regional cerebral blood flow, is therapeutically useful. However, if low-frequency rTMS is employed, which has the effect of decreasing regional cerebral blood, the symptoms of depression actually increase (Speer *et al.*, 2000).

Recent research has suggested that the orbitofrontal cortex plays a major role in decision making, especially that involving the evaluation of rewards and punishment. This region of the brain is the place where neural pathways connect limbic, visceral and autonomically responsive areas of the brain with higher cortical centres in the prefrontal cortex. Individuals with lesions to orbitofrontal regions will pick a deck of cards that can provide a big individual pay-off but on average yields a loss over that which provides smaller individual pay-offs but on average yields a gain (e.g. Bechera *et al.*, 2000). Likewise, activation of these regions as assessed via PET predicts performance on a gambling task (Grant *et al.*, 1999). Given the linkage of the orbitofrontal cortex to the evaluation of reward, it is not surprising that this brain region has been implicated in substance abuse (e.g. Volkow and Fowler, 2000).

Medial frontal regions, especially the anterior cingulate, have been implicated in error detection and monitoring. In particular, there is an ERP component known as the error-related negativity (ERN) that occurs 80–150 milliseconds after the production of an incorrect response (Gehring *et al.*, 1993), whose source appears to be the anterior cingulate (DeHaene *et al.*, 1994). This component is reduced in individuals with obsessive–compulsive disorder (Gehring *et al.*, 2000) and may be heightened in individuals with negative affect (e.g. Luu *et al.*, 2000).

As this chapter illustrates, the frontal regions of the brain are involved in a vast array of behaviours. Rather than being important for specific domains of cognitive activity, such as language, spatial processing or object recognition, frontal regions provide us with executive capabilities that are used across a vast number of domains and allow for flexible and adaptive behaviour.

The Parietal Lobe

The parietal lobe of the cortex plays a role in (1) integrating information from various sensory modalities, (2) integrating information from the sensory world with information stored in memory, and (3) integrating information about an individual's internal state with information from the external sensory world. Because this integrative function can occur in various ways, the deficits observed after parietal lobe damage are often diverse and difficult to conceptualize as all falling under the same rubric. However, if the role of the parietal lobe is conceptualized as associating different forms of information, then the array of functions performed by the parietal lobe will not seem so disjointed. As an example, single-cell recordings in monkeys indicate activity in parietal areas when a conjunction of conditions occurs. For example, a cell in the parietal lobe might fire only when a banana is in view *and* that banana is within the monkey's reach. Alternatively, another cell in the parietal lobe might not fire at the sight of the banana alone but would fire only if the animal was also hungry. Thus, in both cases the cell would fire in response to some conjunction of attributes: the visual stimulus of the banana and its position in space, or the visual stimulus of the banana and the animal's internal state (e.g. Lynch, 1980).

In humans, the role of the parietal lobe in multimodal integration is seen in many syndromes that occur after damage to this region, including agnosia, alexia, agraphia and apraxia. Agnosia is a modality-specific deficit in recognizing objects, meaning that the individual cannot recognize an object in one sensory modality but can recognize it in other modalities. For example, if an individual has a visual agnosia, he or she will be unable to identify an item as a rose merely by looking at it. However, if the person is pricked by a thorn or smells the flower, he or she will recognize it instantly. An important point to note about agnosia is that the deficit can be attributed neither to the inability to perform basic sensory processing nor to a memory deficit. Persons with visual agnosia are not blind. They can distinguish light from dark and they can discriminate basic shapes (e.g. square from rectangle) (Warrington and James, 1988). So, for example, when looking at a rose, a person with visual agnosia can see that an object is there, describe its colour, and maybe even crudely describe its shape, but they cannot use this information to gather a visual impression of a rose. Furthermore, memory for the item is intact. So, for example, if asked what kinds of flowers compose the garland that is placed around the neck of the horse that wins the Kentucky Derby, or which popular flower with thorns is associated with romance, then the person with visual agnosia could easily answer 'rose'.

Agnosias can occur in all modalities. In auditory agnosia, an individual knows that a sound has occurred but they do not know its significance; for example, they cannot identify a particular sound as a car horn, even though he or she knows that some sound has just occurred. Likewise, in tactile agnosia, objects cannot be recognized by touch.

What is common to all these agnosias is that basic sensory processing in the affected modality is intact, as are memory processes. Tactile agnosia is most often associated with parietal damage, visual agnosia with parieto-temporal damage, and auditory agnosia with temporal lobe damage.

Two other deficits common after parietal lobe injury are alexia and agraphia, which are, respectively, the inability to read and the inability to write as a result of brain damage. A parietal locus for these functions is consistent with the operations that are involved in reading or writing. Psychological research has suggested that there are two distinct manners by which reading and writing occur. In the phonological route, a linkage is made between each letter and a sound (e.g. 'bat' ⇒ /ba/ /a/ /ta/), and then the sound pattern is linked to information stored in memory (/ba/ /a/ ta/ ⇒ 'a mammal that flies around and eats insects; seen most often at dusk'). The

alternative route bypasses sound entirely because a pattern of letters is linked directly with meaning (e.g. 'dog' ⇒ a favourite household pet). For both routes, a person must take a visual form and arrive at meaning via association. These deficits, as might be expected, are observed only after left, not right, hemisphere damage.

Notice that the localization of these written language functions is quite distinct from those that produce aphasia (the inability to produce spoken language). As discussed earlier, Broca's aphasia, which is characterized by an inability to produce fluent output in the face of intact production, is associated with damage to frontal regions (the frontal operculum, or Brodmann area 44), while Wernicke's aphasia, which is characterized by an inability to comprehend language in spite of fluent output, is associated with damage to the parieto-temporal junction. Hence, the neural machines that control spoken and written language are quite distinct. Interestingly, it also appears that the neural substrates for reading written language are distinct from those required to read music (e.g. Sergent, 1993). Musical notation relies more on the appreciation of spatial relationships between symbols than do standard language systems.

Still another deficit observed after damage to parietal regions is apraxia, which is the inability to perform skilled motor movement in an abstract manner. Basic motor control is intact, and the individual is not paralysed, yet these people have trouble linking motor movement to a representation. People with apraxia can usually make certain voluntary movements without difficulty, but they cannot pantomime them. For example, an individual with apraxia might be able to put a spoonful of sugar into his or her coffee cup, but when asked to pantomime the same gesture, they might use one finger to represent the spoon rather than positioning the hand as appropriate for stirring sugar into coffee. Apparently, individuals with apraxia lack the capacity to programme the motor sequences that allow for the representation of an act, but these people have the capacity to perform the act itself. Apraxia may also involve the inability to perform gestures that have symbolic significance. For example, an individual may not be able to make the sign of the cross, the victory symbol or the 'OK' sign. Once again, these deficits are most prominent after left hemisphere damage.

Other abilities affected by parietal lobe damage include disturbances in spatial processing. Damage to parietal regions disrupts the ability to localize points in space, to know the angle of lines, and to understand spatial relations between items. Parietal regions of the brain appear to contain the map of space that we use for navigation, for placing our limbs in the correct position for the manipulation of objects, and for knowing what part of space to explore. It was originally thought that most aspects of spatial processing are performed by the right hemisphere. However, more recently it has been noted that the right hemisphere may be involved in those spatial processes involved in the metric or distance aspects of spatial processing (e.g. knowing that two items are 5 cm apart), whereas the left hemisphere may be more involved in spatial processing that involves categorization of spatial relations (e.g. knowing that the glass is to the left of the pen) (Chabris and Kosslyn, 1998). Notice that knowing the distance between two items provides no information on their categorical relationship (e.g. which is to the left and which is to the right), nor does their categorical relationship provide information about the distance that separates them. This is but another example of how the hemispheres may work in parallel in service of cognitive processes.

Right parietal regions have also been implicated in overall arousal and attention. Damage to parietal regions, more so than damage to other regions of the brain, leads to disruption in attention and attentional regulation. This can vary from disruptions in mood states (e.g. decreased arousal in depression; e.g. Heller *et al.*, 1995) to disruptions in the ability to attend to more than place in the visual world (i.e. Balint's syndrome). Typically, such deficits are observed after right, rather than left, hemisphere damage. Probably the most common manifestation of dysregulation in attention is that of hemi-neglect, which typically occurs after a stroke to the right parietal regions. Individuals with this disorder will ignore information on the contralateral side (typically the left); they will forget to eat food on the left side of the plate, ignore people on their left, not shave the left side of the face, and so forth. It can be demonstrated that when stimuli are particularly potent (e.g. a loud noise), individuals can process information contralesionally. Thus, they do not have a sensory deficit that impedes their ability to process the information; rather, they have a limited arena of space to which they can direct their attention, as it is restricted to only one side. In the most severe form of hemi-neglect, almost all contralateral information is ignored. As the neglect abates, as typically occurs with time from onset, the ability to attend to contralesional information improves. Yet, if there is competition between information on the contralesional and ipsilesional side (i.e. bilateral stimulation), then only the ipsilesional information is apprehended whereas that situated contralesionally is extinguished. It has been suggested that this extinction phenomenon occurs because attention gets captured by ipsilesional stimuli and cannot be disengaged to process contralesional stimuli. Support for such a notion comes from studies demonstrating that disrupting activation of the intact hemisphere by TMS or other means ameliorates the hemi-neglect (e.g. Olivieri *et al.*, 1999).

As evidenced by this chapter, damage to the parietal regions can cause a heterogeneous array of syndromes. In general, however, they are all syndromes in which sensory information cannot be integrated across modalities and/or with internal representations or memories.

The Temporal Lobe

Temporal regions of the brain are associated with four main functions: memory, visual item recognition, auditory processing and emotion. Classically, the temporal lobes have been associated with memory function, as documented most clearly in the famous case of H.M., who in early adulthood underwent bilateral removal of anterior portions of the temporal lobe for the relief of intractable epilepsy. Although the surgery was successful in reducing his seizures, he was left with the inability to learn almost all types of new information, even though most of his memories from the years before the operation were intact. This case was pivotal in demonstrating that specific regions within the temporal lobe, most notably the hippocampus, are critical for the formation of new long-term memories. Additional research by Milner and colleagues demonstrated that the memory deficit tends to be greater for verbal material after removal of only the left temporal lobe (e.g. Frisk and Milner, 1990) and greater for spatial information after removal of only the right temporal lobe (Smith and Milner, 1981).

In addition to being important for the formation of new long-term memories, temporal regions of the brain play important roles in visual processing, contributing to visual item recognition. Electrical recordings from single cells in the inferior temporal lobes of monkeys have revealed that these cells respond only to highly specific visual stimuli. Unlike cells in the primary visual cortex, which respond to bars of light oriented at particular angles and moving in particular directions, the cells of the inferior temporal lobe respond to very specific shapes, such as a hand, a brush or a face (Gross *et al.*, 1972). In fact, some of the cells may respond only to the faces of particular people or certain features on a face (e.g. eyes) (Perrett *et al.*, 1987). This specificity of visual processing in temporal regions appears to be a characteristic of the mammalian nervous system. For example, certain cells in the temporal cortex of sheep respond to horned sheep but not unhorned sheep, whereas other cells respond only to sheepdogs but not wolves or dogs with pointed ears (because they resemble wolves) (Kendrick and

Baldwin, 1987). In humans, damage to temporal regions can lead to deficits such as the inability to recognize common objects like cars or chairs (Farah and Feinberg, 2000) or that a given face belongs to a specific individual (De Renzi, 2000). Thus, at least for visual items, temporal regions appear to be important for item identification.

This specialization of temporal regions for visual item processing reflects a segregation of the processing of visual information in the mammalian brain into two streams or systems. On the basis of neuroanatomical, neurophysiological and behavioural work with animals, Ungerleider and Mishkin (1982) suggested that visual information leaving primary occipital areas bifurcates into two pathways, one of which courses dorsally to the parietal lobe, which is important for processing the locations of items, and the other of which travels ventrally to the temporal lobe, which is important for processing the shapes of items.

Because auditory processing areas are located in the temporal lobe, damage to this region of the brain can have consequences for the processing of auditory material. For example, damage in the temporal lobe can lead to auditory agnosia or to difficulties in the appreciation of certain aspects of music, such as pitch (e.g. Zatorre, 1998). Brain-imaging studies also indicate activation of temporal regions, especially that of the right hemisphere, when a tune is running through someone's mind (e.g. Halpern and Zatorre, 1999).

Temporal regions of the brain have also been implicated in the processing of emotional information. The temporal lobe contains structures that are portions of the limbic system, which act to integrate information from the sensory world with internal urges (e.g. urges for food, sex, etc.). One prominent structure in the limbic system located in the temporal lobe is that of the amygdala, which has been implicated in modulating fear responses, especially when incoming information is ambiguous (e.g. Whalen, 1998). It is also implicated in anxiety disorders (e.g. Rosen and Schulkin, 1998), such as panic disorder (e.g. Goddard and Charney, 1997). Moreover, disruptions of temporal lobe functioning can have emotional consequences. For example, some investigators have suggested that temporal regions may be dysfunctional in a certain proportion of people with schizophrenia, most notably those suffering from delusions (Schroder *et al.*, 1995).

SUMMARY

Neuropsychology has much to contribute to understanding a broad number of psychiatric disorders. Damage or dysfunction of a specific brain region has multifaceted consequences, some of which affect mainly cognitive processes while others affect emotional processes. It is the interrelationship between these two sets of consequences that has many implications for our understanding of psychiatric disorders, ranging from depression (e.g. Heller and Nitschke, 1997) to substance abuse (Volkow and Fowler, 2000). The classic approach borrowed from neuropsychology to understanding psychiatric disorders has been that of the lesion method, which makes linkages between dysfunction of specific regions of brain tissue and particular mental consequences. Although such an approach has been fruitful for certain disorders, there are many psychiatric disorders that are produced by more diffuse rather than focal brain dysregulation, schizophrenia being a good example. Nonetheless, neuropsychology will continue to play an important role in understanding the mechanisms of psychiatric disorders and the evaluation of the efficacy of new treatments due to a plethora of new methodologies that can be used singly or in tandem. Evaluation of drug therapies via fMRI, treatment of depression with rTMS, and insights into obsessive–compulsive disorders with ERP recordings are but a few examples of the promise that a neuropsychological approach holds for psychiatry.

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XI

Brain Imaging

Steven Laureys, Philippe Peigneux and Serge Goldman

INTRODUCTION

While philosophers have, for centuries, pondered upon the relation between mind and brain, neuroscientists have only recently been able to explore the connection analytically — to peer inside the black box. This ability stems from recent advances in technology and emerging neuroimaging modalities. It is now possible not only to produce remarkably detailed images of the brain's structure (i.e. anatomical imaging) but also to capture images of the physiology associated with mental processes (i.e. functional imaging). We are able to see how specific regions of the brain 'light up' when activities such as reading this book are performed, and how our neurons and their elaborate cast of supporting cells organize and coordinate their tasks. As demonstrated in the other chapters of this book, the mapping of the human mind (mostly by measuring regional changes in blood flow, initially by positron emission tomography (PET) and recently by functional magnetic resonance imaging or (fMRI)) has provided insight into the functional neuroanatomy of neuropsychiatric diseases.

Amazingly, the idea that regional cerebral blood flow (rCBF) is related intimately to brain function goes back more than a century. As is often the case in science, this idea was initially the result of unexpected observations. The Italian physiologist Angelo Mosso first expressed the idea while studying pulsations of the living human brain that keep pace with the heartbeat (Mosso, 1881). These brain pulsations can be observed on the surface of the fontanelles in newborn children. Mosso believed that they reflected blood flow to the brain. He observed similar pulsations in an adult with a post-traumatic skull defect over the frontal lobes. While studying this subject, a peasant named Bertino, Mosso observed a sudden increase in the magnitude of the 'brain's heartbeats' when the church bells signalled 12 o'clock, the time for a required prayer. The changes in brain pulsations occurred independently of any change in pulsations in the forearm. Mosso understood that the bells had reminded Bertino of his obligation to say a silent Ave Maria. Intrigued by this observation, Mosso then asked Bertino to perform a mental calculation; again, he observed an increase in pulsations and, presumably, blood flow as the subject began the calculation and a second rise just as he answered. This was the first study ever to suggest that measurement of cerebral blood flow might be a way of assessing human cognition.

Charles Roy and Charles Sherrington characterized this relationship further at Cambridge University. Based on animal experiments, they suggested that 'the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity'. Their observations were fundamental, and their brilliant deductions have dominated the brain-imaging field since. One of the most extraordinary examples of the relationship posited by Roy and Sherrington was observed in Walter K., a German-American sailor who consulted Dr John Fulton at Boston's neurosurgery clinic for headache and failing vision. Walter also reported to hear a humming noise in his head. Fulton, when listening with a stethoscope at the back of his patient's head, confirmed this bruit and organized an exploratory intervention. Dr Harvey Cushing performed the neurosurgery and found a large arteriovenous malformation overlying the visual cortex. An attempt to remove the vascular malformation failed and left Walter with a bony defect overlying his visual cortex. His physicians could now hear the bruit even more clearly. During the course of his stay, Walter mentioned that the noise in his head became louder when he was using his eyes. As Fulton (1928) published later in the journal *Brain*:

> It was not difficult to convince ourselves that when the patient suddenly began to use his eyes after a prolonged period of rest in a dark room, there was a prompt and noticeable increase in the intensity of his bruit . . . Activity of his other sense organs, moreover, had no effect upon his bruit. Thus, smelling tobacco or vanilla did not influence it, straining to hear the ticking of a distant watch produced no effect, and ordinary quiet conversation was without demonstrable influence.

In order to document his remarkable observations for others to appreciate, Fulton recorded the sounds of the bruit while his patient 'was allowed to lie on his stomach in a comfortable position, with his chin resting on the edge of a chaise-longue in such a way that he could close his eyes and open them to read a newspaper lying on the floor'. Within 20–30 seconds after Walter began to use his eyes to read the newspaper, there was a noticeable increase in the intensity of the bruit. If the lights were put out, the bruit continued for nearly a minute afterward; then it gradually subsided, and at the end of two minutes it had returned almost to resting level. Recent research capable of recording changes in blood flow within milliseconds has provided remarkable confirmation of these pioneer observations made with only a stethoscope and a simple recording device. After many such studies, Fulton 'gained the impression' that it was the effort of trying to discern objects that were just at the limit of his patient's acuity that brought on the increases of the bruit. Merely shining light into his eyes when he was making no mental effort had no effect. This was, indeed, a remarkable observation, the significance of which would not be appreciated for many years. It was probably the first ever recorded result of top-down influences on sensory processing (Posner and Raichle, 1994).

In what follows, we will introduce the vast area of anatomical brain imaging (X-ray computed tomography (CT) and magnetic resonance imaging (MRI)) and functional brain imaging (PET, single-photon emission tomography (SPECT), fMRI, electroencephalography (EEG), event-related potentials (ERPs), magnetoencephalography (MEG), magnetic resonance spectroscopy (MRS),

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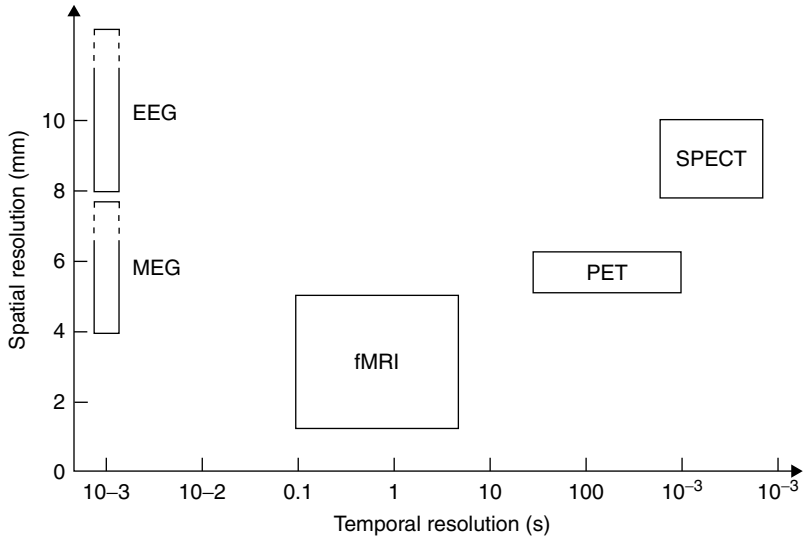

Figure XI.1 Approximation of the resolution in time and space of the most commonly employed functional neuroimaging techniques based on measurements of haemodynamic (fMRI, PET, SPECT) and electrical (EEG, MEG) activity of the brain

transcranial magnetic stimulation (TMS), and near-infrared spectroscopy (NIRS)). Each technique provides different information and has its own advantages and disadvantages in terms of cost, safety and temporal and spatial resolution (Figure XI.1). After briefly discussing their history and basic principles, we will present a short overview of study design and methods to process and analyse functional neuroimaging data.

COMPUTERIZED TOMOGRAPHY

History

The modern era of medical imaging began in the early 1970s, with the introduction of a remarkable technique called X-ray computed axial tomography, now known as CAT, X-ray CT or just CT. The theories that underlie tomographic imaging have existed since 1917, when the German mathematician Radon published his work dealing with reconstruction from image projections. Extension of this work by the South African physicist Allan Cormack in the early 1960s, and its practical applications by the British engineer Sir Godfrey Hounsfield, resulted in the construction of the first CT scanner. Both investigators received the Nobel Prize in 1979 for their independent contributions. Crucial in the development of X-ray CT was the emergence of clever computing and mathematical techniques to process the vast amounts of data necessary to reconstruct the images.

The development of X-ray CT had two consequences. First, it changed forever the practice of medicine because, for the first time, clinicians could non-invasively clearly view living human tissue such as the brain (standard X-rays reveal only bone and some surrounding tissues). Second, it immediately stimulated engineers and scientists to consider alternative ways of creating images of the body's interior using similar mathematical and computerized strategies for image reconstruction (e.g. SPECT and PET) (Posner and Raichle, 1994).

Despite its wide availability, CT has been replaced by the more sensitive MRI as the procedure of choice for cerebral imaging. CT is useful mainly when rapid information about the state of the brain is desired. In particular, it helps in making the choice between surgical and medical management of patients with sudden onset of neurological symptoms; such conditions include head trauma and stroke (where a differentiation between haemorrhage and infarction is important). CT is also used widely for the evaluation of lesions that involve bone (e.g. fractures or bone metastases) and calcifications within lesions of the brain.

Basic Principles

X-rays (i.e. a form of electromagnetic radiation travelling at the speed of light and carrying a large amount of energy) are capable of knocking an electron out of its orbit, therefore they are a form of ionizing radiation. A CT scanner delivers a narrow beam of X-rays that pass through the head. The exiting beam is then collected by a set of detectors, converted into digital data, and fed into a computer for image reconstruction. As the beam travels through the brain, it undergoes attenuation due to interaction with the various tissues it encounters. The degree of attenuation depends on the tissue density: very dense tissue, such as bone, attenuates lots of X-rays, cerebral grey matter attenuates some X-rays, and fluid attenuates even fewer. X-ray detectors positioned in a circle around the head collect attenuation readings as the beam is delivered from multiple angles. A computerized algorithm reconstructs a slice from these multiple readings. Thus, the contrast in a CT image is due to differences in X-ray attenuation among various tissues.

The use of exogenous contrast media in CT (and MRI) of the brain improves the sensitivity of detection and delineation of pathological structures, such as tumours, inflammation and ischaemia. CT contrast agents, like all commonly employed radiographic contrast agents, utilize substances with a high electron density (typically iodated agents) to absorb X-rays and thus produce a contrast effect. In both CT and MRI, a disruption of the blood–brain barrier results in accumulation of the intravenously administered contrast material in the extravascular space, leading to signal enhancement.

MAGNETIC RESONANCE IMAGING

History

MRI comprises a vast and varied array of techniques that use no ionizing radiation and provide an enormous range of information.

From an established ability to provide high-quality structural information, magnetic resonance techniques are advancing rapidly and providing other clinically relevant physiological information, such as spectroscopic studies illuminating the details of biochemical status (MRS; see below), blood oxygenation level allowing functional activation studies (fMRI; see below), cerebral blood compartment (magnetic resonance angiography or (MRA)); perfusion (perfusion-weighted imaging (PWI)), water molecular diffusion (diffusion-weighted imaging (DWI)), cerebral microstructure and fibre tracking (using diffusion anisotropy effects measured by diffusion tensor imaging (DTI)), magnetization transfer imaging, etc.

MRI derives from a potent laboratory technique, nuclear magnetic resonance (NMR), which was designed to explore detailed chemical features of molecules. In 1946, Felix Bloch of Stanford University and Edward Purcell of Harvard University discovered independently the phenomenon of NMR. They were awarded the Nobel Prize 6 years later. In the period between 1950 and 1970, the use of NMR was limited to chemical and physical molecular analysis. In 1971, Raymond Damadian showed that the nuclear magnetic relaxation times of tissues and tumours differed, thus motivating scientists to consider magnetic resonance for the detection of disease. NMR moved from the laboratory to the clinic when Paul Lauterbur found that it could form images by detecting protons. He used a back-projection technique similar to that used in X-ray CT to generate these images. Protons are interesting because they are abundant in the human body; by acting as little compass needles, they respond sensitively to magnetic fields. In 1975, Richard Ernst proposed MRI using phase and frequency encoding and the Fourier transform. This technique is the basis of current MRI techniques. It resulted in excellent images of the anatomy of the brain and other organs that far surpassed in detail those produced by CT. Six years later, Ernst was rewarded the Nobel Prize. In 1977, Peter Mansfield developed the echo-planar imaging (EPI) technique, which would permit the production of images at video rates (30 ms per image). By 1986, the NMR microscope was developed, allowing 10-mm resolution. In 1987, Charles Dumoulin perfected MRA, which could image flowing blood without the use of contrast agents. fMRI was developed in 1993 (see below). Clearly, the magnetic resonance technique is powerful and continuously expanding. Because of the negative connotations associated with the word 'nuclear' in the late 1970s, NMR is now commonly known as magnetic resonance imaging.

At present, MRI is the procedure of choice for the structural imaging of the brain. However, it is susceptible to movement artefacts, and patients who are on life-support systems, have gunshot wounds, or who have implanted MRI-incompatible material (pacemakers, prostheses, etc.) still represent problems. The main limit on the wealth of diagnostic information that can be obtained for each patient is in the duration of the procedure. Approximately 1 hour of magnetic resonance examination time is the practical limit of patient discomfort and cooperation for routine clinical studies. As scan times decrease through the use of new sequencing techniques, the available examination time can be used to improve the quality of the data or to obtain additional types of data. A range of magnetic resonance physiological measurement techniques is poised to fill any reclaimed imaging time. It is expected that further refinements of fMRI, MRA, MRS, PWI, DWI, DTI and other magnetic resonance techniques will allow them to fit into routine clinical practice.

Basic Principles

Some atoms, such as hydrogen (^{1}H), behave like spinning charges. Spinning nuclei will induce microscopic loops of electric current. As a result, they generate a very small magnetic field. MRI depends on the fact that these spinning atoms (mainly hydrogen nuclei, i.e. protons) act as little compass needles in the presence of a magnetic field. A strong magnetic field (approximately 10 000 times stronger than the earth's magnetic field) can align most of the atoms. By applying radiowave pulses to the tissue, the atoms can be perturbed in a precise manner. As a result, they emit detectable radio signals unique to the number and state of the particular atoms in the tissue. The strength of the MRI signal (i.e. the signal emitted when nuclei return to their equilibrium state) depends primarily on three parameters: the proton density in a tissue (the higher the density of protons, the larger the signal), the T_1 relaxation time (longitudinal magnetization), and the T_2 relaxation time (transverse magnetization). These properties are variable among tissues and are the predominant factors responsible for contrast among tissues. The signal intensity of one tissue compared with another (contrast) can be manipulated by varying the time elapsed between application of the radiofrequency pulse and sampling of the emitted signal (Pykett, 1982). The signal in magnetic resonance images is high or low (bright or dark) depending on the pulse sequence used and the type of tissue under study. On a T_1-weighted image, fat, subacute haemorrhage, melanin protein-rich fluid, slowly flowing blood, and paramagnetic substances (e.g. gadolinium) will appear bright. On a T_2-weighted image, increased water, as in oedema, tumour, infarction, inflammation, infection or subdural collection will appear bright (dark on T_1-weighted images). MRI contrast involves the use of relatively non-toxic paramagnetic agents, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA). Unlike CT contrast agents, MRI contrast enhancement is indirect. Indeed, paramagnetic substances will act as relaxation centres for other nuclei in the local microenvironment and shorten the magnetic relaxation times of the surrounding hydrogen nuclei. MRI is a more sensitive method of detection of contrast, requiring dosages in the range of one-twentieth of that for CT.

The techniques used in MRI of the brain depend on the primary goal of its procedure (e.g. anatomical detail is best obtained using T_1 sequences, while inflammation and oedema are better visualized using T_2 sequences). As mentioned previously, MRI can provide a wealth of information not only about intrinsic T_1 and T_2 relaxation properties and spin density but also regarding blood and cerebrospinal fluid flow, bulk motion, diffusion, diffusion anisotropy, perfusion, local oxygenation, local iron content, membrane permeability, temporal dynamics of contrast agent interaction, etc.

POSITRON EMISSION TOMOGRAPHY

History

PET has its roots in tissue autoradiography, a method used for many years in animal studies to investigate organ metabolism and blood flow. In tissue autoradiography, a radioactively labelled compound is injected into a vein. After the compound has accumulated in the organ under interest (such as the brain), the animal is sacrificed and the organ removed for study. The organ is sectioned carefully and the individual slices are laid on a piece of film sensitive to radioactivity. This X-ray film records the distribution of radioactively labelled compound on each slice of tissue. After film development, a picture of the distribution of radioactivity within the organ is obtained, hence regional information on the organ's specific functions can be deduced. The injected radioactive compound determines the type of information. Radioactively labelled water, for example, measures cerebral blood flow. In the late 1940s, Seymour Kety developed this technique for autoradiography in laboratory animals. Accumulation of a radioactively labelled form of glucose measures cerebral metabolism because glucose is the primary source of energy for neurons. In 1977, thanks to such a tracer, Louis Sokoloff introduced a now widely used autoradiographic method for the regional investigation of neuronal activity.

Researchers in the field of tissue autoradiography became fascinated when CT was introduced in the 1970s. They realized that if the anatomy of an organ could be reconstructed by passing an X-ray beam through it, then the distribution of a previously administered radioisotope could also be reconstructed *in vivo*. They simply had to measure the emission of radioactivity from the body section. With this insight was born the idea of autoradiography of living human subjects. A crucial element was the choice of the radioisotope. A class of radioisotopes was selected that emitted positrons (i.e. particles identical to electrons, except that they carry a positive charge). A positron will combine immediately with a nearby electron. They will annihilate each other, emitting two gamma rays in the process. Because the gamma rays travel in opposite directions, detectors around the sample can detect the gamma rays and locate their origin. The crucial role of positrons in human autoradiography gave rise to the name positron emission tomography (Ter-Pogossian *et al.*, 1980).

Throughout the late 1970s and early 1980s, PET was developed rapidly to measure various activities in the brain, such as glucose metabolism, blood flow (see below), oxygen consumption, and uptake of drugs. Although PET is primarily a research tool for brain imaging, its increasing availability in medical centres for oncology and cardiac imaging makes likely its more widespread application to neuropsychiatric diseases. The most frequently performed PET studies measure resting regional cerebral metabolic rates for glucose (rCMRGlu) or changes in rCBF as indirect indices of neural synaptic activity (Magistretti and Pellerin, 1999). Recent developments are PET/CT combined imaging (offering improved attenuation correction and co-registration or fusion of the functional PET image with a high-anatomic-resolution CT image) and improved detector materials, such as lutetium oxyorthosilicate (LSO) scintillators (offering higher stopping power and counting rates).

Basic Principles

PET scanning involves the administration of positron-emitting radionuclides with short half-lives in which particle disintegration is captured by multiple sensors positioned around the head. The radiotracer is administered into a vein in the arm and is taken up by the brain through the bloodstream. After a course of a few millimetres, the positron will interact with an electron in the brain tissue and produce two high-energy photons at approximately 180 degrees apart from each other. In the PET scanner, a ring of detectors around the patient's head can detect these coincident photons. As the radioactive compound accumulates in different regions of the brain, and positron annihilations occur, the scanner detects the coincident rays produced at all positions outside the head and reconstructs an image that depicts the location and concentration of the radioisotope within a plane of the brain. This emission scan is then corrected by comparison with the attenuation image made from a transmission scan of the subject's head. PET studies involve the use of a cyclotron to produce the radioactive tracers. The type of information on the PET image is determined by the administered radiolabelled compound. Oxygen-15, fluorine-18, carbon-11 and nitrogen-13 are common radioisotopes that can combine with other elements to create organic molecules that can substitute for natural substances, such as water, glucose, levodopa (L-dopa), benzodiazepine-receptor ligands, etc. Using different compounds, PET can assess regional blood flow, oxygen and glucose metabolism, and neurotransmitter and drug uptake in the tissues of the working brain. PET can sample all parts of the brain with equal resolution and sensitivity. Typically, it can locate changes in activity with an accuracy of about 6 mm. PET measurements of radioligand binding are discussed elsewhere (see Chapter XIX.10).

Cerebral Metabolic Rate for Glucose

To study regional cerebral glucose utilization, a positron-labelled deoxyglucose tracer is used, [^{18}F]fluorodeoxyglucose (FDG) (Huang *et al.*, 1980). This tracer is taken up by active brain regions as if it was glucose. However, once inside the cell, FDG is phosphorylated by hexokinase to FDG-6-phosphate, which is not a substrate for glucose transport and cannot be metabolized by phosphohexoseisomerase, the next enzyme in the glucose metabolic pathway. Thus, labelled FDG-6-phosphate becomes metabolically trapped within the intracellular compartment. The amount of radioactive label that eventually remains in each discrete region of the brain is related to the glucose uptake and metabolism of that particular region. An FDG-PET scan summates approximately 30 minutes of cerebral glucose metabolism and allows assessment of regional variations. However, given the half-life of ^{18}F (2 h), it is less suited for brain-activation studies.

Cerebral Blood Flow

Most PET activation studies rely on the administration of radioactively labelled water, specifically hydrogen combined with oxygen-15, a radioactive isotope of oxygen ($H_2{}^{15}O$). The labelled water emits copious numbers of positrons as it decays (hydrogen isotopes cannot be used because they do not emit positrons). In just over a minute after intravenous injection, the radioactive water accumulates in the brain, forming an image of blood flow. The radioactivity of the water produces no deleterious effects. Oxygen-15 has a half-life of only 2 minutes, and an entire sample decays almost completely in about 10 minutes (five half-lives) into a nonradioactive form. The rapid decay substantially reduces the exposure of subjects to the potentially harmful effects of radiation. Moreover, only low doses of the radioactive label are necessary. The fast decay and small amounts permit many measures of blood flow to be made in a single experiment. In this way, $H_2{}^{15}O$-PET can take multiple pictures of the brain at work in different experimental conditions. Each picture represents the average neural activity of about 45 seconds. The total number of scans that can be made per subject (typically about 12 images) is limited by the exposure to radiation.

In the last decade, PET has been the technique used most widely to assess the neural substrates of cognitive processes at the macroscopic level. At present, it is superseded by fMRI for many applications (see below). It remains, however, a powerful tool in receptor imaging (e.g. assessment of neurotransmitter or drug uptake) and molecular imaging (e.g. assessment of gene expression or protein synthesis) in both normal and pathological states (Phelps, 2000).

SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY

History

The history of SPECT parallels that of PET. The development of nuclear tomographic techniques paralleled the advances in X-ray CT in the early 1970s (see above). In 1977, Keyes constructed the first rotating gamma camera SPECT system. In this technique, multiple angular projections are accumulated as the camera turns in orbit around the subject's head. (The mathematically equivalent technique of holding a gamma camera head steady while rotating the patient in a chair in front of the camera has been tried successfully but has not caught on commercially.) Hybrid SPECT systems, including coincidence cameras (which can perform both SPECT and PET imaging), and hybrids that incorporate a CT

scanner along with a SPECT system (which offer improved attenuation correction and perfect co-registration or fusion of the SPECT scan with a high-anatomical-resolution CT scan), are currently being developed (Groch and Erwin, 2001).

In general, SPECT tracers are more limited than PET tracers in the kinds of brain activity they can monitor, but they are longer lasting. Thus, SPECT does not require an on-site cyclotron. However, most SPECT technology is relatively nonquantitative, does not permit measured attenuation correction, and has a spatial resolution inferior to that of PET. On the other hand, SPECT is less expensive and more widely available.

Basic Principles

Similar to PET, SPECT also uses radioactive tracers, but it involves the detection of individual photons (low-energy gamma rays) rather than positrons emitted at random from the radionuclide to be imaged. A SPECT scanner uses two or three cameras that rotate around the patient to record data at different angles. Images of active brain regions are then reconstructed by combining a finite number of projections. Typical radionuclides include technetium-99m (^{99m}Tc) and iodine-123 (^{123}I) with half-lives of 6 and 13 hours, respectively. On average, SPECT acquisition times are 20–30 minutes.

Frequently used radiolabelled agents for brain perfusion SPECT are: (1) Tc-99m-hexamethyl propylamine oxime (Tc-99m-HMPAO), a lipid-soluble macrocyclic amine with a rapid brain uptake (reaches its maximum within 10 min of injection, and its distribution remains constant for many hours post-injection); (2) Tc-99m-bicisate ethyl cysteinate dimer (Tc-99m-ECD), with rapid uptake and very slow clearance from the brain (blood clearance is also rapid, resulting in high brain-to-soft-tissue activity ratios); (3) I-123-isopropyliodoamphetamine (I-123-IMP); and (4) the inert gas xenon-133, which permits the quantitative measurement of rCBF without the need for arterial sampling (however, this requires a technically difficult inhalation technique, and it has a poor spatial resolution). The long half-life, rapid brain uptake and slow clearance of most radiolabelled agents for brain perfusion SPECT offer the opportunity to inject the tracer at a time when scanning is impossible (e.g. during an epileptic crisis) and to scan (post-event) the associated distribution of activated brain regions.

In addition to their use in determining perfusion, radiotracers can also be used to determine biochemical interactions, such as receptor binding. Iodine-123-labelled ligands, such as iodo-hydroxy-methoxy-N-[(ethyl-pyrrolidinyl) methyl]-benzamide (IBZM), have been developed for imaging the dopamine receptor system and are used in studies of movement disorders and schizophrenia (IBZM is a D2 receptor agonist that shows high uptake in the striatum and can be displaced by haloperidol). Iodine-123-quinuclidinyl-iodobenzilate (I-123-QNB), an acetylcholine muscarinic antagonist, has been used to image these receptors in the brains of normal subjects and patients with Alzheimer's disease. Other iodine-123 labelled ligands have been used for imaging the benzodiazapine and serotonin receptors. These radiotracers, however, have not yet reached the stage of routine clinical practice, as a consensus on measuring receptor binding using single-photon tracers has not yet been reached, nor have diagnostic clinical strategies been identified.

FUNCTIONAL MAGNETIC RESONANCE IMAGING

History

In 1935, Nobel Laureate Linus Pauling discovered that the amount of oxygen carried by haemoglobin affects its magnetic properties. In 1990, Sejji Ogawa and his colleagues at AT&T Bell Laboratories demonstrated that MRI could detect these small magnetic fluctuations. Several research groups realized immediately the importance of this observation. By the middle of 1991, it was shown that MRI could detect the functionally induced changes in blood oxygenation in the human brain. This ability has led to the term 'functional MRI'. The ability of fMRI to monitor the oxygen signal in real time is limited not by technique but by physiology. Indeed, the stumbling block is the speed of neural activity with the rate of change of oxygenation levels. Signals from one part of the brain can travel to another in 10 ms or less. Unfortunately, changes in blood flow and blood oxygenation are much slower, occurring hundreds of milliseconds to several seconds later. Hence, fMRI is not able to keep up with the 'conversations' between brain areas. For the time being, the only methods that respond quickly enough are electrical recording techniques such as EEG and MEG (see below).

fMRI is taking the place of ^{15}O-labelled-water-PET as the procedure of choice for haemodynamic functional activation measurements. It has several advantages over PET activation studies. First, it does not require the injection of radioactive tracers as the signal comes directly from functionally induced changes in brain tissue (i.e. changes in venous oxygen concentration). Second, the spatial resolution is better, distinguishing parts as small as 1 or 2 mm (better than the PET resolution of about 6 mm). Third, the temporal resolution is better, monitoring changes in blood-flow-induced oxygen signal in real time, using echo-planar imaging (better than $H_2{}^{15}O$-PET resolution of about 45 s). Fourth, MRI provides both anatomical and functional information in each subject, hence permitting a more accurate structural identification of the active regions.

Some concerns have been raised about the intensity of the magnetic field to which the tissues are exposed in MRI, but so far there are no known harmful biological effects. The largest limiting factor is the claustrophobia some subjects may suffer as in most instrument designs the entire body must be inserted into a relatively narrow tunnel. Other limiting drawbacks are its susceptibility to the subject's movement artefacts and artefacts related to the use of metal-containing devices in the magnet (e.g. EEG electrodes, electrical wires).

Basic Principles

fMRI can detect an increase in oxygen that occurs in an area of heightened neuronal activity. The basis for this capacity comes from the way neurons make use of oxygen. Functionally induced increases in blood flow are accompanied by alterations in the amount of glucose the brain consumes but not in the amount of oxygen it uses. Indeed, despite the presence of abundant oxygen, the normal brain resorts to anaerobic metabolism during spurts of neuronal activity. Apparently, this physiological behaviour relies on tactics similar to those present in a sprinter's muscles. It is not understood fully why the brain acts in this way. Additional blood flow to the brain without a concomitant increase in oxygen consumption leads to a heightened concentration of oxygen in the small veins, draining the active neural centres. The reason for this is that supply has increased but the demand has not. Therefore, the extra oxygen delivered to the active part of brain simply returns to the general circulation by way of the draining veins.

The commonest form of fMRI is blood-oxygenation-level-dependent (BOLD) imaging (Ogawa *et al.*, 1990). The BOLD signal depends on the ratio of oxygenated to deoxygenated haemoglobin. In regions of neuronal activity, this ratio changes as increased flow of oxygenated blood temporarily surpasses consumption, decreasing the level of paramagnetic deoxyhaemoglobin. These localized changes cause increases in the magnetic resonance signal, which are used as markers of functional activation. Ultrafast scanning can measure these changes in the signal, which are mapped directly on to a high-resolution scan of the subject's anatomy. fMRI studies require magnets with field strengths superior to 1 tesla.

ELECTROENCEPHALOGRAPHY

History

EEG detects spontaneous brain electrical activity from the scalp. In 1848, the German physiologist Dubois-Reymond demonstrated that an externally recordable electrical signal occurred concomitantly with passage of a nerve impulse along a peripheral nerve. This discovery led the English physiologist Richard Caton to explore the possibility that nerve impulses flowing within brain cells might also produce electrical signals. In 1875, Caton published in the *British Medical Journal*, 'feeble currents of varying direction pass through the multiplier when one electrode is placed on the grey matter and one on the surface of the skull'. Caton demonstrated that the cerebral cortex had a tonic level of oscillatory electrical activity, and that additional phasic electrical activity could be evoked in response to peripheral sensory stimulation. The diagnostic potential of EEG was first hinted at in 1912 by the discovery of Kaufman that abnormal neuroelectric discharges could be recorded in experimentally induced epilepsy in animals. Despite the exciting developments in animal EEG, it was not until 1929 that Hans Berger, a German neuropsychiatrist, recorded the first human EEG. Hereafter, there was an explosion of human EEG studies with (of particular interest) the discovery of 'the EEG in epilepsy and in conditions of impaired consciousness' (Gibbs *et al.*, 1935). Today, EEG is considered a routine clinical procedure of substantial diagnostic value in neurology, neurosurgery and psychiatry, and it is proving to be a useful measure of brain physiology in neuroscientific research.

EEG provides temporal resolution in the millisecond range. However, traditional EEG technology and practice provide insufficient spatial detail to identify relationships between brain electrical events and structures and functions visualized by fMRI or PET. Recent advances help to overcome this problem by recording EEGs from more electrodes (experimental laboratories use 32–64 or even 120 or more electrodes), by registering EEG data with anatomical images, and by correcting the distortion caused by volume conduction of EEG signals through the skull and scalp. In addition, statistical measurements of sub-second interdependences between EEG time series recorded from different locations can help to generate hypotheses about the instantaneous functional networks that form between different cortical regions during mental processing. Physiological and instrumental artefacts (e.g. subject's eye or head movements, heartbeats, poor electrode contacts) can contaminate the EEG (and MEG), so care must be taken to correct or eliminate such artefacts before further analyses are performed.

Basic Principles

Scalp-recorded EEGs in the waking state in healthy adults normally range from several to about 75 μV. The EEG signal is largely attributable to graded postsynaptic potentials of the cell body and large dendrites of vertically oriented pyramidal cells in cortical layers 3 to 5. These are synchronized by rhythmic discharges from thalamic nuclei, with the degree of synchronization of the underlying cortical activity reflected in the amplitude of the EEG. Most of the EEG signal originates in cortical regions near the recording electrode. The columnar structure of the cerebral cortex facilitates a large degree of their electrical summation rather than mutual cancellation. Thus, the EEG recorded at the scalp represents the passive conduction of currents produced by summating activity over large neuronal aggregates. Regional desynchronization of the EEG reflects increased mutual interaction of a subset of the population engaging in 'cooperative activity' and is associated with decreases in amplitude.

To measure the EEG, electrodes are attached to the scalp with a conducting paste. Each electrode is connected with an electrically neutral lead attached to the ear, nose, chin or chest (i.e. reference montage) or with an active lead located over a different scalp area (i.e. bipolar montage). Differential amplifiers are used to record voltage changes over time at each electrode. These signals are then digitized with 12 or more bits of precision, and are sampled at a rate high enough to prevent aliasing of the signals of interest. EEGs are conventionally described as patterns of activity in five frequency ranges: delta (less than 4 Hz), theta (4–7 Hz), alpha (8–12 Hz), beta (13–35 Hz; sometimes subdivided into beta1 at 13–20 Hz and beta2 at 21–35 Hz), and gamma (above about 35 Hz).

EVOKED POTENTIALS

History

An evoked potential (EP) or ERP is the time-locked average of the EEG in response to a specific sensory, motor or cognitive event. Because of their low amplitudes, especially in relation to the background EEG activity, a number of stimuli have to be recorded and averaged with a computer in order to permit their recognition and definition. The background EEG activity, which has no fixed temporal relationship to the stimulus, will be averaged out by this procedure. As mentioned above, Richard Caton reported a clear reactivity of rabbit EEG to sensory stimuli in the form of a cortical-event-related response in 1875.

Basic Principles

Sensory evoked or 'exogenous' potentials are recordings of cerebral or spinal potentials elicited by stimulation of specific sensory pathways, e.g. visual evoked potentials (VEPs) elicited by monocular stimulation with a reversing chequerboard pattern; brainstem auditory evoked potentials (BAEPs) elicited by monaural stimulation with repetitive clicks; and somatosensory evoked potentials (SEPs) elicited by electrical stimulation of a peripheral nerve. They are an important and routinely used means of monitoring the functional integrity of these pathways.

Certain EP components depend on the mental attention of the subject and the setting in which the stimulus occurs, rather than simply on the physical characteristics of the stimulus. Such 'event-related' or 'endogenous' potentials are related in some manner to the cognitive aspects of distinguishing an infrequently occurring target stimulus from other stimuli occurring more frequently. For clinical purposes, attention has been directed particularly at the so-called P_{300} or P_3 component of the ERP (named after its positive polarity and latency of approximately 300–400 ms after onset of an auditory target stimulus).

As a research tool, ERPs can provide valuable information about the precise timing and cortical distribution of the neuroelectrical activity generated during mental activity. An averaged EP waveform consists of a series of positive and negative waves; a significant difference in latency, amplitude, duration or topography of one or more of these waves between experimental conditions that differ in one specific cognitive factor is assumed to reflect the mass neural activity associated with that cognitive factor. Measurements of changes in the amplitudes and timings of peaks in the series of EP waves allows inferences to be made about the sequence and timing of task-associated processes, such as prestimulus preparation, encoding of stimulus features, operations such as matching or comparison of stimulus codes and memory codes, evaluation of the meaning of the stimulus, and response selection and execution. However, it is important to bear in mind that the averaged EP method assumes that the component subprocesses comprising a cognitive behaviour do not vary in time from trial to trial (Gevins, 1998).

MAGNETOENCEPHALOGRAPHY

History

MEG and magnetic source imaging (MSI) measure the magnetic fields generated by electrical activity within the brain. In the early nineteenth century, Hans Christian Oersted, a Danish physicist, discovered that electrical currents generate magnetic fields. The first measure of magnetic fields generated by human bioelectric currents was a magnetocardiogram recorded by a 2-million-turn hand-wound induction coil magnetometer at room temperature (Baule and McFee, 1963). Five years later, using a similar conduction coil and signal-averaging techniques, the brain's alpha rhythm (generating a magnetic field 100 times weaker than that of the heart) was recorded in a specially designed magnetically shielded room constructed at the Massachusetts Institute of Technology (MIT) (Cohen, 1968). The subsequent development of point-contact superconducting quantum interference devices (SQUIDs), operating at liquid helium temperatures of -269°C, improved the sensitivity of the magnetometers, and the first stimulus-elicited cerebral magnetic fields could be recorded (Brenner *et al.*, 1975). The early technical difficulties were overcome in the late 1980s, such that instruments covering the whole scalp and containing more than 100 channels are now available commercially. Nowadays, magnetic field tomography (MFT), a technique based on distributed source analysis of MEG data, makes possible the three-dimensional reconstruction of dynamic brain activity in humans with a temporal resolution better than 1 ms and a spatial accuracy of 2–5 mm at the cortical level (which deteriorates to 1–3 cm at depths of 6 cm or more) (Ribary *et al.*, 1991).

The major advantage of techniques based on the measurements of cerebral electrical activity (i.e. EEG and MEG) is their uncompromised time resolution. Their major drawback, however, is their limited spatial resolution. Indeed, accurate localization of the source of brain activity remains difficult. Furthermore, the resolution becomes poorer the deeper into the brain we attempt to image. The main advantages of MEG over EEG are its superior spatial accuracy and ease of use, particularly when a large number of channels are involved. On the other hand, EEG complements MEG in detecting source components not detected by MEG (i.e. radially oriented sources) (Naatanen *et al.*, 1994). For the time being, MEG, MFT and MSI remain expensive and largely experimental research tools unavailable to most clinical settings.

Basic Principles

Electric currents in the brain produce a change in the magnetic field that can be detected outside the head by SQUID magnetometers. The signals measured can be used to compute the distribution of cerebral activity as a function of time. This method is related closely to EEG, in which the electric field pattern on the scalp is measured.

Electrical currents generate magnetic fields. The direction of the magnetic field is described by a simple right-hand rule. When the thumb of the right hand is pointed in the direction of current flow, the fingers curl in the direction of the surrounding magnetic field. This is true not only for currents flowing within power lines but also for all bioelectric currents, such as those flowing within neurons. Biomagnetic fields directly reflect electrophysiological events of the brain and pass through the skull without distortion. Hence, currents initiated at the synapses, and guided postsynaptically by cell structure, produce the magnetic field detectable outside the head. Magnetic field lines encircle the flow path of this primary current, extending outside the skull. Because pyramidal cells are predominantly oriented perpendicular to the cortex, the direction of the primary current is also perpendicular to the cortex. MEG is therefore most sensitive to activity in the fissural cortex, where the current is oriented parallel to the skull, whereas it does not detect sources that are oriented exactly radially to the skull. Because MEG detects only the tangential component of the primary current, amplitude comparison between differently oriented sources is possible only if source orientations can be estimated, e.g. on the basis of MRI.

The average electromagnetoencephalogram is about 10 picotesla (10^{-12}T) in amplitude, nine orders of magnitude smaller than the earth's steady magnetic field and seven orders of magnitude smaller than the magnetic fields generated by power lines, cars and elevators. The magnetic field produced by a single postsynaptic potential is too weak to be detected outside the head. Instead, what is detected is the macroscopic coherent activity of thousands of neurons. Still, cerebral magnetic fields are so weak that measurements are preferably performed inside magnetically shielded rooms. Sensitivity to such weak signals requires the use of cryogenic technologies. Current MEG instruments consist of an induction loop of niobium wire, which becomes superconducting (i.e. loses its resistivity to the flow of electrical currents) at approximately -258°C. Recording neuromagnetic signals has been compared to listening for the footsteps of an ant in the middle of a rock concert. Noise cancellation is improved by measuring gradients of the magnetic field instead of the field itself. In quiet environments, disturbances can be compensated for sufficiently by using elaborated compensation methods to enable MEG studies to be carried out even without shielded rooms.

Inverse Problem

As for EEG, MEG data have to be subjected to an inverse problem algorithm to obtain an estimate for the distribution of the activity in the brain. Similar to PET, fMRI and EEG, these can then be displayed on cross-sectional anatomical images (obtained by MRI) of the same subject. The inverse problem relates to the difficulty of determining internal sources on the basis of measurements performed outside the head. The most common way to tackle this problem is to determine the single-source current element (dipole) that explains most completely the EEG or MEG pattern. This can be done with a computer algorithm that starts from a random dipole position and orientation and keeps changing these parameters as long as the field pattern computed from the dipole keeps approaching the observed EEG or MEG pattern. When no further improvement is obtained, a minimum in the cost function has been reached; a source corresponding to this solution is called the equivalent current dipole (ECD). In most cases, however, the EEG or MEG data pattern cannot be explained accurately by a single source. In these cases, two or more dipoles could be used to explain the data, but this leads to computational difficulties in trying to determine the best multisource solution. Alternatively, continuous solutions, such as the minimum norm estimate, might also be constructed (Nenonen *et al.*, 1994). When interpreting EEG or MEG results, it should be borne in mind that the inverse problem is fundamentally non-unique. This means that even if the complete electric and magnetic field around the head could be measured precisely, an infinite number of current distributions in the brain could still be constructed that would explain the measured fields. It is always possible that some sources are missed, whatever the measurement set-up. For example, MEG alone is insensitive to radially oriented sources, but even when combined with EEG, silent sources are possible. Full use of available techniques requires the use of estimation theory to derive optimal solutions based on all available information, including MRI, PET and fMRI.

TRANSCRANIAL MAGNETIC STIMULATION

History

TMS is a tool for the non-invasive stimulation of the superficial cortex. In 1980, Merton and Morton surprised neuroscientists by

showing that it was possible to stimulate the motor areas of the human brain electrically through the intact scalp (transcranial electrical stimulation (TES)). They used a brief, high-voltage electric shock to activate the motor cortex and produce a relatively synchronous muscle response, the motor evoked potential (MEP). The problem was that TES was painful because of activation of pain fibres in the scalp. In 1985, Barker and colleagues showed that it was possible to stimulate both nerve and brain using external magnetic stimulation (TMS) with little or no pain. TMS is now used commonly in clinical neurology to study central motor conduction time. Depending on stimulation parameters, TMS can excite or inhibit the arbitrary sites of the superficial cortex, allowing functional mapping and creation of transient functional lesions (Hallett, 2000).

In neuropsychology, the classical paradigm is that of studying the effects of brain lesions on behaviour. With TMS, this paradigm can be applied in spatially and temporally restricted fashion to healthy volunteers. It is now used widely as a research tool to study aspects of human brain physiology, including motor function, vision, language, and the pathophysiology of brain disorders. Combined with other brain-imaging techniques, such as PET, EEG and fMRI, it can be used to evaluate cortical excitability and connectivity (Paus, 1999). It may also be useful in treating various neuropsychiatric disorders, most notably depression.

TMS is still a relatively young technique, and questions remain unanswered regarding its impact on brain function. Nonetheless, recent work with TMS has demonstrated that it allows the investigation of the relationship between focal cortical activity and behaviour to trace the timing at which activity in a particular cortical region contributes to a given task, and to map the functional connectivity between brain regions (Pascual-Leone *et al.*, 2000).

Basic Principles

A brief, high-current pulse is produced in a coil of wire, called the magnetic coil, placed above the scalp. A magnetic field is produced, with lines of flux passing perpendicular to the plane of the coil. An electric field is induced perpendicular to the magnetic field. Magnetic coils may have different shapes: round coils are relatively powerful; figure-of-eight-shaped coils are more focal, producing maximal current at the intersection of the two round components. The precise extent of neuronal activation is not known, but it varies with the intensity of stimulation. Ordinarily, TMS does not activate corticospinal neurons directly; rather, it activates them indirectly through synaptic inputs. Single-pulse TMS, which is very safe, has been used most commonly. Devices are now available that can deliver high-frequency (1–30 Hz), repetitive TMS (rTMS). This has greater effects than single-pulse TMS, but it also has the potential to cause seizures, even in normal individuals. Intracortical inhibition (ICI) and intracortical facilitation (ICF) are obtained using paired-pulse studies, and reflect the activity of interneurons in the cortex. Safety guidelines have been published that should prevent problems (Wassermann, 1998).

rTMS can produce effects that last after the stimulation period. The mechanisms of these changes are not clear, but the analogies to long-term potentiation (LTP) and long-term depression (LTD) of individual synapses in the central nervous system are apparent. Therapeutic effectiveness depends on the exact site of stimulation, intensity, and the precise pattern of pulses, including rate, train length, intertrain interval, and number of trains. This is clearly difficult to clarify and is currently an active area of psychiatric research (George *et al.*, 1999).

MAGNETIC RESONANCE SPECTROSCOPY

History

The fundamentals of NMR spectroscopy were studied and developed at least 30 years before being extended to clinical imaging. During this time, it was a major tool in physical and organic chemistry for determining molecular structure. As MRS technology evolved to allow the study of larger samples at higher field strengths, a logical step was to compare the spectral characteristics of normal and pathological tissue specimens. Following the discovery that normal and cancerous tissue samples had different NMR signals (Damadian, 1971), the first clinical NMR scanning machine was patented in the early 1970s. Hence, MRS has been in use much longer than clinical proton MRI. MRI is generally associated with the signals from hydrogen nuclei (i.e. protons) because of the large amounts of hydrogen atoms in human tissue and brain and the strong signals they provide. However, MRS makes measurements not only of protons but also of nuclei, such as phosphorus (^{31}P), carbon (^{13}C) and fluorine (^{19}F) (Dacey *et al.*, 1991). MRS offers the potential of assessing brain function at metabolic and molecular levels. At present, much of the work in this area is experimental.

Basic Principles

This technique uses natural emissions from atomic nuclei activated by magnetic fields to measure the concentration of endogenous molecules. Potential nuclei include ^{31}P, ^{13}C, ^{23}Na and ^{7}Li, in addition to ^{1}H. The ^{31}P magnetic resonance spectrum can detect tissue concentrations of the phosphomonoesters phosphocholine and inorganic orthophosphate, the phosphodiesters glycerol-3-phosphoethanolamine and glycerol-3-phosphocholine, the triphosphate adenosine triphosphate (ATP), and other phosphorus-containing molecules, including phosphocreatinine. ^{1}H spectroscopy offers the ability to measure lactate concentrations and neuronal markers such as *N*-acetyl aspartate. MRS permits quantitative analysis of these compounds *in vivo*, with the potential of three-dimensional resolution within the brain.

NEAR-INFRARED SPECTROSCOPY

History

NIRS and event-related optical signals (EROS) are relatively new methods to measure *in vivo* changes in cerebral haemodynamics and oxygenation. Changes in the intrinsic optical properties of the tissue are associated with changes in the level of physiological activity in neuronal tissue. As a consequence, it is possible to optically monitor neuronal activity without the use of dyes or other contrast-enhancing agents. Such optical techniques have been applied in the laboratory for more than 50 years. In previous studies of exposed brain tissue, optical imaging of brain activity has been achieved at high temporal and microscopical spatial resolution. Now, using near-infrared light, which can penetrate biological tissue reasonably well, it has become possible to assess brain activity in human subjects through the intact skull non-invasively. Recent developments in NIRS and intraoperative optical imaging have suggested a number of clinically important applications of this technology. After early studies employing single-site NIRS, first near-infrared imaging devices are being applied successfully for low-resolution functional brain imaging (Villringer and Chance, 1997).

Basic Principles

Brain activity is associated with changes in optical properties of brain tissue. Optical measurements during brain activation can assess haemoglobin oxygenation, cytochrome-c-oxidase redox state, and two types of changes in light scattering, reflecting either membrane potential (fast signal) or cell swelling (slow signal). The physiological basis of the most common form of NIRS is wavelength-specific absorption of photons by oxygenated and deoxygenated haemoglobin. Thus, the contrast mechanism for NIRS signals is related closely to that of intrinsic optical imaging of exposed cortex using visible light. The much lower baseline absorption levels at the longer wavelengths used in NIRS allow the light to travel further through skin, skull and brain tissue, thus allowing non-invasive imaging of haemodynamics, albeit with lower spatial resolution. Although it is possible to sample optical signals quite rapidly (>1 kHz), the effective temporal resolution is limited by the indirect nature of the coupling of the haemodynamic processes affecting the optical signals and the underlying neuronal electrical activity. However, there is some evidence that it may be possible to detect optical signals related more directly to neuronal activation (i.e. EROS). The physiological basis of EROS is not well understood, but it may include cell swelling or membrane polarization associated with neuronal activity, resulting in local light-scattering changes. Thus, optical imaging may provide insights into both the electrophysiological (fast) and haemodynamic (slow) processes underlying other brain-imaging signals. However, the spatial resolution afforded by optical methods alone is limited by the diffuse nature of photon transport through tissues.

Although promising, the application of NIRS in functional brain imaging is in an early experimental state. Advantages of the optical methods include biochemical specificity, a temporal resolution in the millisecond range, the potential of measuring intracellular and intravascular events simultaneously, and the portability of the devices enabling bedside examinations. Caveats of cerebral NIRS include insufficient light shielding, optode displacement, and a sample volume including muscle or the frontal sinus mucous membrane.

FUNCTIONAL NEUROIMAGING STUDY DESIGN

History

Mapping the human brain is distinct from the assumptions held by phrenologists of the nineteenth century. According to the German physician Franz Josef Gall, thought processes are localized in single brain areas identified by bumps on the skull. Gall posited that complex behavioural traits (e.g. ideality, cautiousness, imitation, self-esteem, calculation) could be related to the size of these bumps. Although the 'bumps theory' was fanciful, the idea of a functional segregation of the brain was not. In 1861, by carefully studying the brain of a man who had lost the faculty of speech after a left inferior frontal lesion, Paul Broca became convinced that different functions could be localized in different parts of the cerebrum. Now, more than a century of neuropsychological investigations in brain-damaged patients has confirmed that a cortical area can be specialized for some aspects of perceptual or sensorimotor processing, and that this specialization is segregated anatomically in the cortex. In our current vision on brain function, however, functional segregation holds for simple processes rather than for complex behaviours or traits, such as those described by phrenologists. Now, the view is that the cortical infrastructure supporting a single function (and a fortiori a complex behaviour) may involve many specialized areas that combine resources by functional integration between them. Hence, functional integration is mediated by the interactions between functionally segregated areas, and functional segregation is meaningful only in the context of functional integration, and vice versa.

In this framework, the foundation for most functional neuroimaging studies is that complex behaviours can be broken down into a set of constituent mental operations. In order to read this book, for example, you must recognize that a string of letters is a word; then recognize the meanings of words, phrases and sentences; and finally create mental images. The methodological challenge is first to separate each of these tasks from a cognitive perspective, and second to determine those parts of the brain that are active and those that are dormant during their performance. In the past, cognitive neuroscientists have relied on studies of laboratory animals and patients with localized brain lesions to gain insight into the brain's functions. Imaging techniques, however, permit us to visualize, safely, the anatomy and the function of the human brain in both normal and pathological conditions.

It is amazing that the strategy used most widely for functional neuroimaging over the past 15 years is based on an idea first introduced to psychology in 1868. Indeed, Franciscus C. Donders, a Dutch ophthalmologist and physiologist, then proposed a general method to estimate cognitive processes based on a simple logic. He subtracted the time needed to respond to a light (with, say, a press of a key) from the time needed to respond to a particular colour of light. He found that discriminating colour required about 50 ms more than simply responding to the light. In this way, Donders (1969) was the first to isolate a basic mental process and to obtain a measure of the time needed by the brain to perform this specific process.

Basic Approaches

The current strategy in functional neuroimaging is designed to accomplish a similar subtraction but in terms of the brain areas implementing the mental process. In particular, images of neural activity (be it blood flow measured by PET or fMRI or electrical activity measured by EEG or MEG) taken before a task is begun can be compared with those obtained when the brain is engaged in that task. The two periods are referred to as the control state and the task state. It is important to choose carefully each state so as to isolate as best as possible a limited number of operations. Subtracting neural activity measurements made in the control state from each task indicates those parts of the brain active during a particular task. To achieve reliable data, averages are made of many experimental trials in the same person (e.g. fMRI) or of responses across many individual subjects (e.g. PET). Averaging enables the detection of changes in neural activity associated with mental activity that would otherwise be confused easily with spurious shifts resulting from noise.

It is important to stress that this methodological approach, known as the cognitive subtraction paradigm, has an important drawback. Indeed, in order to isolate the neural substrate of a given cognitive component of interest, it must be assumed that the only difference between the control state and the task state is the component of interest to the exception of any other stimulus- or task-related processes. Unfortunately, this cannot always be guaranteed easily and fully. Analytic strategies, however, have been devised to circumvent this problem (see below), and cognitive subtraction designs remain the foundation of most functional neuroimaging experiments.

ANALYSING BRAIN IMAGING DATA

History

Regional differences among brain scans have long been characterized thanks to hand-drawn regions of interest (ROIs). This approach reduced the information from hundreds of thousands of voxels (volume elements that in three dimensions correspond to a pixel with a given slice thickness) to a handful of ROI measurements, with a somewhat imprecise anatomical validity. The development of more powerful voxel-based statistical methods has made these ROI analyses obsolete. Although several solutions are in use in neuroscience laboratories, one of the most popular methods for the analysis of neuroimaging data is statistical parametric mapping (SPM). This is a standardized method that refers to the construction and assessment of spatially extended statistical processes used to test hypotheses about neuroimaging data (mainly PET, SPECT and fMRI). Statistical parametric maps can be thought of as 'X-rays' of the significance of an effect, which can be projected on a three-dimensional representation of the brain. These ideas have been instantiated in software (version SPM99 at time of going to press) by Karl Friston and coworkers at the Wellcome Department of Cognitive Neurology in London (http//www.fil.ion.ucl.ac.uk/spm). SPM has become the most widely used and validated method for analysing functional neuroimaging data. Since its first description in 1990, over 1500 citations now refer to its use.

Basic Approaches

As described above, there are two basic approaches when analysing and interpreting functional neuroimaging data. They are based on the distinction between functional segregation and integration.

Functional Segregation

Using a functional specialization concept of the brain, the following sets of approaches are based on detecting focal differences. They generally fall into one of three broad categories: (1) The subtractive or categorical designs are predicted on the assumption that the difference between two tasks can be formulated as a separable cognitive or sensorimotor component, and that the regionally specific differences in brain activity identify the corresponding functional area (i.e. the cognitive subtraction paradigm). Its utilization ranges from the functional anatomy of word processing to the functional specialization in visual cortex, an application that has been validated by electrophysiological studies in monkeys (Zeki, 1993). (2) The parametric or dimensional design assumes that regional physiology will vary systematically with the degree of cognitive or sensorimotor processing. Parametric designs may avoid many of the shortcomings of 'cognitive subtraction'. A fundamental difference between subtractive and parametric designs lies in treating a cognitive process not as a categorical invariant but as a dimension that can be expressed to a greater or lesser extent in relation to the brain's regional activity. (3) Factorial or interaction designs are also well suited to avoiding the drawbacks of simple subtraction paradigms. Two or more factors can be combined in the same experiment, and the interaction term will assess the effect of one factor while excluding the effect of the other.

Functional Integration

The functional role played by any component (e.g. a neuron or a specific brain area) of a connected system (e.g. the brain) is defined largely by its connections. Connectionist approaches to understanding the integration of brain functions are well established (Hebb, 1964). The nature and organizational principles of intracortical (Goldman-Rakic, 1988) and subcortical (Mesulam, 1990) connections have provided a basis for mechanistic descriptions of brain function, referring to parallel, massively distributed and interconnected (sub)cortical areas. Anatomical connectivity, determined mainly by neuroanatomic tracer experiments in animals, is a necessary underpinning for these models. The concepts of functional and effective connectivity were developed in the analysis of separable spike trains obtained from multi-unit electrode recordings. However, the neurophysiological measurements obtained from functional neuroimaging have a very different timescale (seconds versus milliseconds) and nature (metabolic or haemodynamic versus spike trains) than those obtained from electrophysiological studies.

Only recently have analytical tools become available to assess the functional or effective connectivity between distant cerebral areas (Friston *et al.*, 1997). Functional connectivity is defined as the temporal correlation of a neurophysiological index (e.g. blood flow) measured in different remote brain areas, whereas effective connectivity is defined as the influence that one neural system exerts over another (Buchel and Friston, 1997). In this context, a psychophysiological interaction can be assessed in the framework of the general linear model, as employed by SPM (Friston *et al.*, 1997), to explain the activity in one cortical area in terms of an interaction between the influences of another area in a given experimental context. Put simply, the statistical analysis will identify brain regions that show condition-dependent differences in the way their activity relates to the activity in another (chosen) area.

Preprocessing the Data

Voxel-based analyses require the data to be in the same anatomical space. This is obtained by realigning the data. Indeed, in functional neuroimaging experiments, movement-related variance components represent one of the most serious confounds. Therefore, scans from each subject are realigned using an optimization procedure minimizing the residual sum of squares (Friston *et al.*, 1995).

In a second step, the realigned images are normalized. They are subject to nonlinear warping so that they match a template that already conforms to a standard anatomical space approximating those described by Talairach and Tournoux (1988). Pooling neuroimaging data from grossly different individual brains requires a procedure to spatially normalize the individual brains to an idealized or standard brain for the purpose of achieving overlap between corresponding anatomical and functional areas in different subjects. The Talairach and Tournoux atlas was initially developed — and has proven very useful — for anatomical normalization required for neurosurgical procedures, particularly those at brain sites close to the origin of the reference system (e.g. the anterior and posterior commisures). Each point within Talairach space into which brains are transformed is defined using three coordinates (expressed in millimetres). The first coordinate defines the position in x, i.e. from left (negative) to right (positive), with 0 mm corresponding to the interhemispheric line. The second coordinate defines the position in y, i.e. from posterior (negative) to anterior (positive), with 0 mm corresponding to the anterior commisure. The third coordinate defines the position in z, i.e. from bottom (negative) to top (positive), with 0 mm corresponding to the plane through the anterior and posterior commisures. This standard coordinate system facilitates the reporting of results in a conventional way, and facilitates comparisons between peak voxels obtained in experiments from different laboratories.

After spatial normalization, images need to be smoothed (i.e. convolved with an isotropic Gaussian kernel). Smoothing individual

images before a statistical analysis offers (1) an improved signal-to-noise ratio; (2) conditioning of the data, so that they conform more closely to the Gaussian field model, which lies at the basis of the correction procedure for multiple statistical comparisons; and (3) a better overlap between the localization of anatomical and functional brain areas from different subjects, which permits intersubject averaging.

Statistical Analysis

The data obtained after preprocessing consist of a matrix of many hundred-thousandths of voxels for each subject and for each condition. Each of these voxels is characterized by the x, y and z spatial coordinates in the standard space and a value representing the functional activity in that voxel (e.g. blood flow, glucose metabolism, BOLD signal). The statistical analysis corresponds to modelling the data in order to partition observed neurophysiological states or responses into components of interest, confounds of no interest, and an error term. This partitioning is effected using the framework of the general linear model to estimate the components in terms of parameters associated with the design matrix. The analysis of regionally specific effects uses the general linear model to assess differences among parameter estimates (specified by a contrast) in a univariate sense, by referring to the error variance. The significance of each contrast is assessed with a statistic with a Student's t distribution under the null hypothesis for each and every voxel (i.e. SPM{t}). The SPM{t} is transformed to the unit normal distribution to give a Gaussian field or SPM{Z}.

Statistical Inference

The final stage is to make statistical inferences on the basis of the SPM and characterize the responses observed using the fitted responses or parameter estimates. On one hand, with an a priori anatomically constrained hypothesis about effects in a particular brain location, the Z value in that region in the SPM{Z} can be used to test the hypothesis (i.e. uncorrected P value). On the other hand, if an anatomical site cannot be predicted a priori, a correction for multiple non-independent comparisons is required. Therefore, the theory of Gaussian fields (Friston, 1997) provides a way for correcting the P value for the multiple non-independent comparisons implicit in the analysis. This correction depends on the search volume, the residual degrees of freedom due to error, and the final image smoothness estimate. The obtained corrected and uncorrected P values pertain to different levels of inference in terms of (1) the significance of the effect in a particular voxel, (2) the significance of the coactivation of a cluster of voxels in a specific region, and (3) the significance of the coactivation of several clusters in the whole brain. Importantly, only in cases of well-documented prior neuroanatomical knowledge about the expected result can uncorrected P values be accepted. By specifying different contrasts, one can test for the variety of effects described above, and the significance values above a chosen threshold are represented comprehensively in an SPM map, where each voxel is represented at its proper location on the brain template, and where the T value in this voxel for a given contrast is represented by use of a colour-intensity code.

FUTURE DIRECTIONS: MULTIMODALITY INTEGRATION

As discussed above, fMRI and $H_2{}^{15}O$-PET measure local changes in brain haemodynamics induced by cognitive or perceptual tasks. These measures have a uniformly high spatial resolution of millimetres or less but poor temporal resolution (about 1 s at best). Conversely, EEG and MEG measure instantaneously the current flows induced by synaptic activity, but the accurate localization of these current flows remains an unsolved problem. Recently, techniques have been developed that, in the context of brain anatomy visualized with structural MRI, use both haemodynamic and electromagnetic measures to get estimates of brain activation with higher spatial and temporal resolution. These methods range from simple juxtaposition to simultaneous integrated techniques. However, further advances in multimodality integration will require an improved understanding of the coupling between the physiological phenomena underlying the different signal modalities (Dale and Halgren, 2001).

The combination of TMS with PET or EEG permits the assessment of connectivity and excitability of the human cerebral cortex. PET and fMRI, working in a combination yet to be determined, can define the anatomy of the circuits underlying a behaviour of interest; electrical recording techniques can reveal the course of temporal events in these spatially defined circuits. Parallel information from different imaging modalities is beginning to be used to constrain the EEG or MEG inverse solutions discussed above to limited regions of the cerebrum. This approach provides optimal combined spatial and temporal resolution by exploiting the best aspects of each technology. Combining various techniques offers a more complete characterization of the different aspects of brain activity during cognitive processing. This is even more so regarding our understanding of transitory neuropsychiatric phenomena (e.g. single hallucinations).

Regardless of the particular mix of technologies that will ultimately be used to image human brain function, the field demands extraordinary resources. MRI, PET and MEG equipment cost from €1–4 million, require large teams to be run, and are expensive to maintain. Furthermore, success requires close collaboration within multidisciplinary teams of scientists gathering knowledge and expertise in basic sciences (physics, informatics, chemistry, mathematics, etc.) and neuroscience (neurology, psychiatry, psychology, neuropharmacology, neurobiology, etc.). Few institutions are fortunate enough to have the technical and human resources necessary to master these technologies. Such institutions should optimally make them available to all expert scientists with valid questions in order to increase our knowledge of all aspects of human mental activity. In contrast, EEG data can be collected with a modest amount of compact, lightweight and easy-to-use equipment. Clinical EEG recordings from ambulatory subjects have been made routinely for many years. In the future, then, it appears likely that EEG will become an important tool for studying the neurophysiology of cognition outside the laboratory in naturalistic settings.

Functional neuroimaging experiments provide a vast amount of information. Recent efforts to create neuroscience databases could organize and disseminate quickly such a repository of data. As demonstrated in many chapters of this book, wise use of these powerful tools and the information they produce can aid our understanding and management of many neuropsychiatric diseases. Clearly, neuroimaging is heading us towards a much richer grasp of the relation between the human mind and the brain.

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XII

Neurogenetics

Anita Thapar and Michael O'Donovan

INTRODUCTION

Technological advances have revolutionized modern genetics, and completion of the Human Genome Project will undoubtedly represent a historical landmark. The aim of this chapter is to outline and explain the key areas of genetics that are most relevant to psychiatry. We begin by dealing with the theoretical background to quantitative and then molecular genetics, before moving on to discuss the main research methods that are being used to understand the genetic basis of psychiatric disorders. We end by discussing current molecular genetic techniques and new technologies.

THEORETICAL BACKGROUND

Although it is only relatively recently that molecular methods have allowed us to identify specific genes, the concept of a gene as a basic unit of inheritance long predated these technological advances. Gregor Mendel, an Augustinian monk, originally developed a particulate theory of inheritance. Although Mendel's experiments on pea plants and the rules of inheritance that he deduced by observation are now well recognized, his work published in 1866 was ignored during his lifetime. It was only much later at the beginning of the twentieth century that scientists recognized the importance of Mendel's findings. We now know that the units of inheritance described by Mendel are genes, that alternative forms of a gene are alleles, and that genes lie along chromosomes. It was around this time that the term 'genotype' was used to describe the genetic endowment according to the combination of alleles; the observed characteristic was described as the 'phenotype'.

Patterns of Inheritance

Mendel's Laws of Heredity

Mendel's laws are based on the observations he made from his experiments on pea plants. Mendel studied the transmission of dichotomous characteristics, such as whether the seeds were smooth or wrinkled skinned. He first noted that when plants with smooth seeds were crossed with plants characterized by having wrinkled seeds, the offspring or first generation (F1) all had smooth seeds rather than showing an intermediate phenotype. Mendel then found that when the F1 generation was intercrossed, wrinkled seeds reappeared in the subsequent offspring or second generation (F2). The ratio of plants with smooth seeds to those with wrinkled seeds was three to one.

From these results, Mendel deduced that the uniformity in the F1 offspring was the result of a unit of inheritance for each characteristic being inherited from each parent, with one being dominant and the other being recessive. We now term the different forms of these units 'alleles' and can label the dominant allele as 'A' and the recessive allele as 'a'. Those plants with two of the same alleles (AA or aa) are homozygotes, and those with two different alleles (Aa) are heterozygotes. Mendel concluded that when the F1 heterozygotes were interbred, the F2 generation consisted of AA, Aa, aA and aa plants (Figure XII.1). Thus, for a dominant characteristic, only one dominant unit or allele is needed to display the trait, whereas for a recessive trait to be manifest, both alleles have to be recessive. This is Mendel's law of segregation. He also found that the pattern of inheritance for one trait was not affected by the transmission of another trait, i.e. they show independent assortment. We now know that when genes for two traits are located near to each other on a chromosome, there is not independent assortment, and it is this phenomenon of linkage that is utilized in the search for genes (see later).

Patterns of Inheritance for Human Traits and Disorders

Single-Gene Inheritance

The group of disorders that are transmitted in a predictable fashion according to Mendel's laws are sometimes termed 'Mendelian' or 'Mendelizing' disorders. These disorders are characterized by single-gene defects, which, although rare in the general population, are usually associated with severe clinical consequences. Only one copy of the defective gene is required for autosomal dominant disorders such as Huntington's disease to manifest clinically. That is, both heterozygotes (who carry one disease allele) and homozygotes (who carry two copies of the disease allele) will be clinically affected. Thus, if one parent has an autosomal disorder, half of the offspring will be affected and half will be unaffected (Figure XII.2). Genes for many of these conditions, including Huntington's disease, have been located. Thus, it is now possible to test specifically for whether an individual carries the defective gene.

Individuals affected by autosomal recessive disorders such as cystic fibrosis inherit two copies of the gene abnormality. Carriers for these disorders may be fairly common in the general population, but both parents must be carriers for there to be a risk of the disorder manifesting in the offspring. On average, one in four of the offspring of two heterozygote parents will be clinically affected, half will be unaffected carriers, and one in four will be unaffected (Figure XII.2). Autosomal recessive disorders occur more commonly among offspring of consanguineous (genetically related) relationships, as the risk of both parents carrying the same recessive genes is increased.

The final group of single-gene disorders consists of sex-linked disorders. Strictly speaking, these disorders do not appear to show a typical pattern of Mendelian inheritance. However, we now understand that this is because of the inheritance of sex chromosomes. X-linked disorders, such as fragile X syndrome,

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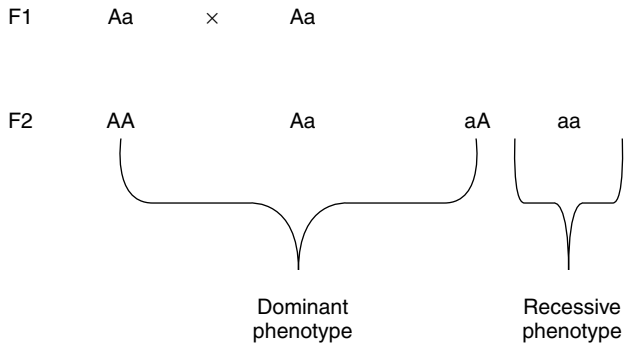


Figure XII.1 Mendel's law of segregation

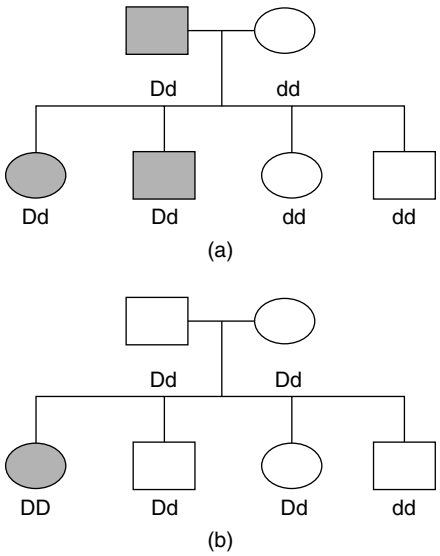


Figure XII.2 Single-gene inheritance. (a) Autosomal dominant inheritance; (b) autosomal recessive inheritance D, disease allele; d, non-disease allele

occur more commonly in males. Males inherit one X chromosome from their mother and one Y chromosome from their father (XY), whereas females have two copies of the X chromosome (XX). Thus, for X-linked recessive conditions, if a father is affected, then all the daughters will be carriers. If a mother is a carrier (X*X) and the father is unaffected, then because the sons must inherit their X chromosomes from their mother, half of the sons will be affected (X*Y) and half will be unaffected (XY). Half of the daughters would be unaffected carriers (X*X).

Atypical Patterns of Mendelian Inheritance

Although these disorders show classical patterns of Mendelian inheritance, the clinical manifestation and severity of many single-gene disorders can vary enormously due to variable expression. For example, conditions such as neurofibromatosis, an autosomal dominant disorder, can manifest with either full-blown clinical symptoms or simply a few café au lait spots. Penetrance is the probability that a particular phenotypic characteristic of a disorder will be manifested, given a specific genotype. Although for Mendelian disorders this should be 1 or 0, single-gene disorders can show an irregular pattern of inheritance due to incomplete penetrance. Penetrance also varies with the definition of disorder.

Variable expression and penetrance have been used to explain the observed clinical manifestations of single-gene disorders. Interestingly, molecular genetic techniques are now providing explanations for other atypical patterns of inheritance for disorders that are otherwise due to single-gene defects. In this section, we focus on the atypical patterns of inheritance. The molecular genetic bases of these observed phenomena are explained later in the chapter.

Heritable Unstable Nucleotide Repeat Sequences

Disorders such as Huntington's disease and fragile X syndrome had previously been observed to show greater clinical severity and an earlier age of onset in subsequent generations, a phenomenon known as anticipation. These are examples of disorders caused by heritable unstable nucleotide repeat sequences. These are unstable repeat sequences of DNA (deoxyribonucleic acid, the molecule that encodes genetic information; see later) that expand in length when transmitted from parent to offspring and manifest as a clinical disorder when the sequence reaches a critical length.

Imprinting

Imprinting is another phenomenon that results in an atypical pattern of inheritance. Here, the phenotypic manifestation of a genetic defect varies according to whether it originates from the mother or father. Angelman syndrome and Prader–Willi syndrome are examples of imprinted disorders where it appears that although most cases are accounted for by a deletion in the same region on chromosome 15 (15q11-13), very different clinical manifestations arise depending on whether the deletion originates from mother or father. Angelman syndrome is characterized by severe mental retardation, ataxia (awkward gait), seizures and a cheerful sociable affect; the deletion originates from the mother. In contrast, Prader–Willi syndrome is characterized by mild mental retardation, short stature, almond-shaped eyes, a high palate, hypogonadism, overeating (hyperphagia) associated with obesity, and increased rates of behavioural difficulties, such as temper tantrums, skin picking and hyperactivity; the deletion is inherited from the father.

Chromosomal Anomalies

Finally, an important group of disorders are explained by chromosomal changes. Humans carry their genetic information on 23 pairs of chromosomes, and medical disorders and mental retardation can arise from anomalies of specific chromosomes. Further explanations are given later in the section on molecular genetics. Until recently, cytogenetic techniques enabled us to detect major chromosomal anomalies such as Down's syndrome, characterized by an additional chromosome 21 (trisomy 21), and Turner's syndrome, in which there is an absence of one of the X chromosomes (XO). High-resolution methods such as fluorescent *in situ* hybridization (FISH) are now available to detect more subtle chromosomal deletions. Microdeletion syndromes include disorders such as velo-cardio-facial syndrome (chromosome 22q11 microdeletion), Angelman syndrome and Prader–Willi syndrome. Some of these syndromes that are known to be associated with increased rates of psychiatric disorders (e.g. velo-cardio-facial syndrome and psychosis) are of particular interest, as they may provide clues about potential susceptibility genes, i.e. genes within the deleted region are potential candidate genes for molecular genetic studies. Although chromosomal anomalies such as Down's syndrome are clearly genetic, they are not inherited and most arise spontaneously.

Polygenic Inheritance

So far, we have considered Mendel's laws, which were based on observations of discrete characteristics (i.e. smooth *v.* wrinkled seeds) influenced by a single gene, and we have briefly discussed disorders caused by single genes. Clearly, many human characteristics, such as height and blood pressure, are distributed continuously

Figure XII.3 Phenotype measured on a continuous scale that results from a single gene with two alleles, A1 and A2. © Oxford University Press 2000. Reprinted from *New Oxford Textbook of Psychiatry*, Volume 1 edited by M.G. Gelder, Juan J. López-Ibor Jr., and Nancy C. Andersen (2000) by permission of Oxford University Press

in the population rather than being simple discrete phenotypes. Moreover, the majority of human traits and diseases, including psychiatric disorders, do not appear to be attributable to single-gene defects. Does this mean that Mendel's laws do not apply for such traits and disorders? This does not appear to be the case.

Let us begin by considering the influence of a single gene A with two alleles A1 and A2 on a trait measured on a continuous scale (Figure XII.3). There are three possible genotypes, A_1A_1, A_1A_2 and A_2A_2. If we describe the phenotypes of each of these genotypes in terms of scores on a continuous scale, then A_1A_1 has a value of $-a$, A_2A_2 has a value of $+a$, and the heterozygote A_1A_2 shows a value of d. When d is equal to zero, then the contribution of A_1 and A_2 is additive, thus the phenotype of A1A2 lies exactly halfway between that of A1A1 and A2A2. When $d = +a$, then A2 is dominant to A1, and when $d = -a$, then A2 is recessive to A1.

In a given population, if we assume that there are no dominance effects, selection, migration, inbreeding or selection, and that p is the frequency of allele A1, q is the frequency of allele A2, and $p + q = 1$, then the frequency of genotypes will be as follows:

A_1A_1	A_1A_2	A_2A_2
p^2	$2pq$	q^2

This is known as Hardy–Weinberg equilibrium. Simplifying further so that $p = q = 0.5$, then we can see that the phenotypic values of A_1A_1, A_1A_2 and A_2A_2 would be distributed in the ratio of $1:2:1$. What happens when we allow for the effect of multiple genes? We next consider a phenotypic trait that results from two genes that have an additive action and two alleles of equal frequency. Now there are five possible phenotype values with relative frequencies of $1:4:6:4:1$. Thus, it can be seen that as the number of genes (n) increases, the number of phenotype values also increases ($2n + 1$), and the distribution of phenotypes begins to approximate a normal distribution. Clearly, Mendel's laws can be applicable for traits that show a continuous distribution. It is thought that the transmission of most of these traits will prove to be explained by the additive action of many genes. This is known as polygenic inheritance.

In general, it is assumed that in most instances, such traits are influenced by the action of multiple genes and environmental factors, otherwise termed as a multifactorial mode of inheritance.

Multifactorial/Polygenic Liability Threshold Models

Most common human diseases, including psychiatric disorders, are considered to be complex disorders, in that they are not transmitted in a classical Mendelian fashion. Although we have shown the effects that multiple genes can have on the distribution of phenotype values, many complex disorders must be considered not as continuous variables but rather as 'present' or "absent". Nevertheless, for those who are affected, clinical severity may be considered along a continuum. A liability threshold model allows us to extend this further by assuming that there is an underlying liability to the disorder that is distributed continuously in the general population; if this liability is influenced by multiple genes, then we can assume that the distribution will be distributed approximately normally. The clinical features of the condition are manifest beyond a particular threshold (Figure XII.4). The liability distribution of relatives of those affected by the disorder will be shifted to the right compared with that of the general population, and a greater proportion of relatives will lie above the threshold. This sort of model can be used to calculate the correlation in liability between pairs of relatives (see the section on twin studies later).

Figure XII.4 Polygenic or multifactorial liability threshold model. © Oxford University Press 2000. Reprinted from *New Oxford Textbook of Psychiatry*, Volume 1 edited by M.G. Gelder, Juan J. López-Ibor Jr., and Nancy C. Andersen (2000) by permission of Oxford University Press

So far, we have assumed that psychiatric disorders are inherited in a multifactorial, polygenic fashion. With the advent of molecular genetic studies, we now know that for some seemingly complex disorders, e.g. Alzheimer's disease and familial breast cancer, Mendelian subforms exist. Although these disorders appear to show a mixed model of inheritance, for most psychiatric disorders it has become increasingly evident that if Mendelian subforms exist, then they are extremely rare. Oligogenic models assume the coaction of a small number of genes, and provide a plausible model of transmission for many psychiatric disorders. These issues can now be addressed directly by molecular genetic studies.

Human Variation and its Components

Quantitative genetics focuses on the observed variation of a given trait (phenotype) within a specified population. This is known as phenotypic variation (V_P); we will next consider the influence of genes and environment on this variation. We begin by assuming a simple model in which phenotypic variation can be partitioned into a proportion explained by genetic factors (V_g) and a component accounted for by environmental influences (V_e):

$$V_p = V_g + V_e$$

Environmental variance can be subdivided further into those influences that result in family members becoming more alike, known as common or shared environmental variance (V_c), and a remaining proportion, termed non-shared environmental variance (V_e). The non-shared environment component includes those environmental influences that serve to make family members dissimilar as well as error variance.

Genetic variance can also be separated further into additive genetic influences (V_a) and dominance effects (V_d). A term that is commonly used to describe genetic variance is 'heritability'. Strictly speaking, heritability describes the proportion of total variance attributable to genetic factors (V_g/V_p). More commonly, the term is used to describe 'narrow-sense heritability', which is the proportion of total phenotype variance explained by additive genetic variance:

$$h_n^2 = V_A/V_P$$

Although heritability and variance explained by shared and non-shared environments provide useful estimates, the limitations of these terms also need to be appreciated. First, heritability and environmental variance estimates apply to populations rather than individuals; for example, a heritability of 50% for antisocial behaviour does not mean that half of a particular individual's antisocial behaviour is accounted for by genes.

Second, heritability estimates refer only to the population that has been studied, and do not apply to different populations or age groups; for example, the magnitude of genetic influences on traits such as IQ and antisocial behaviour appears to be greater in adults than in children (Plomin *et al.*, 2001). Similarly estimates of heritability and environmental variance are likely to have little meaning for selected groups, e.g. those exposed to severe environmental adversity may not be represented in the study sample.

Third, heritability estimates do not account for changes in a given trait over time. Thus, for example, although height has been shown to be highly heritable, there is clear evidence that in the last century, there has been a marked increase in the height of the general population, which is clearly attributable to environmental factors, such as a better diet, rather than to a sudden change in genes.

Finally, in common with other epidemiological study methods, association cannot be equated with cause. Clearly the proportions of variance attributable to genetic and environmental factors cannot tell us about underlying mechanisms and pathways to specific disorders.

These limitations must be brought to mind to avoid overinterpretation of findings. Nevertheless, although there has been much criticism of the concept of heritability, the estimates of genetic and environmental variance provide a useful initial guide for the researcher as to whether genes influence a given trait and to indicate the relative contribution of genes and environment.

Other Influences that Contribute to Phenotypic Variation

Non-Additive Genetic Effects

So far, we have made the simplistic assumption that genes and environment act in a additive fashion. We have already discussed genetic dominance, whereby different alleles within a given locus have a differential effect. The effect of one gene can also have an non-addictive effect when combined with the effects of another locus. This is known as gene–gene interaction or epistasis.

Gene–Environment Interaction

Gene–environment interaction is another important influence that needs to be taken account of by those interested in psychopathology (see Chapter XIII for further discussion). It is well established that not only do mice reared in a favourable environment perform better on tasks than those reared in a less enriched environment, but that this environmental effect will vary depending on the genotype of the mouse. Examples of this type of gene–environment interaction are beginning to emerge in the field of human psychopathology. For example, there is evidence that environmental adversity has a much stronger effect on antisocial behaviour where there is also a background of high genetic risk for such behaviours (Bohman, 1996). Similarly, in both adults and children, the impact of life's stresses on depression varies according to genetic susceptibility (Kendler *et al.*, 1995; Silberg *et al.*, 2001).

Gene–Environment Correlation

So far, we have considered genes and environment as distinct influences. There is increasing evidence that this does not appear to be the case. Gene–environment correlation is observed when a person's genotype is correlated with their environment. There are three forms of gene–environment correlation: passive, active and evocative. Passive gene–environment correlation arises where the parents not only pass on genes to their offspring that increase the likelihood of a particular trait, but also provide an environment that is correlated with the parental genetic make-up. For example, sociable parents may endow their children not only with genes that predispose them to being sociable but also with an environment that increases the likelihood that they will show sociable behaviour. Active gene–environment correlation arises where, for example, a child actively seeks out situations that serve to make him or her more sociable. Finally, evocative gene–environment correlation occurs where responses evoked in others or the environment are correlated with the individual's genotype, e.g. a sociable child who evokes friendly responses in others.

The interesting point about gene–environment correlation is that even where traits or disorders are found to be highly heritable, environmental influences may still be important mediating factors. Again, there are examples from adoption studies of antisocial behaviour that suggest that observed adversity, such as negative parenting, may be due in part to the child's genetic make-up. For example, two studies have found that adoptive parents appear to show increased negative parenting when their adopted children have an increased genetic risk of antisocial behaviour (i.e. their biological parents showed antisocial behaviour) (Ge *et al.*, 1996; O'Connor *et al.*, 1998).

Assortative Mating

Quantitative genetics theory assumes random mating. This, of course, may not hold true for many human traits where like may be attracted to like. This is known as positive assortative mating. Negative assortative mating occurs where opposites attract. There is some evidence that to some extent there is positive assortment for height and, to a lesser extent, cognitive ability.

There has also been reasonably consistent evidence that assortative mating also occurs with antisocial behaviour. Assortative mating may be influenced by social factors, e.g. finding a partner of similar cognitive ability at university, or meeting with an antisocial partner within a delinquent peer group.

The net result of positive assortative mating for twin studies is that genetic similarity amongst the parents leads to increased genetic sharing amongst siblings. This results in an inflated fraternal twin correlation, which would lead to an inflated estimate of the shared environment component. However, so far this has not been shown to cause a serious problem to twin studies.

MOLECULAR GENETICS

So far, we have focused on quantitative genetic theory without considering the molecular basis to inheritance. We now know that genetic information is encrypted in a macromolecule called DNA. The purpose of this section is to explain the organization and structure of DNA, and how information is encrypted within, decoded from, and transmitted by this molecule.

Chromosomes

In humans, DNA is organized into 23 pairs of chromosomes, each of which has a unique appearance. One pair consists of the sex chromosomes, which, as mentioned earlier, come in two forms, X and Y. Females carry two copies of X while males carry one X and one Y. The Y form determines gender, resulting in male differentiation. The other pairs are called autosomes, numbered 1 through to 22. Each chromosome has two arms joined by a central region known as the centromere. The centromere is not positioned exactly in the middle of the chromosome, which therefore appears

to have a long and a short arm, called q and p, respectively. The free end of each arm is called a telomere. The arms are divided further into numbered regions, with those nearest the centromere (cen) having smaller numbers than those near the telomere (tel). Thus, 22q1 designates a region on the long arm of chromosome 22, which is nearer the centromere than 22q2.

The term 'karyotype' is used to describe the chromosome content of a cell. The normal male karyotype is 46,XY and the normal female karyotype is 46,XX. However, occasionally there are abnormalities of chromosome number. Thus, the Turner's syndrome (females with only a single X chromosome) karyotype is 45,X, while the Klinefelter's syndrome karyotype (males with an extra X) is 47,XXY. The usual karyotype of males with Down's syndrome is 47,XY,+21, where +21 indicates an extra copy of chromosome 21. Autosomes are each present in duplicate, with one complete set inherited from each parent. Pairs of the same type are called homologous chromosomes, and each member of a pair is virtually identical at the DNA level.

DNA

The microstructure of DNA is largely responsible for carrying genetic information. DNA is a polymer consisting of a chain of sugar monomers each linked by a phosphate bridge. There are four types of monomer, which differ according to the type of base molecule anchored to each sugar: these are adenine (A), guanine (G), cytosine (C) and thymine (T). A sequence of DNA is usually described by the order of bases in a 5′–3′ direction (5′ and 3′ describe the start and end of the molecule, respectively, as it is synthesized). Thus, a stretch of DNA may be represented as 5′-AGCTTTGGCA-3′.

The entire DNA content of an organism is called its genome, and the complete sequence of the human genome is defined by around 3×10^9 bases. DNA in its natural state usually exists as a double-stranded structure (dsDNA) consisting of two linear DNA molecules held together by hydrogen bonding between the bases in the opposing strands. Pairs of bases in opposite strands do not align randomly. Thus, the adenine is always paired with thymine, and cytosine with guanine. This is called base complementarity, and two strands displaying this property are said to be complementary. Consequently, if the sequence of bases in one strand of DNA is known, then the complementary sequence of bases in the other strand is also known, according to the above simple rule. This permits the dsDNA sequence to be described by one of the two strands. Thus, the dsDNA sequence above would be:

5′-AGCTTTGGCA-3′

3′-TCGAAACCGT-5′

Cell Division

At conception, we begin with a single cell containing 46 chromosomes. By adulthood, each of us consists of around 10^{14} cells. This increase in cell number occurs by mitosis. The production of gametes (sperm and eggs/ova) arises from a rather different process called meiosis.

During meiosis, pairs of homologous chromosomes migrate within the cell to lie together side by side to form a structure called a bivalent (Figure XII.5). Each chromosome is replicated to form pairs of sister chromatids. Because homologues are aligned and each homologue consists of a pair of sister chromatids, each bivalent contains four dsDNA molecules. Chromosomal material is then exchanged almost randomly between homologues, a process called recombination (Figure XII.5). After recombination, the chromosomes are therefore no longer identical to those originally

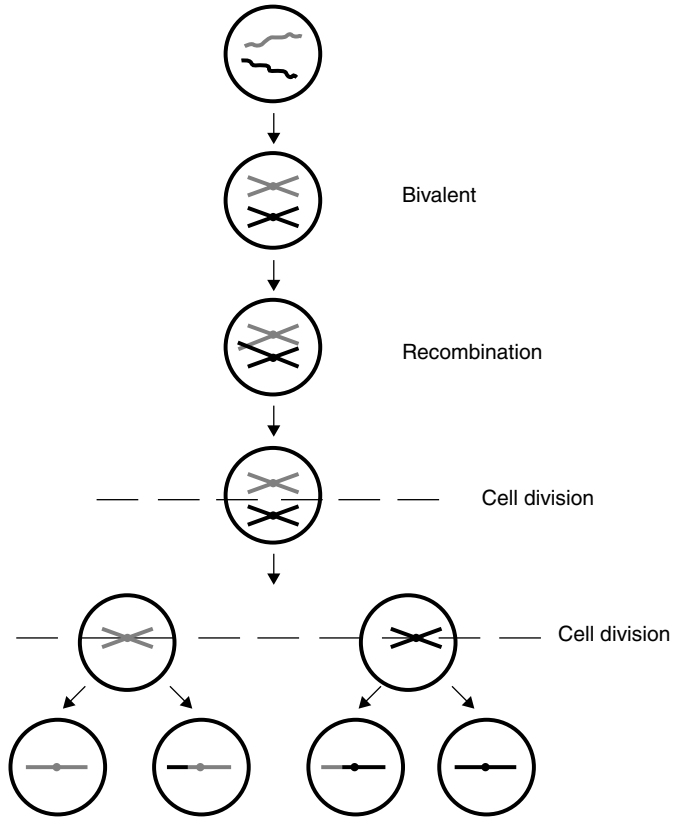


Figure XII.5 Meiosis

inherited from the parents. Furthermore, pairs of sister chromatids are not identical. Instead, each contains a different mixture of genetic material originating from the father and the mother. Recombination is central to the understanding of genetics, particularly of studies that make use of the phenomena of linkage and association (see later).

After recombination, a series of cell divisions occurs, with the final result that each gamete contains only a single copy of each chromosome. Thus, in spermatogenesis, instead of a diploid 46,XY karyotype, each of the four spermatozoa has a haploid 23,X or 23,Y chromosome complement. Furthermore, because of recombination, each sperm is genetically distinct. By a similar process, the product of oogenesis is one large haploid 23,X egg gamete.

Storage of Information in DNA

DNA molecules are organized as genes, which are the stretches of sequence carrying the information required to synthesize RNA (ribonucleic acid) molecules, which in turn code for amino acids that constitute proteins. Only a small proportion of the genome contains this kind of genetic information (~1%). However, there are other functional elements of DNA, including sequences that alter gene expression. In order to express the information encoded in genes, DNA must first be transcribed. During transcription, a segment of DNA corresponding to a gene acts as a template for the synthesis of a complementary strand of messenger RNA (mRNA). In order to initiate transcription, a number of transcription factors bind to DNA at the 5′ end of the gene at a region called the promoter. Whereas the promoter region is located in the immediate region flanking the 5′ end of the gene, there are other regions that can either enhance or repress the rate of transcription. These

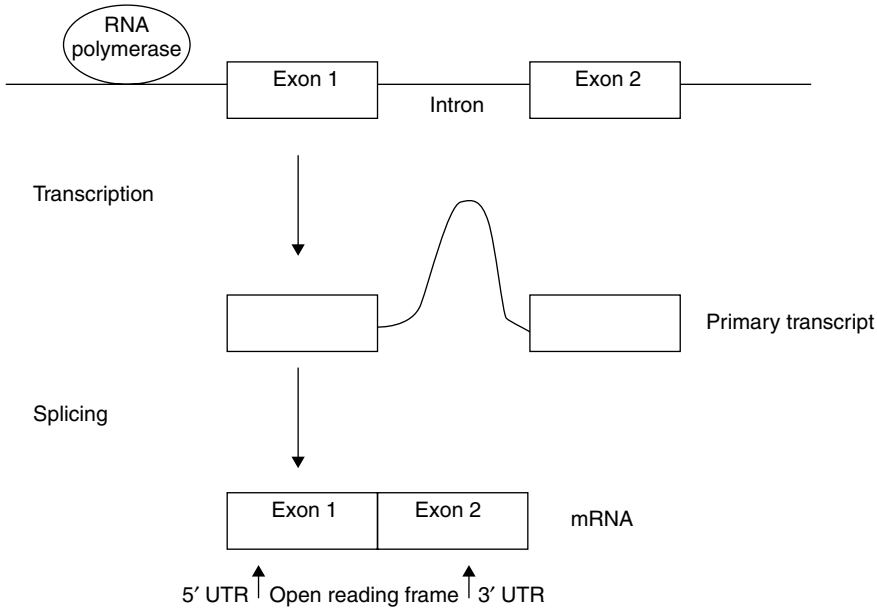


Figure XII.6 Formation of mRNA

are called enhancers and silencers, respectively; unlike promoter elements, their position relative to the gene is unpredictable. The immediate product of transcription does not constitute a functional mRNA molecule. Instead, this primary transcript must be subjected to post-transcriptional processing. There are many post-transcriptional steps, but for our purposes the most important of these is RNA splicing (Figure XII.6).

In humans, the sequence in a gene that is represented by complementarity in a mature mRNA molecule is interrupted by stretches of DNA sequence that is not. These coding and non-coding sequences are called exons and introns, respectively. The intron sequence is removed from the primary transcript by a process called RNA splicing. RNA splicing is of importance for several reasons. First, by altering the pattern of splicing, a single gene may yield several different mRNAs containing different exons and that therefore encode different polypeptides (constituents of proteins). Many genes encoding neuro-receptors display this. Second, mutations in the gene sequence may cause either failure of splicing (read-through) or excessive splicing (exon skipping). Clearly, this can alter the sequence of the mature mRNA and may result in disease.

Codons, Amino Acids and Polypeptides

The amino acid sequence of any given protein is specified by the sequence of bases in the mRNA transcribed from the gene encoding that protein. The unit of information in mRNA encoding an individual amino acid is the codon, which consists of three adjacent bases. The order of codons in the mRNA molecule serially code for each amino acid in its corresponding protein. For example, the amino acid threonine is encoded by the codon ACA, while CAG encodes glutamine. Thus, the sequence ACACAG will specify a segment of a protein where the adjacent amino acids are containing threonine and glutamine. The process of assembling a polypeptide (the units of which are amino acids) based upon the information encoded in mRNA is called translation.

During translation, mRNA molecules first bind to ribosomes, which are large complexes of ribosomal RNA (rRNA) and protein. Another species of RNA called transfer RNA (tRNA) is then responsible for actually reading the genetic code of the mRNA and bringing together the specific sequence of amino acids that is coded for.

Specific subspecies of tRNA bind specific amino acids. Each species of tRNA also has a specific three-base anticodon sequence at one end, which reads the genetic code by binding to the codons of mRNA according to the rules of complementarity. Thus, specific tRNA molecules (and therefore amino acids) base-pair with any given mRNA in an order or sequence that is specific to that mRNA. After base-pairing, the tRNAs yield their captive amino acid for inclusion in the lengthening protein. This is the mechanism by which linear genetic information encoded in DNA results in linear information encoded in a polypeptide (sequence of amino acids; the constituents of proteins).

In addition to encoding specific amino acids, a specific codon sequence (ATG) initiates translation; the sequences TAA, TAG and TGA terminate translation. mRNA species are also preceded by a number of bases that are not translated, called the 5′ UTR (untranslated region), and several hundred bases at the 3′ end that are also not translated (3′UTR). The exonic sequence between these sequences that actually encodes amino acids is called an open reading frame (ORF) because it contains no termination codons.

The codon sequence that is actually translated is called the reading frame. Any stretch of sequence in an mRNA molecule has three potential reading frames, depending on whether the first complete codon begins at base 1, 2 or 3 (see example below).

ACC AGG AUG GGG CAG CUG = thr arg met gly gln leu

A CCA GGA UGG GGC AGC UG = pro gly trp gly ser

AC CAG GAU GGG GCA GCU G = gln asp gly ala ala

Clearly, the order of amino acids specified is dependent on which base forms the start of a codon unit. This is an important concept to grasp because mutations that alter the reading frame can have profound functional consequences.

Just as primary transcripts are modified to produce mature mRNA, so polypeptide products of translation are often modified

before a functional protein is produced. Common modifications include the removal of an *N*-terminal signal sequence, glycosylation, phosphorylation and cleavage.

Genes, Chromosomes and Variation

In 2000, the Human Genome Project delivered the first complete rough draft, covering about 90% of the human genome. Since the information is still incomplete, we can only estimate the total number of genes in the human genome. However, best estimates based on the number of unique mRNA molecules detected so far place this at around 30 000–40 000.

The sequence of any given chromosome is virtually identical in all humans (barring major chromosomal anomalies). However, although they are similar, they are not identical. The science of genetics is essentially the study of this variation. Without DNA variation, every human would carry identical genetic information. All phenotypic (i.e. observable) variation would therefore be due to the effects of the environment.

The term 'locus' is used to describe a sequence at a defined position on a chromosome. If the DNA sequence at a given locus shows variation, then the locus is polymorphic, and each variant of that polymorphism is called an allele. As described earlier, if an individual contains the same pair of alleles at a given locus in a pair of homologous chromosomes, then that person is said to be homozygous at that locus. If an individual carries different allelic forms at a given locus, then that individual is described as heterozygous at that locus. Females are also homozygous or heterozygous at X chromosomal loci because they have two copies of X. However, males are hemizygous because they carry only a single copy of each sex chromosome.

Sequence Variation

The most common types of variation are single-base substitutions (replacement of one base by another) and single-base insertions and deletions (gain or loss of one or more bases relative to a reference sequence). These are often termed single-nucleotide polymorphisms (SNPs). Such changes are extremely common in the human genome. On average, the sequence in a pair of homologous chromosomes carried by any individual is expected have an SNP every 1000 bases, most of which occur in 'junk DNA' and are therefore without phenotypic consequences. Mutations in coding sequence are, however, much more likely to have phenotypic effects. These can be classified as follows:

- *Nonsense mutations* change an amino acid codon to a termination codon, resulting in a truncated protein. Such changes can often be expected to have a dramatic effect, at least on the protein.
- *Missense mutations* change the amino acid specified by a single codon. These are also called non-synonymous substitutions.
- *Silent substitutions* do not alter the amino acid specified by a codon. These are also called synonymous substitutions.
- *Frameshift mutations* are small insertions and deletions in coding sequences, which may have dramatic phenotypic consequences. Because the genetic code is based on the three-base codon system, the deletion or insertion of a single base will cause the entire downstream sequence to be read out of frame, resulting in a dramatically different polypeptide.

Most variation outside coding sequence is expected to have no phenotypic effect, but this is not always so. Variants in promoters, enhancers, repressors, UTRs and introns may result in altered gene expression. Other silent changes may alter mRNA processing. Thus, it should not be assumed that just because a variant does not result in a change in amino acid, it has no phenotypic consequences.

Chromosomal Abnormalities

The most common numerical increases in chromosomal number result from possession of an extra copy of a single chromosome. This is called trisomy; the best known example of this is trisomy 21 or Down's syndrome (male karyotype 47,XY,+21). The converse situation, in which an entire chromosome is missing, is called monosomy; with the exception of Turner's syndrome females, who carry only a single X chromosome (45,X), monosomy of an entire chromosome is lethal and is therefore not found.

If the chromosomal abnormality arises by the failure of a pair of homologous chromosomes to separate during cell division in embryogenesis rather than in the production of sperm and eggs, some cells in the body will have a normal chromosomal number while others will not. This is called mosaicism; the phenotypic consequences of this can vary dramatically, depending on the proportion of cells with the abnormal number of chromosomes.

Other chromosomal abnormalities occur as a result of breakage of chromosomes. These include (1) deletion, in which a large stretch of DNA is lost; (2) inversion, in which a piece of chromosome breaks and becomes reattached in the opposite orientation (consequently, there is no loss of DNA, although individual genes may be interrupted); (3) duplication, in which a segment of chromosome is duplicated; and (4) translocation of segments from one chromosome to another (non-homologous) chromosome. Translocations can be categorized into balanced or unbalanced. For example, if an individual has part of chromosome 1 attached to chromosome 2, and vice versa, then the offspring may be phenotypically normal if both abnormal chromosomes are transmitted simultaneously; this is said to be a balanced translocation. The situation in which offspring receive only one of the abnormal chromosomes is unbalanced, and will result in simultaneous partial monosomy and partial trisomy.

Occasionally, both members of a pair of homologues carried by an individual with a normal karyotype originate from the same parent, rather than one being passed on from the mother and the other from father. This is called uniparental disomy. It might be thought that if a child possesses a pair of chromosomes from a phenotypically normal parent, then this should not pose a problem; however, this is not the case because regions of chromosomes are sometimes 'marked' or imprinted as they are transmitted from a parent to the offspring. Where this occurs, the chromosomes from the mother are not equivalent to those from the father. Abnormalities can then occur when an individual does not receive an imprinted sequence from both parents. Classic examples of relevance in psychiatry are Prader–Willi syndrome and Angelman's syndrome. We have already discussed how these syndromes arise from microdeletions on chromosome 15. These disorders can also arise in the absence of a microdeletion but when both copies of chromosome 15 originate from the mother or the father.

The Mitochondrial Genome

So far, we have focused on chromosomal DNA. However, mitochondria contain their own small genome of around 16 500 bases, which encodes a small number of proteins and RNA molecules required for oxidative phosphorylation. Sperm contribute mitochondria only very rarely during fertilization and, consequently, diseases of the mitochondrial genome are almost always transmitted maternally. There are several thousand mitochondrial DNA molecules per cell rather than two homologous chromosomes, therefore the concepts of homo-, hemi- and heterozygosity do not apply. Instead, what is relevant is the proportion of DNA molecules carrying a variant mutation. This may be 100%, 0% (homoplasmy) or any intermediate ratio (heteroplasmy). Furthermore, because mitochondria are dispersed randomly into daughter cells during cell division, the ratios of heteroplasmy can vary between mother and child and

between individual cells. This has an impact on the severity of the phenotype of diseases caused by mitochondrial mutations.

Mitochondrial DNA has a particularly high mutation rate, therefore mutations commonly accumulate in an individual's somatic cells, leading to a reduction in the efficiency of oxidative phosphorylation. This has been proposed to be important in the aetiology of some neurodegenerative diseases, in particular Parkinson's disease and Alzheimer's disease.

Unstable DNA

We tend to think of DNA as a relatively stable structure that is transmitted reliably from parent to offspring and from cell to cell. However, as mentioned earlier, DNA sequences containing repetitive sequences are often unstable and are prone to extremely high rates of mutation, which may be pathogenic. A particular class of repeat — the trinucleotide repeat — has emerged as playing an important role in human neurological disorders. Trinucleotide repeats are repeat elements in which the unit of DNA sequence that is repeated consists of three bases, e.g. CAGCAGCAG. At a given locus, trinucleotide repeat sequences are often polymorphic in repeat number; when they are located within genes, possession of a relatively large copy number of repeats (called an expanded trinucleotide repeat) can sometimes lead to disease. Expanded trinucleotide repeats were first identified as pathogenic in classic fragile X syndrome; since this discovery, many other disorders have been attributed to expanded trinucleotide repeats, including Huntington's disease and numerous forms of ataxia.

The mechanisms by which trinucleotide repeats lead to disease are still not understood. Some with very large repeats in the promoter, untranslated region or intronic regions appear to exert their effects by silencing transcription. However, the largest group of identified trinucleotide repeat diseases are neurodegenerative disorders (e.g. Huntington's disease) caused by moderate-sized CAG repeats encoding polyglutamine sequences. The pathogenic mechanisms of this group are essentially unknown, and although neurones in people with these diseases have characteristic protein deposits called nuclear inclusions, these are unlikely to provide a primary explanation (Cummings and Zoghbi, 2000).

Progression in age at onset and/or phenotypic severity as a disease transmits through a pedigree is called anticipation. This only known genetic mechanism for anticipation is the propensity of pathogenic expanded repeats to increase in size during transmission from parent to child. As age at onset and/or severity are often related to the repeat size, unstable transmission results in anticipation. Many modern studies generally note that patterns of transmission of major mental disorders are consistent with anticipation, and although biases cannot be ruled out, this has led to the hypothesis that schizophrenia, bipolar affective disorder and even anxiety disorders may, at least in part, be attributed to one or more pathogenic trinucleotide repeats. However, to date, the evidence for this is entirely circumstantial.

Methylation, Imprinting and Epigenetic Inheritance

So far, our discussion has concerned the genetic information carried by the linear DNA sequence. However, there are mechanisms by which variation in the rate at which a gene is expressed (i.e. mRNA is produced) can be inherited in the absence of a change in the primary DNA sequence. This is often referred to as epigenetic inheritance. Although our understanding of epigenetic phenomena is rudimentary, we do have a limited understanding of several important mechanisms. One involves methylation of bases at the 5′ end of genes. This leads to changes in chromatin structure that make the DNA unavailable for transcription and thus silence the methylated gene.

The DNA surrounding some genes is in a different state of methylation in sperm and eggs. This results in a variation in the activity of even identical copies of a gene, depending on the transmitting parent. This phenomenon is called imprinting. Genetic information can also be altered by other influences. For example, gene activity can be dependent upon the precise chromosomal localization of the gene (position effects), and therefore the activity of a gene may be altered by translocation, even if the gene itself is not disrupted by the chromosomal breakage.

METHODS IN PSYCHIATRIC GENETICS

Family, Twin and Adoption Studies

Family Studies

The family study design involves examining whether the rate of disorder is increased amongst relatives of affected individuals compared with the rate amongst relatives of those with either no disorder or some other psychiatric disorder. Thus, family study findings inform us as to whether a disorder is familial. Familial clustering of a disorder can, of course, be explained by environmental influences shared by different family members as well as by genetic factors. Nevertheless the relative risk to first-degree relatives (λ_r) is often used not only as a measure of familiality but also when considering how worthwhile molecular genetic studies may be.

There are two types of family study methods. The family history method involves obtaining information on relatives from the proband (affected individual). Although this is a practical and economical method, given that many individuals may know little about their relatives' health, this type of study can result in an underestimate of the rate of diagnoses in relatives. A preferable, although expensive, method is the family study method, which involves direct interviews with all relatives.

It is rarely possible to include all affected individuals from a defined population (complete ascertainment), and most family studies are, by necessity, based on selected samples, e.g. patients that have been referred to hospital. The selection processes and factors influencing referral patterns, such as comorbidity and help seeking, should ideally be taken into consideration when analysing the data. Similarly, all probands in a family study should be ascertained in an independent fashion, although for common disorders it may arise that different members of the same family are included. This is known as multiple ascertainment. Another difficulty that needs to be considered by those carrying out family studies is that the rate of disorder will depend on the age of the individual, and whether they have passed through the risk period for the disorder being studied. Some relatives will not have reached the age of risk, some will become affected at a later point in time, and others may have died before becoming affected. Thus, the rate of disorder in relatives has to be adjusted using an age correction, and must be expressed as the morbid risk or lifetime expectancy.

Twin Studies

Twin studies allow for the disentangling of genetic and shared environmental effects. Genetically identical or monozygotic (MZ) twins arise from the same fertilized egg and thus share 100% of their genes in common. Non-identical or dizygotic (DZ) twins come from two fertilized eggs and, like full biological siblings, share on average 50% of their genes.

Given certain assumptions (see later), where there is greater MZ twin similarity compared with DZ twin similarity for a given trait or disorder, this suggests a genetic contribution. For continuous

traits, such as height, weight and IQ test score, similarity between the twins is stated as an intraclass correlation coefficient that can be expressed in terms of heritability and shared environmental variance:

$$r_{mz} = h^2 + c^2$$
$$r_{dz} = 0.5\ h^2 + c^2$$

Although we can calculate h^2 and c^2 by solving these simultaneous equations, and e^2 is the remaining variance $(1 - h^2 - c^2)$, most researchers now use formal methods of model fitting (see later).

For categorically defined characteristics, e.g. those with a disorder and those who are unaffected, similarities are expressed as concordance rates. The pairwise concordance rate is calculated by dividing the total number of twin pairs who both have the disorder by the total number of pairs. Proband-wise concordance rates are used when the twins have been ascertained systematically, e.g. from a twin register. Here, we calculate the concordance rate as the number of affected twins divided by the total number of co-twins.

Equal Environments Assumption

The twin study design is based on the assumption that MZ and DZ twins share environment to the same extent. There has been much criticism of this assumption, and indeed where questionnaire measures of environmental sharing had been used, there is evidence of greater MZ environmental sharing than DZ twin sharing.

However, greater MZ environmental similarity may arise as a consequence of their greater genetic similarity. Criticisms of the equal environments assumption have been examined in three main ways. First, some studies have used questionnaire measures of environmental sharing that include items such as whether the twins were dressed alike as children, were treated alike, shared a room and shared friends. It is then examined whether the greater MZ twin environmental sharing is associated with increased MZ twin phenotypic similarity. Studies that have used this approach suggest that greater similarity in childhood environment does not account for greater MZ twin similarity in cognitive ability, personality, depressive symptoms or depressive disorder.

Another method has been to examine twins who have been mistaken about their zygosity. Again, studies that have used this method suggest that perception of zygosity appears to have little influence on twin similarity.

The most powerful method of examining this issue is to investigate reared-apart twins. This allows for examining the effects of environmental sharing apart from those factors that are influential when the twins are very young or when they are in the womb. Although reared-apart twin studies have inevitably been based on highly selected samples, they have yielded findings that are consistent with those of reared-together twins and shown a genetic contribution for traits such as cognitive ability, personality and psychosis.

Comparability of Twins

Another important criticism of the twin method is that twins may not be representative of the general population in that they differ from singletons in some important respects. DZ twinning is influenced by factors such as maternal age, multiparity, family history of twins, and the use of fertility drugs. Twins experience greater intrauterine and perinatal adversity and are clearly unusual in their experience of being brought up as a twin. Moreover, there is also some evidence to suggest that depression is more common in mothers of young twins than in mothers of singletons. However, twins do not appear to differ markedly from singletons for most types of behaviours and disorders, other than showing increased rates of early language delay.

Ascertainment

Many of the older twins studies suffered from bias in that they were based on affected twins referred to the study or on volunteer samples. These groups tend to include more female monozygotic twins who are concordant. More recent studies have been based on either hospital registers or population-based samples. Hospital registers have the advantage of including those with definite disorders, but clearly findings for some disorders may be biased given the process of selection for referral.

Population-based samples overcome these difficulties, but clearly extremely large numbers are required to study less prevalent disorders.

Zygosity

Ideally, zygosity should be assigned utilizing genotyping methods. However, this may be impractical and too expensive for very large population-based studies. Twin similarity questionnaires are used widely in research; they include questions on hair colour and eye colour, and whether the twins look alike and are mixed up by family and strangers. This simple method has over 95% accuracy in distinguishing between MZ and DZ twin pairs.

Adoption Studies

Adoption studies, like twin studies, allow for disentangling the effects of genes and shared environment. They provide a particularly powerful method for examining gene–environment interaction and gene–environment correlation. The basis of the method involves examining the similarity between individuals who have been adopted and their adoptive relatives and biological relatives. This type of method has added to twin evidence in demonstrating the contribution of genetic effects to disorders such as schizophrenia and traits such as IQ.

There are three main types of study design. First, the adoptee study involves examining the rate of disorder among the adopted-away offspring of affected biological parents and comparing this with the rate among controls consisting of adopted-away individuals whose biological parents are unaffected. The second type of study is the adoptee's family study. Here, the adoptee is affected by a specific disorder and the rate of disorder in the biological and adoptive relatives is compared. Finally, the cross-fostering study involves a more complex design but allows for further examination of gene–environment interaction. The rate of disorder in adoptees with affected biological parents and unaffected adoptive parents (high genetic risk, low environmental risk) is compared with the rate of disorder in adoptees with well biological parents and affected adoptive parents (low genetic risk, high environmental risk).

Adoption studies are becoming increasingly difficult to conduct, and they clearly suffer from a number of drawbacks. Adoption is an unusual event, and adoptees are not placed randomly. Adoption may occur more commonly where there is increased early environmental and perinatal adversity, and where there is increased genetic risk for some disorders. Moreover, recent work suggests that adoption studies may lack power in identifying important environmental influences due to a 'restricted range' of environments (Stoolmiller, 2000). Nevertheless, adoption studies have yielded important findings and, in particular, have provided empirical evidence of gene–environment correlation. For example, as mentioned earlier, two adoption studies have shown that the adoptive parents of children with an increased genetic risk of antisocial behaviour (because the biological parents showed antisocial behaviour) show increased rates of negative parenting (Ge *et al.*, 1996; O'Connor *et al.*, 1998).

Similarly adoption study findings have demonstrated evidence of gene–environment interaction, whereby the effects of environmental adversity are increased when there is also genetic susceptibility. In an adoption study of criminality (Bohman, 1996), the

rate of criminality amongst adoptees was 6.7% in those exposed to increased environmental risk alone and 12.1% in those at increased genetic risk. This rate rose to 40% in adoptees with a background of both genetic and environmental adversity.

Other Designs

Finally, an increasing number of mixed designs are being used to examine the contribution of genetic and environmental factors to behavioural traits and psychiatric disorders. Studies involving combinations of twins, siblings and stepfamilies have been used. These all utilize the same principle of examining the phenotypic similarity of pairs of relatives who are biologically related to a different degree and those who are biologically unrelated.

Methods of Statistical Analysis used in Family, Twin and Adoption Studies

Path Analysis

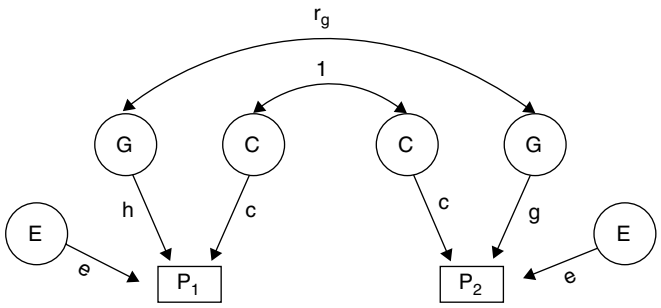
Earlier, we described the resemblance between twin pairs in terms of the proportions of variance attributable to genetic and environmental effects. The resemblance between relatives can be shown diagrammatically using a path diagram (Figure XII.7). It can be seen that the phenotype variance for each relative (P) is influenced by a genetic path (h), a common environmental path (c) and a non-shared environmental path (e), where h, c and e are path coefficients. Path analysis provides a simple method for deriving the equations we presented previously. Using the tracing rules of path analysis (see Li, 1986), the correlation between the relatives (r_{rel}) is obtained by identifying all the connecting paths and multiplying the path coefficients for each of the connecting paths. From Figure XII.7, we obtain two connecting paths: a genetic path, $h \times r_g \times h$, and a common environment path, $c \times c$.

The correlation is then obtained by summing these coefficients, so that:

$$r_{rel} = h^2 r_g + c^2$$

For monozygotic twins, $r_g = 1$, and for full biological siblings and dizygotic twins, $r_g = 0.5$. Thus, we obtain the equations that we presented earlier:

$$r_{mz} = h^2 + c^2$$
$$r_{dz} = 0.5h^2 + c^2$$



C, common/shared environment; E, non-shared environment; G, genotype; P_1, twin 1 phenotype; P_2, twin 2 phenotype; r_g, genetic correlation, 1 for MZ twins, 0.5 for DZ twins.

Figure XII.7 Basic path model of the sources of variance and resemblance between twins

Genetic Model Fitting

Although we can solve the equations presented above to obtain the different components of phenotype variance, most family and twin studies now utilize structural equation model-fitting techniques. This method has the advantage of allowing us to test formally the goodness of fit of a specific model, compare different models, and analyse more complex data sets.

Computer packages such as Mx (Neale, 1997) involve the investigator providing the raw observed data and initial starting values for the unknown parameters (h, c and e for a basic ACE model, incorporating additive genetic (A), shared environmental (C) and non-shared environmental (E) influences). The program iterates using different parameter estimates until expected values are obtained that result in the optimization of fit (i.e. minimizing the difference between the observed and expected values). Different fit functions, such as the maximum likelihood function, are available (see Neale and Cardon, 1991 for details). Acceptability of the tested model is then assessed, usually on the grounds of goodness of fit and parsimony (accepting the simplest model). One method of assessing fit is to examine the chi-squared goodness of fit, where a smaller value indicates a more satisfactory fit. The fit of reduced models, in which the additive genetic influence is dropped (CE), the common environmental influence is dropped (AE), or both A and C are dropped (E), can then be compared against the full ACE. We can subtract the chi-squared value of the full model (χ^2_f) from the reduced model (χ^2_r), and if there is no significant change in goodness of fit (i.e. $P > 0.05$), then we can select the more parsimonious model. Akaike's information criterion (AIC) provides a measure of both goodness of fit and parsimony, and models can be chosen using this method whereby the model with the most negative AIC value is chosen as providing the most satisfactory explanation of the data (Neale and Cardon, 1991).

More complex models incorporating other parameter estimates, such as dominance effects, rater contrast effects, and sibling interaction, as well as measured variables, e.g. marital conflict, can also be examined, provided that the number of unknown parameters does not exceed the number of equations.

So far, we have considered analysis of a single phenotype, which is known as univariate genetic analysis. Multivariate model fitting allows for examining the genetic and environmental influences on two or more observed phenotypes. For example, if we examined anxiety and depression and found that the cross-twin cross-trait correlation was higher in MZ than DZ twins, then this would suggest a common genetic influence on these two phenotypes. Indeed, twin studies utilizing multivariate model fitting have shown that common genetic factors account for much of the overlap of anxiety and depression symptoms and disorder (Kendler *et al.*, 1987; Thapar and McGuffin, 1997; Eley and Stevenson, 1999). Multivariate genetic methods are also being used increasingly to examine longitudinal data and the relationship between environmental risk factors and psychopathology.

DeFries and Fulker Twin Regression Analysis

A simple but elegant method of analysing continuously distributed twin data is based on simple regression analysis. This is commonly known as the DeFries–Fulker (DF) analysis. The method is particularly useful in that it can be used to test whether the genetic aetiology of extreme scores differs from aetiological influences across the normal range. For a trait that is influenced genetically, we will expect the MZ co-twins of the proband to regress less towards the population mean than the DZ co-twins of affected DZ probands. Using regression analysis, estimates of heritability and shared environment for individual differences (across the normal range), as well as for extremes, can be obtained. DF analysis

from several twin studies has, for example, suggested that attention deficit hyperactivity disorder (ADHD) behaves as a continuum, with genetic influences being as important for high scores as for across the range (Thapar *et al.*, 1999).

MOLECULAR GENETIC RESEARCH METHODS

The key technologies in molecular genetic analysis are those that aim to detect and measure DNA sequence variation. How are these techniques used to map susceptibility genes for human disease? The two methods of mapping genes are linkage and association studies. In this section, we will explain linkage and association methods that involve examining whether specific DNA variants (polymorphisms/markers) can be identified as being related to a given disorder. An outline of how DNA sequence variation is measured in the laboratory is then given.

Linkage Studies

Linkage studies involve examining the co-inheritance of the disease and a set of genetic markers within families. Where the disease susceptibility locus and marker locus are located close together on a chromosome, they will tend to be co-transmitted more often than expected by chance. This phenomenon is known as linkage. The estimated distance between the marker and disease locus is defined in terms of the recombination fraction θ. Where there is independent assortment of two loci, $\theta = \frac{1}{2}$; where the two loci are close so that recombination occurs infrequently, θ approaches zero. The advantage of linkage methods is that they can be used for hypothesis-free research. That is, no knowledge of disease pathogenesis is required, as it is feasible to undertake whole genome linkage studies rather than focusing on candidate genes. The main drawback is that unlike association methods (see later), this approach only detects genes of moderate to large effect size. The two main linkage study methods are the lod score method and the affected sib pair method.

Lod Score Method

The traditional approach of linkage analysis for mapping Mendelian disorders is the lod score method. This involves the collection of families with multiply affected individuals, and calculating lod scores for a range of values for θ. The lod score is calculated as follows:

$$\text{lod} = \log_{10} \frac{\text{probability that } \theta = \theta'}{\text{probability that } \theta = 0.5}$$

where θ' is a given value of θ. The final estimate of θ is obtained when the maximum lod score is achieved. A lod score of 3 or more is taken as indicating significant linkage.

The main problem with this method is that specific models and the mode of inheritance need to be specified. Although single-gene subforms exist for some complex disorders, e.g. Alzheimer's disease, it is becoming increasingly apparent that for most psychiatric disorders, major genes do not exist or at least are extremely rare. Thus, there has been more interest in non-parametric methods of linkage analysis.

Affected Sib Pair Method

This approach involves collecting pairs of siblings of whom both are affected by the disorder. The approach is being used widely as a strategy for mapping susceptibility genes for disorders such as schizophrenia, bipolar disorder and autism. If a marker allele is linked to the disorder, then we would expect affected siblings to share that particular allele more often than would be expected. Given that the probabilities of siblings sharing 0, 1 or 2 alleles that are identical by descent from the parents should be $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{1}{4}$, then we can test formally for statistically significant departures from these expected proportions. The affected sib pair method does not require specification of the mode of transmission, and it is thus better suited to mapping susceptibility genes for complex disorders.

The sib pair method has also been adopted for studies of quantitative (continuously distributed) traits, such as reading disability. Several approaches based, for example, on regression and variance components methods (Sham, 1998) have been developed, although extremely large sample sizes (thousands) are required to detect genes of moderate effect size. Nevertheless, strategies such as sampling sibling pairs who are highly discordant and highly concordant for scores on a particular trait measure and including a greater number of siblings can increase power. This method has been used for identifying quantitative trait loci (QTL) for reading disability, but given the increasing appreciation of the importance of genes of small effect size, association approaches also need to be considered.

Association Studies

Association studies are like case–control studies, in that they involve comparing a group of affected individuals with a control group consisting of people without the disorder. The frequencies of alleles for a given marker among cases and controls are then compared using contingency table analysis. If one particular marker allele is significantly more common among cases, then this suggests that the allele is associated with the disorder. The strength of the association is usually expressed as an odds ratio or relative risk. There are three main explanations of association. First, it may be that the polymorphism itself is contributing to susceptibility to the disease. Alternatively, the polymorphism may be extremely close to the disease susceptibility locus, with resulting linkage disequilibrium. Finally, association may arise for spurious reasons if the cases and controls differ in terms of allele frequencies for some other reason (e.g. ethnically distinct groups); this is known as population stratification. This potential for yielding false positive results has been used as an argument by some for not using case–control association studies. Although careful matching of controls is undoubtedly important, another way of overcoming these criticisms is to use family-based association study designs, whereby genetic information from the parents is used to generate an 'internal' control group. The two most commonly used methods are the haplotype relative risk (HRR) method and the transmission disequilibrium test (TDT) (Spielman *et al.*, 1993). The TDT, which requires the collection of DNA from the affected offspring and both parents, is now used widely and is a test of linkage in the presence of association. TDT analysis involves examining families where at least one of the parents is a heterozygote for the polymorphism that is being tested. If a specific allele is associated with the disorder, then we would expect this allele to be transmitted more frequently to the affected offspring than expected. The number of transmissions and non-transmissions for the two alleles are compared statistically using a simple McNemar chi-squared test. The TDT has now been developed further to allow for testing multiple alleles (so far, we have considered a biallelic polymorphism), utilizing siblings when parents are unavailable, and examining quantitative traits.

Association studies provide a statistically more powerful method of detecting genes of small effect size compared with linkage methods. So far, this type of study design has been used when examining polymorphisms within or near candidate genes. These are genes that encode for proteins that are thought to be involved in the aetiology of the disorder. This clearly poses a problem in psychiatry, where the pathogenesis of most disorders remains unknown. Nevertheless, meta-analyses of findings from association studies have

yielded significant findings of association of polymorphisms in the dopamine DRD4 receptor gene with ADHD, and the 5-HT2a receptor with schizophrenia, although effect sizes appear to be small. The alternative approach is to undertake a whole genome linkage disequilibrium or association study, whereby thousands of evenly spaced markers are tested. Although it has been suggested that this represents a useful way forward, at present there are many reservations about the practicality of such an approach, particularly with regard to the issue of multiple statistical testing and the feasibility of such large-scale genotyping. However, techniques such as DNA pooling and high-throughput genotyping may mean that whole genome association studies will become a feasible strategy for the future.

BASIC TECHNIQUES IN MOLECULAR GENETICS

The Polymerase Chain Reaction

Most molecular genetic studies employ the polymerase chain reaction (PCR). This is a method for replication of a small sequence of DNA, typically 200–1000 bases in size. The first step in PCR is to identify the sequence that defines the DNA fragment of interest. A pair of synthetic, single-stranded DNA molecules called primers are then designed. The two primers are identical to the two 5′ extremes of the DNA fragment of interest and are used to prime synthesis of DNA. Genomic DNA is mixed with the primers, a heat-stable DNA polymerase (an enzyme that catalyses the formation of DNA, e.g. Taq polymerase), and a mixture of the individual monomeric nucleotides (A, T, C and G, often referred to as dNTPs). The dsDNA is converted into single-stranded DNA (or denatured) by heating, and then cooled to allow the primers to bind to the single-stranded genomic DNA by base-pairing, a process known as hybridization. Catalysed by the Taq polymerase, primers are then extended from their 3′ ends according to the rule of complementarity by the sequential addition of the appropriately paired dNTPs. This results in a larger DNA molecule that is complementary to the original DNA strand to which the primer is bound. The whole process (denature, hybridize, extend) is repeated many times, with each cycle exponentially increasing the number of copies of the target sequence. At the end of the process, the starting concentration of the target fragment has been increased by $\sim 10^5 - 10^6$ to a concentration that can be detected easily by several techniques (see below).

Genotyping Polymorphisms that Alter the Size of Fragments

Genotyping is the process of determining which alleles are carried by an individual at a given locus. A class of polymorphism commonly used in genetic studies is the short tandem repeat polymorphism (STRP). These contain a certain sequence repeated several times, but the exact number of times a sequence is repeated varies in the population. The repeat is often a dinucleotide repeat, containing multiple copies of the sequence CA. Genotyping is then simply a case of measuring the size of the repeat. This is achieved by first amplifying genomic DNA by PCR in the presence of primers that flank the repeat. One of the primers is usually tagged with a radioisotope or a fluorescent dye. The PCR products are then separated by size by gel electrophoresis. The DNA molecules are placed at one end of a gel-like substance and subjected to a voltage gradient. This causes the PCR products to migrate along the gradient towards the positive terminal at a rate that is related inversely to size. The DNA fragments in the gel can then be visualized by the use of one of several DNA-staining techniques or, if fluorescent dyes or radioisotopes have been used, by a fluorescence scanning device or autoragiography respectively. This allows the size of the amplified molecules to be determined and therefore the alleles present to be identified.

Genotyping Single-Nucleotide Polymorphisms

SNPs are the most common types of polymorphism, and are increasingly thought of as the polymorphisms of choice for genetic studies. At the time of writing, the most common method for genotyping SNPs is based upon enzymes called restriction endonucleases. Such enzymes recognize and cut dsDNA at specific stretches of the DNA sequence of usually between four and eight bases. If a polymorphism changes the recognition sequence for one of these enzymes, then one allelic variant will be cleaved but the other not. After PCR, the reaction products are digested with an appropriate enzyme, which results in two smaller fragments in the presence of the allele that is cleaved by the enzyme, or one large fragment in the presence of an allele that is not cleaved. Polymorphisms like these are called restriction fragment length polymorphisms (RFLPs). As the assay is now based upon fragment size, the genotype can be determined by a process of electrophoresis.

Emerging Methods for Genotyping

There are numerous new techniques on the horizon for genotyping SNPs, some of which have already been installed in a number of laboratories. It is not yet clear which technique will emerge as the method of choice. Here, we consider briefly a few of the most promising techniques.

Mini-Sequencing/Primer Extension

After PCR, a third primer (the extension primer) is added. This extension primer is designed so that it anneals to one of the strands of the PCR product immediately adjacent to the polymorphic base. A second reaction is then performed, which permits the primer to be extended by only a single base. The specific base that is added to the primer will be the base that is complementary to the base at the polymorphic site. If the polymorphism at a given site is either an A or a C, then by complementarity, either a T or a G will be incorporated, respectively. The nature of the incorporated base can then be detected by a variety of methods, including mobility in a gel, fluorescence or mass.

Micro-Arrays (or DNA Chips)

Genotyping by DNA chips may be based on primer extension or allele-specific hybridization. In general terms, a DNA sequence that is a perfect match to another DNA sequence will hybridize more efficiently to that sequence than to a very similar sequence. In the case of the SNP above, amplified alleles containing an A at the polymorphic site will hybridize to a complementary DNA molecule containing a T in the correct position in preference to a molecule that is identical in all respects other than it contains a G instead of a T. The opposite is true for alleles containing a C. This is the principle of allele-specific hybridization.

In one application of allele-specific hybridization, PCR products from a single locus from thousands of subjects are spotted out (arrayed) individually in an area approximately the size of a postage stamp. This micro-array can then be hybridized with two short synthetic DNA molecules, each of which is perfectly complementary to one of the two possible alleles, and each tagged with a different fluorescent dye. After hybridization, the micro-array is analysed by a confocal laser system to determine the colour of the oligonucleotide bound to each spot, which therefore defines the genotype of that individual at that locus. Alternatively, instead of arraying PCR products from thousands of individuals, each spot on

the micro-array can contain a different oligonucleotide, with pairs of spots representing a pair of possible alleles at a given locus. Under this scenario, a chip can be constructed that will allow thousands of loci to be arrayed on a single micro-array. Different PCR products representing thousands of different loci from an individual can then be used to probe the micro-array, and the pattern of binding at each pair of spots then yields the genotype at each locus.

Detecting Unknown DNA Variation

The term 'mutation detection' describes the process of identifying unknown population variation in a defined stretch of DNA. There are currently numerous mutation-detection techniques available. The definitive method is DNA sequencing, which is the process of identifying the complete sequence in a fragment of DNA. Thus, one method for looking for population variation is to sequence the same stretch of DNA in many different people. However, sequencing is expensive and, outside the major public and private genome centres, it is labour intensive. For this reason, prior to sequencing, most laboratories first undertake mutation scanning to identify fragments that are likely to contain variants.

One of the simplest and most widely used of these is single-strand conformation analysis (SSCA) (Orita *et al.*, 1989). Even a single-base change in a single-stranded PCR product can alter its three-dimensional folding pattern (or its secondary structure), particularly if the DNA molecule is small (around 200 bases). This can be detected as altered migration of single-stranded PCR products during electrophoresis. Polymorphisms detected in this way are sometimes called single-stranded conformation polymorphisms (SSCP), and the technique is sometimes also known as SSCP. There are many more mutation-scanning techniques (Cotton, 1997), and the degree of automation is evolving rapidly. For example, in more highly automated laboratories, mutation-scanning analysis is often performed using high-performance liquid chromatography (HPLC) rather than a gel. With this method (called denaturing HPLC or DHPLC), and other semi-automated technologies, (including improvements in sequencing) whereas a few years ago screening a single gene for polymorphisms was a fairly labour-intensive process, it is now possible to contemplate screening hundreds or even thousands of genes in a single laboratory.

Information Technology and Positional Cloning

Positional cloning refers to a set of techniques by which disease-related genes are identified on the basis of their chromosomal location. Classically, positional cloning is achieved by first identifying the approximate chromosomal location of a disease gene by linkage (see earlier). This process is simplified greatly by the availability of fairly detailed 'maps' of polymorphisms called DNA markers, which are sequence variants known to reside at particular locations in the genome. The next step is to identify the specific sequence variations in that region that confer susceptibility to disease. A number of strategies may be used. However, the classic approach requires the creation in the test tube of a complete representation of the linked region in a series of small contiguous cloned fragments of DNA. The next stages are to identify the relatively small proportion of the DNA within the region corresponding to genes, screen these for sequence mutations/polymorphisms, and then test whether any of the detected variants are more common in patients with the disease compared with people who are unaffected. This process involves a great deal of cost and labour, such that analyses of even relatively small genomic regions of 1–2 million bases are large undertakings.

Fortunately, as a consequence of the Human Genome Project, most of these techniques are now in the process of being replaced by cloning 'in silico'. Increasingly, genomic sequence, gene sequence, and even the identification of some polymorphisms can all be obtained by searching computer databases. Thus, genetic researchers increasingly depend at least as much upon expertise with bioinformatics as they do upon 'wet' laboratory experiments.

Candidate Gene and Positional Candidate Gene Analysis

A major benefit of the positional cloning approach is that genes can be identified without any knowledge of disease pathophysiology. However, as we discussed earlier, where there are good clues to the pathophysiology of disease, or where the genetic effects are so small that they are difficult to detect by (at least today's) positional cloning technologies, hypothesis-driven candidate gene approaches are appropriate. Essentially, the task of the candidate gene approach in the laboratory is to select genes with plausible a priori roles in the disease pathogenesis (called functional candidate genes), screen the candidate gene sequences for DNA sequence variation, and test populations of affected and unaffected subjects for evidence that variants in the candidate gene are more commonly associated with affected status. As mentioned above, the technologies that make this approach possible (availability of gene sequence, mutation detection and genotyping) are all undergoing rapid improvements, and whereas testing a single candidate gene was rather time consuming in the past, it is now possible to test many candidate gene hypotheses in relatively short periods of time. Furthermore, although some considerable distance in the future, a catalogue of every human polymorphism in every gene will eventually become available, making the mutation-screening step at least partially obsolete. Ultimately, one can envisage the possibility of buying micro-arrays with either every known common polymorphism represented, or using specific chips that will allow whole systems of candidate genes (e.g. the genes known to be involved in neurotransmission or neurodevelopment) to be tested for association with disease. However, with multiple testing on such a large scale on the horizon, appropriate statistical techniques and adequate sample sizes will clearly be required.

Gene Expression

The function of a cell depends upon its pattern of gene expression, and it follows that altered function as part of a disease process is likely to be accompanied by alteration in the gene-expression profile. This offers the possibility of identifying the pathophysiological pathways or genes involved in a disease process by looking for differences in gene expression in tissues from individuals with and without that disease. Many readers will already be familiar with this broad approach, which is analogous to more traditional studies looking at differences in metabolic enzyme activities or neuroreceptor densities in patients versus controls. Indeed, many of our hypotheses concerning the aetiology of mental disorders have been provided by these methods. The recent development of technology allowing virtually every protein or mRNA in a tissue to be assayed is likely to provide important opportunities for developing new hypotheses concerning the aetiology of mental disorders.

Gene expression can be quantitated at the level of mRNA or the level of translated protein. There are many methods that permit individual mRNA species to be quantitated (e.g. Northern Blotting), but technological advances in the last 5 years have changed beyond recognition the rate at which expression data can be extracted. For example, it is now possible to measure the expression levels of thousands of genes simultaneously using gene-expression chips. These chips are simply micro-arrays containing thousands of DNA sequences, each of which is individually complementary to a different species of mRNA. Total RNA from a cell type or a tissue is reverse-transcribed in the presence of a fluorescent dye to create a complex cDNA mix. This is then hybridized to the micro-array.

Each different arrayed sequence binds a specific mRNA species, and the amount that is bound gives an indication of the abundance of that specific mRNA. By comparing the patterns of hybridization from either different tissues or different individuals, it is possible to create a profile of the genes that are differentially expressed either in different tissues or between subjects. Analogous technologies are also available for quantitating proteins, and technologies are under development that will even allow the function of thousands of proteins to be analysed simultaneously.

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XIII

Gene–Environment Interactions

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INTRODUCTION

Most genetic-epidemiological approaches used in the study of the aetiology of individual differences in psychiatric disease in humans assume that the effects of genes and environment are additive. That is, it is assumed that the effect of an environmental risk factor does not depend on the genotype of the individual, or, stated differently, that the expression of the genotype does not depend on the subject's environment. Kendler and Eaves (1986) discuss several models for the joint effects of genes and environment on the liability to psychiatric illness that extend this simple additive approach. In addition to the additive effects of genes and environment, they consider models for the genetic control of sensitivity to the environment (gene–environment interaction) and models for the genetic control of exposure to the environment (gene–environment correlation). We begin this chapter by briefly introducing the ideas of Kendler and Eaves, and by considering the predictions for risk of illness in relatives when for both probands and relatives their environmental exposure can be assessed. Next, we focus in more detail on genotype $\times$ environment ($G \times E$) interaction, and present some designs, using twin data, to assess its significance. We illustrate these models with published data on depression, disinhibition and alcohol use in Australian and Dutch twins.

Kendler and Eaves (1986; see also Eaves, 1982 and Eaves, 1984) describe three basic models that represent how genes and environment may jointly influence the liability to psychiatric disorder. Liability to psychiatric disorder is considered a quantitative dimension that is unobserved, or latent, and that underlies the probability of becoming affected. Individuals with a high liability have a high probability of illness, while individuals who score low on the liability scale have a low probability. Many traits that vary in a discontinuous manner, but do not show a pattern of simple Mendelian inheritance, may have an underlying continuous liability scale with a threshold that imposes a discontinuity (e.g. affected or unaffected for the disorder) on the visible expression. The variation in liability may be caused by both genetic and environmental influences (Falconer, 1989).

Kendler and Eaves (1986) propose three models that explain variation in liability:

1. Genes and environment contribute additively to liability of a disorder.
2. Genes and environment interact; this interaction model can be thought of as genes controlling sensitivity to the environment, or as the environment controlling gene expression.
3. Genes and environment are correlated: genes alter the exposure to relevant environmental risk factors.

These explanations for the variation in disease liability are not mutually exclusive; part of the variance may be explained by the additive actions of genes and environment and another part by their interaction. The properties of such complex models are a mixture of the characteristics of the three basic models, and we limit ourselves to the presentation of these basic models. In discussing some of the predictions based on these models, it is assumed that the environment can be dichotomized into 'protective' or 'predisposing', and that environmental risk can be assessed in the proband (the index case or patient from whom other family members are identified) and in his or her relatives.

In the additive model (model 1), an individual's liability to disorder is the sum of the contributions of genes and environment. Under this model, the effect of a given environment is the same, regardless of genotype. The risk to relatives to become affected is highest when an affected proband is or was exposed to a protective environment. The risk of developing the disorder is always higher in monozygotic co-twins of probands (who share all their genetic material with the proband) than in their siblings or other first-degree relatives (who share, on average, 50% of their genetic material with the proband).

When there is $G \times E$ interaction (model 2), genes determine the degree to which a subject is sensitive to the environment. Individuals with a 'sensitive' genotype have the greatest increase in scores on the liability scale in the predisposing environment and the largest decrease in the protective environment. In individuals with an 'insensitive' genotype, their scores on the liability scale do not change, or change to a lesser extent, as a function of their environment. *The risk to relatives of affected probands randomly distributed over environments is modestly greater than in the general population*. Under this model, familial resemblance is, in general, lower than under an additive model, especially when the predisposing environment is relatively rare. The risk to relatives is highest when both proband and relative have been exposed to a predisposing environment and risk is higher in monozygotic co-twins than in siblings only when both proband and relative have been exposed to the same kind of environment. If, for example, the proband is exposed to a protective environment and the relative to a predisposing environment, then the risk to siblings may be higher than the risk to monozygotic co-twins.

Genetic control to environmental exposure (model 3) implies that the heritability of illness can be explained (entirely or to a certain extent) by an indirect path of genetic influence on the probability of exposure to a predisposing environment. If the heritability of the disorder is explained entirely by this mechanism, then the liability of disease does not depend on genotype once environmental exposure is taken into account. If this mechanism operates, then it will be very difficult to distinguish it from a pure genetic model (Eaves *et al.*, 1977).

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A CLOSER LOOK AT GENOTYPE × ENVIRONMENT INTERACTION

The models outlined by Kendler and Eaves (1986) offer a first conceptualization of the joint effects of genotype and environment on psychiatric disease liability or on a (quantitative) psychiatric phenotype. We now take a closer look at G × E interaction. As stated above, G × E interaction can be thought of as the effect of an environmental risk factor depending on the genotype of the individual, or as the expression of the genotype depending on the subject's environment. Measures of either the subject's genotype or of a relevant aspect of their environment are therefore required to assess the significance of G × E interaction (Martin *et al.*, 1987). With respect to measures of the genotype that are possibly relevant to the development of psychiatric disease, no strong empirical findings have yet emerged that unambiguously identify susceptibility loci for psychiatric disorder. This is not for lack of trying. Polymorphisms in large numbers of candidate genes have been tested in genetic association studies. These include polymorphisms in pathways of neurotransmitters, such as serotonin, dopamine, noradrenaline and γ-aminobutyric acid (GABA). Not all variants that have been tested, however, have yet been shown to be functional at the transcription or enzyme activity level (Stoltenberg and Burmeister, 2000). Terwilliger and Weiss (1998) present an overview of the results obtained in association studies of candidate genes and psychiatric disorder published in *American Journal of Medical Genetics (Neuropsychiatric Genetics)* and *Psychiatric Genetics* in 1997. The distribution of the 261 reported significance levels is almost uniform between 0 and 1, which leads Terwilliger and Weiss to conclude: 'even the significant results may not be real, as there are just as many P values that are too large as those that are too significant.' The published P values are consistent with the absence of gene effects in all published analyses. It may be that for some candidate genes that have been studied, the relevant variants have not yet been investigated. For example, for both the serotonin transporter gene (SERT) and the dopamine transporter gene (DAT), it has recently been reported that they contain many more variants than the simple repeat polymorphisms that, until now, were used in genetic association studies (Nakamura *et al.*, 2000; Miller *et al.*, 2001). Another possibility to explain the absence of any strong findings of risk genotypes for psychiatric disorder is population stratification. If in case–control studies investigating candidate genes the effect of stratification is in the opposite direction to the effect of a genuine candidate gene, this will lead to false negative results (Cardon, personal communication). Alternative approaches to model the effects of candidate genes that test the association of particular alleles and disorders both between and within families (Fulker *et al.*, 1999) overcome the problem of stratification but have rarely been applied to the study of psychiatric disorders. One example is the study of Lesch *et al.* (1996). In this study, the association between SERT and neuroticism was shown to be equally strong in a sample of unrelated individuals as in a sample of siblings (brothers) with different genotypes for the short/long polymorphism in the transcriptional control region upstream of the SERT coding sequence. Therefore, the association cannot be explained by mechanisms such as population stratification, because siblings belong to the same stratum.

In the absence of solid findings of candidate genotypes increasing susceptibility for psychiatric disorder, we explore the presence of G × E interaction by looking at the expression of unidentified genetic influences conditional on environmental exposure. The approaches suggested by Eaves (1982) to test for the presence of G × E interaction involving a measured dichotomous environmental variable (e.g. presence or absence of a risk factor) and Falconer (1989), involving a longitudinal study design, will be introduced and illustrated with some empirical examples.

In the approach suggested by Eaves (see also Heath *et al.*, 1989 and Heath *et al.*, 1998), the relative influences of genotype (heritability) and environment on a trait are estimated conditional upon environmental exposure. When there is no G × E interaction, the influence of genetic and environmental factors should not differ between subjects with different degrees of exposure. If genetic effects are modified by exposure, such that heritabilities differ significantly between exposure-positive and exposure-negative groups, then this constitutes evidence for G × E interaction. Thus, this type of interaction is detected by testing whether the amount of variance explained by genetic factors differs between exposure-positive and exposure-negative groups. In addition to G × E interactions as indexed by differences in heritabilities between groups, there may also be differences in the amount of variance explained by environmental factors. This phenomenon is called heteroscedasticity (e.g. Heath *et al.*, 1989).

A true difference in heritabilities between subjects from groups differing in environmental exposure constitutes evidence for G × E interaction, but it does not tell us whether the same or different genes are expressed in the different groups. To address this issue, data from twins discordant for environmental exposure or longitudinal data from twins (or other genetically related subjects), are required. If in a longitudinal design the same subjects can be measured under different environmental conditions, then the extent to which the same genes are expressed in different environments can be assessed. Falconer (1989) (see also Lynch and Walsh, 1998) proposed that the same character measured on the same subjects in two different environments can be treated as two different traits. G × E interaction can then be detected in a bivariate genetic analysis from the genetic correlation between the two traits. If the genetic correlation is high, then trait values in two different environments are very nearly determined by the same set of genes. If the genetic correlation is low, then the trait is influenced by different sets of genes in different environments, and this provides evidence for G × E interaction. A genetic correlation across environments of unity need not imply the absence of G × E interaction, because, as was discussed above, this still leaves open the possibility that the relative importance of genetic factors in explaining individual differences is a function of environmental exposure.

Not all traits can be studied in a longitudinal design. Lynch and Walsh (1998) distinguish between 'labile' and 'nonlabile' traits. Labile traits are those for which phenotypic expression can adjust within individuals, through physiological and/or behavioural means, to changes in the environment, e.g. behavioural traits in the presence of competitors or mates, blood pressure or cortisol in response to stress (Boomsma *et al.*, 1998), and behavioural and emotional problems during development (Van der Valk *et al.*, 2001). Nonlabile traits become fixed during some sensitive period of development (e.g. height, age at first major depression) and thus cannot be studied in a longitudinal design that requires the same subjects to participate under different environmental conditions. Both labile and nonlabile traits can show G × E interaction, but whereas for a labile trait the entire reaction norm can be determined at the individual level by measuring the same individual in a number of environments, for nonlabile traits, different individuals need to be studied in different environments. A particularly informative design for nonlabile traits is one that includes twin pairs who are concordant and discordant for a certain environmental exposure. The extent to which the resemblance (expressed as a correlation between trait values of twin 1 and twin 2) in discordant twins differs from the correlation predicted from the correlations in concordant twin pairs gives an estimate of the extent to which the genetic correlation differs from unity. In the next section, we present a brief introduction to the estimation of genetic and environmental parameters (e.g. heritabilities and correlations) and to formal testing of G × E interaction.

STATISTICAL APPROACH

In analysing G × E interaction, we will use structural equation modelling (SEM) or genetic covariance structure modelling (GCSM) to obtain estimates of parameters and to carry out hypothesis testing. These techniques provide a general and flexible framework for the analysis of data gathered in genetically informative samples. In applying GCSM, genetic and environmental effects are modelled as the contribution of latent (unmeasured) variables to the phenotypic individual differences, or to a liability dimension, which itself is also unobserved (e.g. Martin and Eaves, 1977; Neale and Cardon, 1992). The latent genetic and environmental factors represent the effects of many unidentified influences. In the case of a genetic factor, these effects are due to a possibly large, but unknown, number of genes (polygenes). The latent environmental factors can be distinguished into environmental influences common to family members and environmental influences specific to an individual. The contributions of the latent variables are estimated as regression coefficients in the linear regression of the observed phenotype on the latent variables. Given an appropriate design providing sufficient information to identify these coefficients, actual estimates may be obtained using a number of well-known computer programs, such as LISREL (Joreskog and Sorbom, 1995), or Mx (Neale, 1997).

Identification of genetic models, especially the identification of the effect of shared family environment versus shared genes, can be achieved by several designs, such as adoption or twin designs. We focus on the classical twin design (Martin *et al.*, 1997), which includes monozygotic (MZ) and dizygotic (DZ) twins. MZ twins are genetically identical, while DZ twins (and siblings) share on average 50% of their segregating genes. If MZ twins resemble each other more than DZ twins do, then this is evidence for the importance of genetic influences on the trait under consideration. One advantage of structural equation modelling is that this approach is easily generalized to multivariate and longitudinal data. Twin data are used to decompose the variance for a single trait into a genetic and a non-genetic part. Likewise, bi- and multivariate twin data can be used to decompose the covariance between traits, or between repeated measures of the same trait, into a part due to correlated genetic influences and a part due to correlated environmental influences (Martin and Eaves, 1977; Boomsma and Molenaar, 1986). The flexibility of GCSM is also evident in the relative ease with which measured genotypic (e.g. DNA marker data) or environmental information can be incorporated into the analysis.

In both univariate and multivariate genetic analysis, the identification of genetic and environmental parameters depends on a multigroup analysis in which data from MZ and DZ twins are analysed simultaneously. For a univariate phenotype, the phenotype (P) can be expressed as a function of an individual's genotype (G), the environment common to family members (C), and the environment unique to the individual (E):

$$\mathrm{P}_i = g\mathrm{G}_i + c\mathrm{C}_i + e\mathrm{E}_i$$

where $i = 1, \ldots N$ individuals.

The coefficients g, c and e are population parameters that represent the strength of the relation between the measured phenotype and the latent (unmeasured) factors G, C and E. The latent genetic and common environmental factors are correlated in family members. In MZ twins, the genetic factors are correlated 1, and in DZ twins the correlation is 0.5. The common environmental factors are correlated 1 in both MZ and DZ twins, unless the twins are of opposite sex. For DZ opposite-sex (DOS) twins, the correlation of their common environmental factors may be less than 1 if the family environment exerts a different influence on boys and girls. Likewise, the correlation between genetic factors may be less than 0.5 in DOS twins if different genes are expressed in males and females.

Assuming that all latent variables have been standardized to have unit variance, and that the latent factors are uncorrelated (no gene–environment correlation), then the variance of P may be written as:

$$V(P) = g^2 + c^2 + e^2$$

where $V(P)$ is the variance of the phenotype, and g^2, c^2 and e^2 represent the genetic, common environmental and unique environmental variances. The standardized genetic variance (i.e. g^2 divided by the phenotypic variance $V(P)$) is called the heritability of the phenotype, often symbolized by h^2.

The parameters g, c and e may be estimated by maximum likelihood, implemented in, for example, LISREL or Mx. Their significance can be assessed by likelihood ratio tests. For example, the goodness of fit of a model that constrains the genetic variance to be zero can be compared with a model in which the genetic variance is estimated freely. The difference in goodness-of-fit parameters then provides a test of significance for the genetic effect. In multigroup designs with, for example, MZ and DZ twins who were assessed in a protective or a predisposing environment, equality of parameter estimates across exposure groups can also be assessed by likelihood ratio tests (Neale and Cardon, 1992).

Estimates of the genetic correlation between two traits (or between values of the same trait assessed twice in the same twins under different environmental conditions) can be obtained from the bivariate generalization of this model. The correlation (r_p) between two phenotypes P1 and P2 is given by:

$$r_p = h_1 h_2 r_g + c_1 c_2 r_c + e_1 e_2 r_e$$

where r_g, r_c and r_e are the correlations between genetic, common and unique environmental factors that influence phenotype 1 and phenotype 2 (or the same trait measured in environment 1 and environment 2). These correlations are weighted by the square roots of the standardized heritabilities for trait 1 and 2 (h_1 and h_2, respectively) and the square roots of the standardized environmentalities (Plomin *et al.*, 2001). The significance of these correlations may also be tested by likelihood ratio tests, comparing the likelihood of a more restricted model (e.g. $r_g = 0$) with the likelihood of a less restricted model (in which r_g is estimated).

EMPIRICAL EXAMPLES

Information on personality, anxiety and depression, alcohol initiation and several aspects of religion was collected by mailed survey in 1974 Dutch families consisting of adolescent and young adult twins and their parents (Boomsma *et al.*, 1999; Koopmans *et al.*, 1999). These data were used to explore the influence of religion on personality traits and alcohol initiation. First, the influence of different aspects of religion on average scores for personality characteristics, such as neuroticism, extraversion and sensation seeking, and for depression and anxiety was investigated. Several traits were associated significantly with religion. The association between religion and personality was found to be especially strong for the disinhibition scale of the sensation seeking questionnaire (Zuckerman, 1971; Feij *et al.*, 1997). Therefore, this scale was selected to look at the genetic architecture of disinhibition in male and female twins with and without a religious upbringing. The familial resemblance for different aspects of religion — upbringing, religious affiliation and active participation — was large, and genetic factors did not contribute to this familial resemblance.

We tested whether in addition to an effect on means, there was any evidence for an interaction between genotype and environment (G × E) on disinhibition scores. When there is no interaction, the influence of genetic and environmental factors should not differ

Table XIII.1 Twin correlations for disinhibition and alcohol use as a function of religious upbringing in adolescent and young adult Dutch twins

	Disinhibition					Alcohol use				
	MZM	DZM	MZF	DZF	DOS	MZM	DZM	MZF	DZF	DOS
Religious	0.62	0.62	0.61	0.50	0.38	0.93	0.82	0.87	0.90	0.75
Non-religious	0.62	0.35	0.58	0.35	0.30	0.88	0.61	0.95	0.72	0.75

DZF, dizygotic female; DZM, dizygotic male; MZF, monozygotic female; MZM, monozygotic male
Religious: MZM, 149 pairs; DZM, 124 pairs; MZF, 227 pairs; DZF, 169 pairs; DOS, 259 pairs
Non-religious: MZM, 143 pairs; DZM, 123 pairs; MZF, 188 pairs; DZF, 151 pairs; DOS, 214 pairs

Table XIII.2 Percentage of variance in disinhibition and alcohol use explained by genetic factors (G), common environment (C) and unique environment (E) for males (M) and females (F) as a function of religious upbringing. Parameter estimates with 95% confidence intervals in parentheses

	Disinhibition			Alcohol use		
	G	C	E	G	C	E
F, religious	37 (22–55)	25 (9–37)	38 (32–46)	25 (7–48)	67 (46–82)	7 (3–16)
F, non-religious	61 (7–67)	0 (0–48)	39 (32–51)	40 (5–69)	47 (20–76)	13 (6–26)
M, Religious	0 (0–22)	62 (43–69)	38 (31–45)	0 (0–17)	88 (72–92)	12 (7–19)
M, Non–religious	49 (14–69)	11 (0–40)	40 (32–51)	39 (14–66)	56 (29–78)	5 (2–11)

between twins with and without a religious upbringing. If genetic effects are modified by religious upbringing (differ significantly between the religious and non-religious groups), then this constitutes evidence for G × E interaction. In these analyses, religious upbringing is thus considered to be the shared environment, and disinhibition and alcohol use are the phenotype. Table XIII.1 presents the correlations between twins for disinhibition and for alcohol use conditional on religious upbringing. Both for disinhibition and alcohol use, the MZ and DZ correlations do not differ, or differ very little, from each other in twin pairs from a religious background. In contrast, in the twin pairs who were brought up in a non-religious environment, MZ correlations are larger than DZ correlations, though more so for disinhibition than for alcohol use. Table XIII.2 gives the estimates for heritability and the amounts of variance explained by common environmental factors shared by family members and by unique (or idiosyncratic) environmental factors (Boomsma *et al.*, 1999; Koopmans *et al.*, 1999). In males, the difference in heritabilities between religious and non-religious groups is significant for both variables. In females, the differences between religious and non-religious groups are in the same direction as for males, but they are not statistically significant. In subjects with a religious upbringing, the influence of their genotype on disinhibition and alcohol use is much lower than in subjects from a non-religious background. Indeed, in religious males, the point estimates for heritability are zero. These findings suggest that in subjects raised in a religious home environment, the expression of genetic factors is restricted. The influence of genetic factors is larger in adolescents and young adults who have been brought up without religion.

Further examples suggest that the environmental dichotomy of married/partnered versus having no life partner is a powerful modifying factor on expression of genetic variation for a number of traits in females, including alcohol consumption (Heath *et al.*, 1989) and depression (Heath *et al.*, 1998), although since being married successfully is itself under partial genetic control (Jockin *et al.*, 1996), these may not be straightforward cases of G × E interaction. Our first example did not include any twin pairs who were discordant for religious upbringing (all pairs grew up in the same family), but the study on alcohol consumption in adult Australian twins includes a group of older females discordant for marital status. In comparison to the concordant groups, MZ female twins who are discordant for being married resemble each other less. The correlation for alcohol use in discordant DZ twins is near zero, but a similar low correlation was observed in concordant unmarried DZ females. Due to the small number of pairs in some of the groups, a statistically based discrimination between different models of G × E interaction was not possible, but all analyses pointed to the conclusion that the importance of genetic factors is larger in older, unmarried respondents. The estimate for the genetic correlation between alcohol consumption in the married and in the unmarried state was 0.59, indicating that different genes may be expressed under these two conditions.

POWER

The number of twin pairs needed to detect a statistically significant difference in heritabilities between two groups may be quite large. The number depends on the size of the heritabilities in the two groups, on the significance level, and on the power chosen by the researcher. We investigated through simulation (e.g. Neale and Cardon, 1992) the sample sizes required to detect G × E interaction in the classical MZ/DZ twin design. Some results are presented in Table XIII.3 The standardized heritability of the trait was always 60% in one of the exposure groups, and was smaller in the second group (between 0% and 40%). In the second group, there could be an additional contribution of common environment, accounting for 20–60% of the variance. The left-hand part of Table XIII.3 gives the results for the situation in which there is always contribution of common environment to trait variance (in this case, the MZ correlations in both exposure groups are always 0.6, and only the DZ correlations differ, e.g. when $h^2 = c^2 = 0.3$, the DZ correlation is 0.45). The right-hand part of Table XIII.3 gives the required sample sizes when heritabilities differ between groups, but when there is no contribution of common environment. In this case, both MZ and DZ correlations differ from those in the group where $h^2 = 0.6$. The columns labelled N1 give the required number of twin pairs for a design in which the sample consists of equal numbers of MZ and DZ pairs, and the columns labelled N2 for a design in which the sample consists of 40% MZ and 60% DZ pairs, for power equal to 50, 80 or 90%. The test of significant differences in genetic architecture

Table XIII.3 Number of twin pairs required to detect a significant difference in heritability between two groups differing in exposure to an environmental risk factor (df = 3, $P = 0.05$, power = 50, 80 or 90%) for samples consisting of 50% MZ and DZ 50% (N1) pairs and of 40% MZ and 60% DZ (N2) pairs

Group 1: $h^2 = 0.6$ and $c^2 = 0$				Group 1: $h^2 = 0.6$ and $c^2 = 0$			
Group 2:	Power(%)	N1	N2	Group 2:	Power(%)	N1	N2
$h^2 = 0.4$ and $c^2 = 0.2$	50	3169	2639	$h^2 = 0.4$ and $c^2 = 0.0$	50	451	533
	80	5998	4994		80	854	1009
	90	7796	6491		90	1110	1311
$h^2 = 0.3$ and $c^2 = 0.3$	50	1329	1103	$h^2 = 0.3$ and $c^2 = 0.0$	50	229	269
	80	2507	2087		80	433	509
	90	3258	2713		90	563	661
$h^2 = 0.0$ and $c^2 = 0.6$	50	263	219	$h^2 = 0.0$ and $c^2 = 0.0$	50	76	88
	80	498	414		80	143	166
	90	647	539		90	186	215

between groups had three degrees of freedom (df), allowing for differences in the contributions of genetic, common and unique environmental factors, although it could be argued that a 2 df test is more appropriate (which would require smaller sample sizes), because the type of G × E interaction of interest does not include a difference between groups in the contribution of unique environmental factors.

Table XIII.3 shows that for a power of 80% and for relatively small differences in heritabilities between groups (0.3 or smaller), large samples are needed, especially if there is also a contribution of common environment to familial resemblance. When this is the case, a design with 40% MZ pairs and 60% DZ pairs has more power than a design with equal numbers of MZ and DZ pairs (e.g. when for the second group $h^2 = c^2 = 0.3$, a study with an equal number of MZ and DZ pairs requires 2507 pairs, i.e. more than 5000 individuals, whereas an N2 design requires 2087 pairs).

Fewer twin pairs are needed to detect a significant difference in heritabilities between exposure groups, when genes are the only source of familial resemblance and there is no contribution of common environment. In this case, as shown in the right-hand part of Table XIII.3, a design with an equal distribution of MZ and DZ twins has the highest power.

LEVEL GENES AND VARIABILITY GENES

It is possible that there are genes that have no effect on the mean expression level of a trait but, depending on environmental circumstances, have a greater or lesser variance of expression. It is not difficult to think of molecular mechanisms, e.g. promoters of different binding efficiency, to explain the existence of such variability genes. MZ twins offer a unique opportunity to test for the presence of such genes if a measured genotype is available. Most readers will be familiar with the ABO and Rh blood groups, which are polymorphisms in proteins of the red blood cells determined by genes on chromosomes 9 and 1, respectively. The MN blood group is another such polymorphism in a cell-surface protein, which is coded for by a gene on chromosome 4. Magnus *et al.* (1981) showed that the intrapair variance for cholesterol in MZ pairs who were blood group M− (i.e. blood group N) was significantly greater than in MZ pairs who were M+ (i.e. blood groups M and MN); this was replicated by Martin *et al.* (1983). From this observation, Berg *et al.* (1989) introduced the idea of 'variability genes' as opposed to 'level genes'. Level genes affect the mean expression of a trait, or prevalence in the case of a disease, and are the usual target of association studies. Variability genes do not need to influence trait levels, but they determine the influence of the environment on intra-individual variability. Thus, Birley *et al.* (1997) were able to predict from these results that blood group N subjects would have a greater drop in serum cholesterol levels in response to a low-fat diet than MN heterozygotes, and indeed this was observed. Potentially, variability genes can be even more important than level genes. An allele that increases environmental variance by 50% (the approximate effect size of the N allele on cholesterol variability) will increase the proportion of cases above the second standard deviation by more than two-fold, and those above the third standard deviation by more than five-fold. Unfortunately, however, the power of the variance ratio test to detect heterogeneity of variances is not large (Martin, 2000).

If the genes that contribute to sensitivity to the environment are correlated (distribution or action) with genes affecting the mean expression of a trait, then MZ twins offer an opportunity to detect G × E, without the requirement that they are genotyped (Jinks and Fulker, 1970; Eaves *et al.*, 1977). The difference in trait values between MZ twins is an estimate of the environmental effect, whereas the sum of MZ twins' scores is a function of their (shared) genetic deviation and their family environmental deviation. Assuming that there is some genetic contribution to the trait — as evidenced by a greater MZ than DZ correlation — any relationship between the absolute trait differences of MZ pairs and their corresponding sums is evidence of G × E interaction. Jinks and Fulker (1970) suggested that as a preliminary step to any biometrical genetic analysis of twin data, one should therefore regress the MZ absolute pair differences on their pair sums to check for G × E interaction. In practice, this form of G × E is often predictable as a function of the non-normality of the scale distribution and can be removed by an appropriate transformation of the scale of measurement. Thus, positive skewness (tail to the right) produces positive correlations between MZ differences and sums, and negative skewness produces a negative correlation. The often observed 'basement–ceiling' effect in psychometric scales, where there is good discrimination of differences in the middle of the range but a bunching at the low and high extremities, produces an inverse U-shaped quadratic relationship of means and intrapair differences.

DISCUSSION

'No aspect of human behavior genetics has caused more confusion and generated more obscurantism than the analysis and interpretation of the various types of non-additivity and non-independence of gene and environmental action and interaction . . .' (Eaves *et al.*, 1977). This statement is as true today as when it was written. Often, the term 'G × E interaction' is used simply to denote that both

genes and environment are important. A better term to describe this situation is 'genotype–environment coaction' (Martin, 2000). We should reserve the term 'G × E interaction' for its statistical sense of different genotypes responding differently to the same environment, or, viewed from the other end, some genotypes being more sensitive to changes in the environment than others (different reaction ranges). From the extensive literature on crops and domesticated animals, the overwhelming message regarding G × E interaction is that it is extremely common (Lynch and Walsh, 1998), although it may explain only a relatively small proportion of the total trait variance. There are, however, still few data on its significance in natural populations. In a review of gene-mapping strategies, Tanksley (1993) discusses some of the first empirical results for Environment by quantitative trait loci (QTL; genes that influence quantitative traits) interactions in plants. QTLs affecting quantitative traits in maize and tomato in one environment (e.g. the USA) are often active in other environments (e.g. the Middle East) as well. QTLs showing the largest effects in one environment seemed more likely to also be detected in other environments, suggesting little environment by QTL interaction. QTLs with minor effects seemed more likely to show interactions with the environment. If similar results would apply to QTLs influencing personality traits and psychiatric disorders in humans, then this might conceivably explain the sometimes inconsistent results observed in candidate gene studies, in which genes of minor effect are tested. Examples of candidate genes that fail to show consistent replication across different populations include the dopamine receptor DRD4 (Plomin and Caspi, 1998), the SERT polymorphism in the serotonin transporter promotor region (Ebstein *et al.*, 2000), and the NOTCH4 gene in schizophrenia (McGinnis *et al.*, 2001; Sklar *et al.*, 2001). These are all examples of QTLs that, when found to be associated significantly with personality or psychiatric disorder, explain only a small proportion of the variance and can be considered minor QTLs.

We have shown how the analysis of G × E interaction can be conducted in the classical MZ/DZ twin design, using measures of environmental exposure or direct measures of the genotype. If measures of either environment or genotype are not available, i.e. in the majority of the quantitative genetic studies of metric human phenotypes, then the detection of G × E interaction is more difficult. One test for G × E interaction is the test suggested by Jinks and Fulker (1970) discussed above, involving examination of the association between the means and differences of MZ twin pairs. Another approach to test for genotype–environment interaction when measures of the environment or the genotype are not available is based on the analysis of the higher-order moments of genetic and environmental factor scores. Molenaar and Boomsma (1987) and Molenaar *et al.* (1990, 1999) have shown that the effects of certain types of interaction cannot be detected at the level of second-order moments (i.e. variances and covariances), but that they do lead to specific values of the third- and fourth-order moments (i.e. skewness and kurtosis) of genetic and environmental factor scores. These methods do not require measurements of the environment or the genotype, but require multiple indicators of the phenotype for the calculation of factor scores (Boomsma *et al.*, 1990) and the estimation of the higher-order moments of these factor scores. This approach to the detection of interaction factors hinges on the estimation of fourth-order moments. Unfortunately, the sampling variability of these estimates is very high, therefore large samples of phenotypic values are needed in order to secure the reliability of the detection tests. In simulation studies (where it is certain that the generated phenotypic values constitute a homogeneous sample), it was found that estimates of fourth-order moments depend strongly on the extreme phenotypic values in a sample, and that removal of these extreme observations (which are often interpreted as outliers in empirical studies) could lead to severe bias.

When G × E is present, but not modelled explicitly (e.g. because no measures of environmental exposure or genetic sensitivity are available), the interaction terms will be confounded with other terms in the model. For example, an interaction of genotype with unique environmental factors will be confounded with the unique environmental influences and cannot be separated from it in an analysis of second-degree statistics (Eaves *et al.*, 1977). Likewise, an interaction between genes and common environmental factors will be confounded with genetic effects. This might explain why it is so rare to observe a main effect of common environment shared by family members on psychiatric disorders (or many other traits). If the effects of parental rearing style and the shared family environment depend on the genetic constitution of an individual, then these will show up in most analyses as pure genetic effects.

The phenomenon of G × E interaction was introduced in this chapter in a statistical manner. Kandel (1998) outlines some of the biological phenomena underlying gene structure and function that may explain genetic sensitivity to the environment. Genes have dual functions: their template function guarantees reliable replication, and their transcriptional function regulates gene expression in the cell. Although almost all cells of the body contain all genes, in any given cell only a small proportion of genes is expressed. The expression of genetic information takes place when RNA is synthesized from DNA. RNA specifies the synthesis of polypeptides, which form proteins. The manufacture of specific proteins by a subset of genes in any given cell is thus highly regulated, and this regulation of gene expression is responsive to environmental factors. Learning, social interaction, stress and hormones (Strachan and Read, 1999) can alter the binding of transcriptional regulators to enhancer elements of genes, which, together with the promoter, usually lie upstream of a gene's coding region. After transcriptional regulators have bound to the promotor region, RNA synthesis is initiated. As demonstrated by studies of learning in simple animals, environmental triggers and experience can produce sustained changes in neural connections by altering gene expression. Certain environments may produce alterations in gene expression that produce structural changes in the brain, which may underlie psychiatric disorder. It remains an intriguing question as to what causes some individuals to experience more of such environmental triggers than others. As already pointed out by Kendler and Eaves (1986), exposure to certain environments may itself be under genetic control.

In conclusion, one might observe that G × E interaction has been the topic of much loose speculation over the years, with extravagant claims made for its potential importance, and precious few well-documented examples, at least in the human domain. Those presented above are some of the few we know of. One of the most exciting prospects now that we have the human genome sequence, and are on the brink of identifying QTLs for many complex traits, is that we shall at last be able to see just how widespread and important a phenomenon it really is.

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XIV

Gender Issues in Brain Structures and Functions and Their Relevance for Psychopathology

D.F. Swaab

SEX DIFFERENCES IN THE PREVALENCE OF NEUROLOGICAL AND PSYCHIATRIC DISEASES

Sex differences in brain and hormone levels are not only of importance for sexual behaviour, but are also thought to be the structural and functional basis of the often pronounced sex differences in the prevalence of neurological and psychiatric diseases. The proportions of cases range from more than 75% women in Rett syndrome, lymphocytic hypophysitis, anorexia and bulimia nervosa, and hypnic headache syndrome to more than 75% men in dyslexia, attention deficit hyperactivity disorder (ADHD), autism, sleep apnoea, Gilles de la Tourette syndrome, rabies, Kallmann syndrome and Kleine–Levin syndrome (Table XIV.1). Women are more prone to anxiety disorders than men (Seeman, 1997). Not only might the number of cases of disorders show clear sex differences, but the signs and symptoms and the course of the disease might also differ. Male schizophrenic patients have more severe enlargement of the lateral ventricles (Nopoulos *et al.*, 1997). Men suffer from schizophrenia 2.7 times more often than women; they are also prone to a more severe form of this disorder, have a poorer premorbid functioning experience, have an earlier onset, experience more negative symptoms and cognitive defects, and exhibit a greater number of structural brain abnormalities. Relapses are more severe, and their response to neuroleptic medication is less favourable. Women, by contrast, display more affective symptoms, auditory hallucinations and persecutory delusions (Castle and Murray, 1991; Leung and Chue, 2000). Moreover, an interaction with gender was observed in the second trimester of pregnancy when prenatal exposure to maternal stress was studied as a risk factor for schizophrenia (Van Os and Selten, 1998). There may also be a strong effect of age on sex differences in the prevalence of disorders: in depression up to the age of 54, the women : men ratio is 61 : 39, while after the age of 54, the ratio reverses to 35 : 65 (Bebbington *et al.*, 1998). Other examples of sex differences in neurological disease are those following restricted left-hemisphere lesions: 41% of men and 11% of women developed aphasia, whereas manual apraxia was found in 6% of the women and 42% of the men (Kimura and Harshman, 1984). The prevalence of cluster headache in men, and the fact that it is extremely rare before adolescence, indicates that sex hormones circulating in adulthood might be involved in the pathogenetic mechanism (Leone and Bussone, 1993). Changes over the years have also been reported. The men : women ratio of cluster headache decreases from 6.2 : 1 for patients with an onset before 1960 to 2.1 : 1 for patients with an onset in the 1990s. Changes in lifestyle, in particular in employment rate and smoking habits of women, are held responsible for this change (Manzoni, 1998). On the other hand, men still commit 89% of all murders and 99% of all sexual crimes (Spratt, 2000).

There is an excess of men with mental retardation (Turner, 1996). However, middle-aged women with Down's syndrome have an earlier onset of dementia than their male counterparts, and a more severe form of Alzheimer's disease, which correlates with the number of neocortical neurofibrillary tangles rather than density of senile plaques (Raghaven *et al.*, 1994). Age-related sex differences are now reported throughout the brain. Women run a higher risk of developing dementia after the age of 80 than men (Letenneur *et al.*, 1999; Launer *et al.*, 1999; Seeman, 1997). Very pronounced neurofibrillary Alzheimer changes are found in the infundibular nucleus and adjacent median eminence in 80% of the men over 60 years of age and in only 6% of the women (Schultz *et al.*, 1996). With advancing age, there is a loss of neurons in the pars cerebellaris loci coeruli that begins in women around age 40 and in men already at age 20 (Wree *et al.*, 1980).

Whether sex differences in the brain that arise in development ('organizing effects') are indeed the basis for the sex difference in neurological or psychiatric diseases still has to be established. In ADHD, an association with androgen receptor haplotypes was found (Comings *et al.*, 1999). Alternative mechanisms that are mentioned are the immediate effects of differences in circulating sex hormone levels ('activating effects'), caused by sex-hormone-stimulated gene transcription (Torpy *et al.*, 1997), as presumed in, for example, sleep apnoea (see Table XIV.1). A number of the diseases listed in Table XIV.1 are related to changes in catecholaminergic neurons, which are influenced during development by direct somatic effects of sex-specific genes (Pilgrim and Reisert, 1992). In a recent Dutch study, a higher prevalence of various psychiatric disorders was found in homosexual people compared with heterosexual people. These differences seem to be gender specific, with a higher prevalence of substance-use disorders in homosexual women and a higher prevalence of mood and anxiety disorders in homosexual men, both as compared with their heterosexual counterparts. It is not clear at present whether these differences result from biological or social factors (Sandfort *et al.*, 2001), but it does mean that sexual orientation should be included as a possible factor in the study of prevalence in psychiatric and neurological diseases.

SEX DIFFERENCES IN HYPOTHALAMIC STRUCTURES

The Hypothalamus as our Sexiest Part

There is an increasing amount of data concerning morphological and functional sex differences, especially in the various nuclei of the hypothalamus, which we will focus on in this review (Figures XIV.1 and XIV.2). The hypothalamus contains a number of structurally sexually dimorphic structures (Figure XIV.3), such

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Table XIV.1 Ratios for women to men suffering from a selection of neurological and psychiatric diseases

Disease	% women : men	Reference
Rett syndrome	100 : 0	Naidu (1997)
Postoperative hyponatraemic encephalopathy with permanent damage or death	96 : 4	Ayus *et al.* (1992)
Anorexia nervosa	93 : 7	Whitaker *et al.* (1989)
Lymphocytic hypophysitis	90 : 10	Maghnie *et al.* (1998)
Hypnic headache syndrome	84 : 16	Dodick *et al.* (1998)
Bulimia	75 : 25	Whitaker *et al.* (1989)
Senile dementia of the Alzheimer type	74 : 26	Bachman *et al.* (1992)
Post-traumatic stress disorders	66 : 34	Breslau *et al.* (1997), Seeman (1997)
Multiple sclerosis	67 : 33	Sadovnik and Ebers (1993)
Anxiety disorder	67 : 33	Seeman (1997)
Dementia	64 : 36	Naidu (1997)
Unipolar depression, dysthymia	63 : 37	Regier *et al.* (1988)
Whiplash	60 : 40	Karlsborg *et al.* (1997)
Severe learning disability	38 : 62	Castle and Murray (1991)
Substance abuse	34 : 66	Kessler *et al.* (1994)
Stuttering	29 : 71	Castle and Murray (1991)
Schizophrenia	27 : 73	Castle and Murray (1991)
REM sleep behavioural disorder	24 : 76	Schenk *et al.* (1993)
Dyslexia	23 : 77	Castle and Murray (1991)
ADHD	20 : 80	Comings *et al.* (1999)
Autism	20 : 80	Skuse (2000)
Sleep apnoea	18 : 82	Block *et al.* (1979)
Kallmann syndrome	17 : 83	Rugarli and Ballabio (1993)
Cluster headache	13 : 87	Rozen *et al.* (2001)
Rabies	13 : 87	Gómez-Alonso (1998)
Gilles de la Tourette	10 : 90	Caine *et al.* (1988)
Kleine–Levin syndrome	0 : 100	Critchley (1962)

as the sexually dimorphic nucleus of the preoptic area (SDN-POA), which was first described in the rat brain by Gorski *et al.* (1978). Due to differences in perinatal steroid levels, the SDN-POA in the male rat is three to eight times larger than in the female rat (Jacobson *et al.*, 1980). The SDN-POA is located between the dorsolateral supraoptic nucleus (SON) and the mediorostral pole of the paraventricular nucleus (PVN), at the same rostro-caudal level as the suprachiasmatic nucleus. Morphometric analysis revealed that there is a striking sexual dimorphism in the size and cell number in the SDN-POA (Swaab and Hofman, 1984; Swaab and Fliers, 1985; Hofman and Swaab, 1989). Sexual differentiation of the human SDN-POA occurs 4 years postnatally, and only after this age does the nucleus differentiate according to sex (see below). This is due to a decrease in both volume and cell number in women, whereas in men volume and cell number remain unaltered up to the fifth decade, after which a marked decrease in cell number is also observed (see below). Sexual differentiation has also been reported for two other cell groups in the preoptic anterior hypothalamus (INAH2 and 3) (Allen *et al.*, 1989b). These areas (Figure XIV.1) were found to be larger in men than in women, and this was later partly confirmed by LeVay (1991) and Byne *et al.* (2000). Other brain regions with a larger volume in men than in women are the darkly staining posteromedial part of the bed nucleus of the stria terminalis (BST-DSPM), described by Allen *et al.* (1989a), and the central nucleus of the BST (Zhou *et al.*, 1995c). The sex difference in the number of vasoactive intestinal polypeptide (VIP) expressing neurons in the suprachiasmatic nucleus (SCN) is age dependent (Zhou *et al.*, 1995b). The infundibular nucleus shows sex-dependent Alzheimer changes (Schultz *et al.*, 1996), and the size of the anterior commissure is sexually dimorphic (Allen and Gorski, 1991). 5-Hydroxyindoleacetic acid (5-HIAA) levels in the hypothalamus of men were lower than those in women, indicating a higher turnover rate of 5-hydroxytryptamine (5-HT) in the female brain (Gottfries *et al.*, 1974). In addition, we found clear age-dependent sex differences in the activity of the vasopressinergic neurons of the supraoptic nucleus (Ishunina *et al.*, 2000a).

The Sexually Dimorphic Nucleus of the Preoptic Area

There are indications that the SDN-POA area is involved in sexual behaviour. Electrical stimulation of the median preoptic area in the rat induces highly exaggerated stimulation-bound sexual behaviour (Merari and Ginton, 1975). Following lesions of the medial preoptic area, mounting, intromission and ejaculation are eliminated, but the animals do not lose the ability to achieve an erection. Although the median preoptic area does not seem to organize copulatory behaviour, it is crucial for the recognition of sensory stimuli as appropriate sexual targets, and for the integration of this recognition with sexual motivation and copulatory motor programmes. The PVN receives extensive input from the medial preoptic area. The parvocellular oxytocinergic neurons of the PVN project to the spinal cord and synapse on neurons that innervate the penis (McKenna, 1998). However, the exact role of the SDN-POA in these functions of the preoptic area is unclear. Electrophysiological experiments in the rat that showed increased multiple unit activity in the preoptic area during mounting, both in intact males and females (Kartha and Ramakrishna, 1996), do not clarify the exact contribution of the SDN-POA to these activity changes. Also, lesion experiments in rats have indicated that the SDN-POA may be involved in aspects of male sexual behaviour, i.e. mounting, intromission and ejaculation (Turkenburg *et al.*, 1988; De Jonge *et al.*, 1989). However, since the effects of lesions on sexual behaviour were only slight, it might well be that the major functions of the SDN-POA are still unknown at present. Penile erection following medial preoptic area

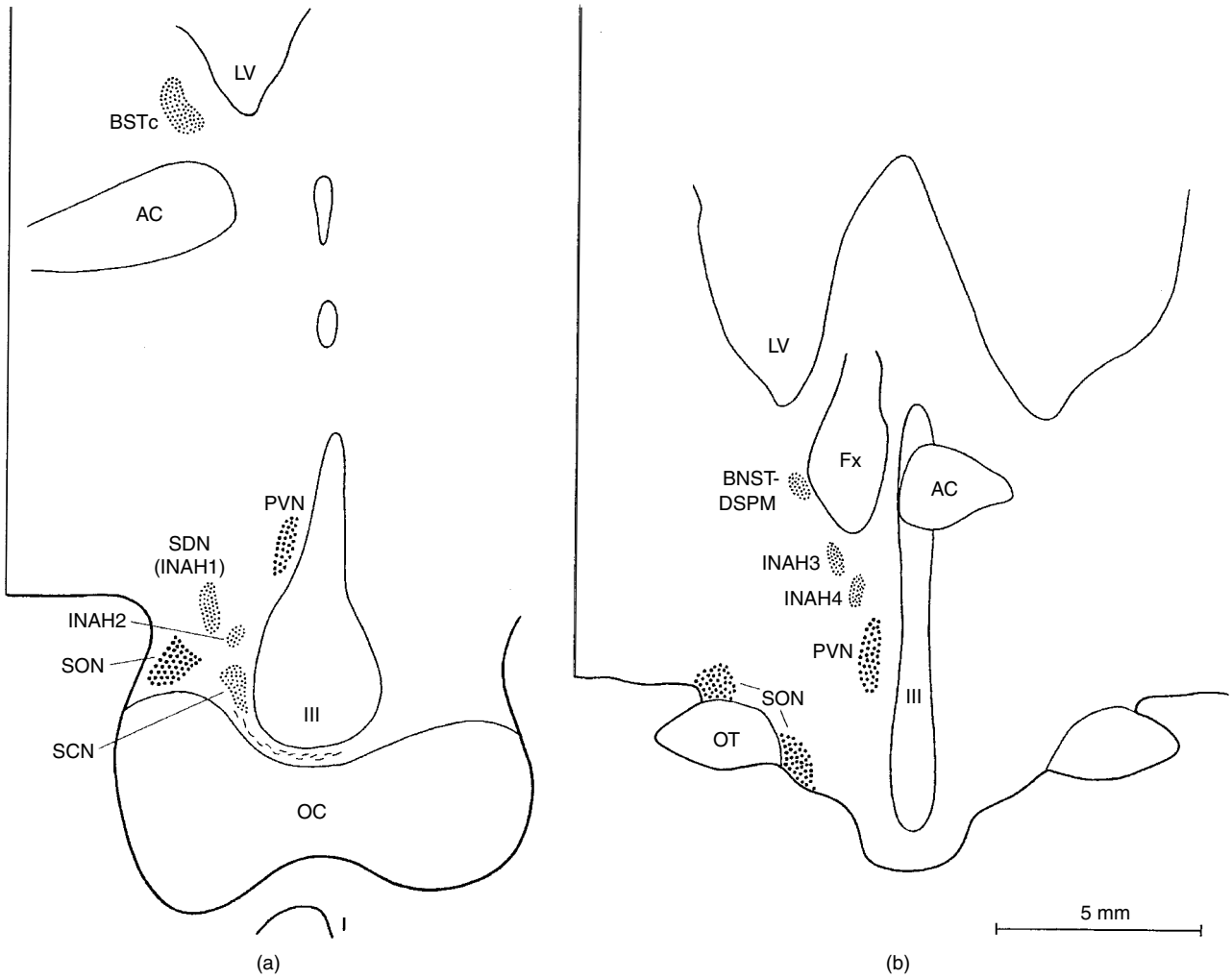
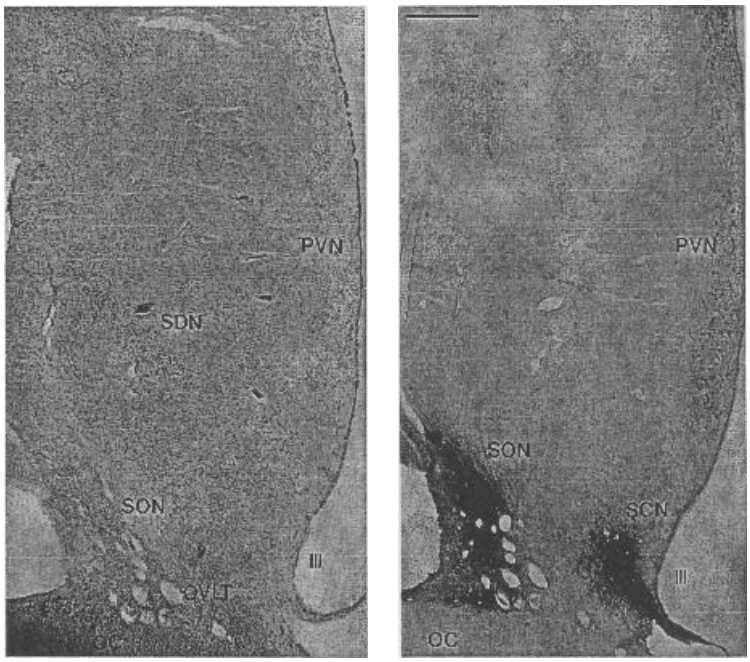


Figure XIV.1 Topography of the sexually dimorphic structures in the human hypothalamus. (a) is a more rostral view than (b). The AC, BSTc, BNST-DSPM, INAH2, 3 and 4, SCN and SDN vary according to sex. The SCN, INAH3 and AC are different in relation to sexual orientation III, third ventricle; AC, anterior commissure; BNST-DSPM, darkly staining posteriomedial component of the bed nucleus of the stria terminalis; Fx, fornix; I, infundibulum; INAH1–4, interstitial nucleus of the anterior hypothalamus 1–4; LV, lateral ventricle; OC, optic chiasma; OT, optic tract; SDN, sexually dimorphic nucleus of the preoptic area (INAH1) BSTc, central nucleus of the bed nucleus of the stria terminates; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; PVN, paraventricular nucleus

stimulation in the monkey and the rat, as reported by Giuliano *et al.* (1996), is considered to be one of such putative functions by some authors, but following lesions of the medial preoptic area, rats do not lose the ability to achieve an erection (McKenna, 1998). On the basis of animal experiments, the SDN-POA area is also presumed to be involved in sexual orientation. Lesions of the area of the SDN-POA in the ferret caused a significant shift in the males' preference from oestrous females to stud males, i.e. from a male-typical pattern of sexual behaviour to a more female-typical pattern (Kindon *et al.*, 1996; Paredes and Baum, 1995). Intact female rats show a preference for interaction with males and a tendency for males to interact with females. After lesion of the medial preoptic area of the anterior hypothalamus (mPOA), the females' preference was not modified. However, mPOA-lesioned male rats changed their partner preference after the lesion, and the coital behaviour of these males was reduced significantly after the lesion (Paredes *et al.*, 1998). Although in our studies of the human SDN-POA we did not find differences in size or cell numbers of this nucleus in relation to sexual orientation (Swaab and Hofman, 1990), this does not exclude a functional involvement of this structure in sexual orientation.

Nomenclature and Homology to the Rat Sexually Dimorphic Nucleus of the Preoptic Area

The SDN-POA in the young adult human brain is twice as large in men ($0.20\,mm^3$) than in women ($0.10\,mm^3$) on one side and contains twice as many cells (Swaab and Fliers, 1985; Figures XIV.3 and XIV.4). The original observations on 13 men and 18 women were extended and confirmed in a subsequent study of 42 men and 38 women (Swaab and Hofman, 1988) and in another independent study (Braak and Braak, 1992). The SDN-POA is also present in rhesus monkey (Braak and Braak, 1992). However, the fact that Byne (1998), who called this nucleus 'the lateroanterior nucleus of the rhesus monkey' did not find a sex difference in its volume makes this comparison complex. Daniel and Prichard (1975) used the term 'preoptic nucleus' for the human SDN-POA, but this name has not been used in the literature since. The SDN-POA is also identical to the 'intermediate nucleus' described by Brockhaus (1942) and Braak and Braak (1987), and to the 'interstitial nucleus of the anterior hypothalamus-1 (INAH1)' of Allen *et al.* (1989b). The term 'intermediate nucleus'



(a) (b)

Figure XIV.2 Thionine- (a) and anti-vasopressin- (b) stained section through the chiasmatic or preoptic region of the hypothalamus III, third ventricle; OC, optic chiasma; OVLT, organum vasculosum lamina terminalis; SDN, sexually dimorphic nucleus of the preoptic area (intermediate nucleus, INAH1) Bar represents 1 mm

Figure XIV.3 Thionine-stained frontal section (6 μm) of the hypothalamus of (a) a 28-year-old man and (b) a 10-year-old girl. Arrows show the extent of the SDN-POA. Note the large blood vessel penetrating the SDN-POA, and note that the SDN of the man is larger than that of the girl. Reproduced by permission from Swaab, D.F. and Fliers, E., 1985. *Science* 228, 1112–1115

is, however, controversial, since Feremutsch (1948) called the scattered vasopressin or oxytocin cells and islands between the SON and PVN the 'intermediate nucleus', and Morton (1961) used this name, by mistake, for the clusters of accessory SON cells, but now referring to the 1942 Brockhaus paper. Judging by the sex differences in the human SDN-POA in size and cell number, rostro-caudal position, cytoarchitecture, peptide and γ-aminobutyric acid (GABA) content (see below), this nucleus is probably homologous to the SDN-POA in the rat of Gorski *et al.* (1978), despite the fact that the rat SDN-POA is located in a more medial position than its human counterpart.

The SDN-POA contains galanin (Gai *et al.*, 1990), named after its N-terminal residual glycine and its C-terminal alanine (Tatemoto *et al.*, 1983), galanin mRNA (Bonnefond *et al.*, 1990), thyrotropin-releasing hormone (TRH) neurons (Fliers *et al.*, 1994), and glutamic acid decarboxylase (GAD) 65 and 67 (Gao and Moore, 1996a; Gao and Moore, 1996b). This supports the possible homology with the SDN in the rat, in which these peptide- and GABA-containing neurons have also been described (Bloch *et al.*, 1993; Gao and Moore, 1996a; Gao and Moore, 1996b). In addition, some scattered substance P neurons are present in the human SDN-POA (Chawla *et al.*, 1997), and moderate substance P cell numbers were found in this area in rat (Simerley *et al.*, 1986). Although we believe that all the data favour the homology between the human and the rat SDN-POA, it should be noted that others have claimed, on the basis of the presence of a sex difference, a possible homology between the rat SDN-POA and the human INAH3 (Allen *et al.*, 1989b; LeVay, 1991; Byne *et al.*, 2000). However, this claim

did not take into account a homology in neuropeptide and GABA content of those nuclei. In men, we recently found a more intense staining than in women for the androgen receptor and for the oestrogen receptor (alpha) in the SDN-POA, further supporting the presence of a sex difference in this nucleus (Fernández-Guasti *et al.*, 2000; Kruijver *et al.*, 2001). Concerning the presence of oestrogen receptors in the SDN-POA, it is relevant to note that an oestrogen response element has been found within the human galanin gene (Howard *et al.*, 1997). It should also be noted that microinjection of galanin into the medial preoptic nucleus facilitates female-typical and male-typical sexual behaviours in the female rat (Bloch *et al.*, 1996).

Development, Sexual Differentiation, Ageing and Alzheimer's Disease

Sexual dimorphism does not seem to be present in the human SDN-POA at the time of birth. At that moment, total cell numbers are still similar in boys and girls, and the SDN-POA contains no more than some 20% of the total cell number found between 2 and 4 years of age. From birth up to this age, cell numbers increase equally rapidly in both sexes (Figure XIV.5). A sex difference in the SDN-POA does not occur until about the fourth year postnatally, when cell numbers start to decrease in girls, whereas in boys the cell numbers in the SDN-POA remain stable until their rapid decrease at approximately 50 years of age. In women, a second phase of marked cell loss sets in after the age of 70 (Figure XIV.4; Swaab and Hofman, 1988; Hofman and Swaab, 1989). The sharp decrease in cell numbers in the SDN-POA later in life might be related to the dramatic hormonal changes that accompany both male and female senescence (Hofman and Swaab, 1989), and to the decrease in male sexual activity around 50 (Vermeulen, 1990). However, it is not clear whether the hormonal changes are related directly to these changes in various functions, either as cause or as effect of the observed cell loss in this nucleus.

The sex difference in the pattern of ageing, and the fact that sexual differentiation in the human SDN-POA only occurs after the fourth year of age (Swaab and Hofman, 1988; Figure XIV.5) might explain why Allen *et al.* (1989b), who had a sample of human adults biased for aged individuals, did not find a significant sex difference in the size of the SDN-POA, which they called INAH1 (Swaab *et al.*, 1992). The age distribution, however, does not explain why LeVay (1991) and Byne *et al.* (2000) could not find a sex difference in the volume of INAH1. Although the numbers of subjects they studied were much smaller than those in our study (Swaab and Hofman, 1988), technical differences such as section thickness may be a possible explanation for the controversy. The finding that nuclear androgen (Figure XIV.6) and oestrogen receptor α staining in the SDN-POA was more intense in men than in women (Fernández-Guasti *et al.*, 2000; Kruijver *et al.*, 2002, submitted) supports the presence of a sex difference in this nucleus.

A prominent theory is that sexual orientation develops as a result of interaction between the developing brain and sex hormones (Dörner, 1988; Gladue *et al.*, 1984). According to Dörner's hypothesis, homosexual men would have a female differentiation of the hypothalamus. Although LeVay's (1991) data on the small female-sized INAH3 in homosexual men are in agreement with this theory, this idea was not supported by our data on the SDN-POA in homosexual men. Neither the SDN-POA volume nor the cell number of homosexual men (Figure XIV.7) who died of AIDS differed from those of the male reference groups in the same age range, nor from those of heterosexuals also suffering from AIDS (Swaab and Hofman, 1988; Swaab and Hofman, 1990). The fact that no difference in SDN-POA cell number was observed between homosexual and heterosexual men, and the large SCN found in homosexual men (Swaab and Hofman, 1990), refutes the general formulation of Dörner's hypothesis that homosexual men would have 'a female hypothalamus' and favours the idea that homosexual men are a 'third sex', i.e. different from heterosexual men and women.

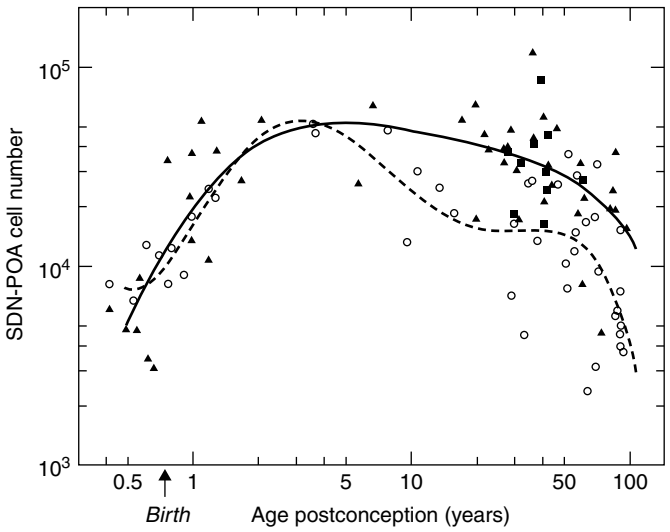


Figure XIV.5 Developmental and sexual differentiation of the human SDN-POA in 99 subjects; (log–log scale). Note that at the moment of birth, the SDN-POA is equally small in boys (▲) and girls (○) and contains about 20% of the cell numbers found at 2–4 years of age. Cell numbers reach a peak value around 2–4 years postnatally, after which a sexual differentiation occurs in the SDN : there is to a decrease in cell number in the SDN of women, whereas the cell number in men remains approximately unchanged up to the age of 50. The SDN-POA cell number in homosexual men (■) does not differ from that in the male reference group. The curves are quintic polynomial functions fitted to the original data for men (full line) and women (dashed line). Adapted, with permission from Swaab, D.F. and Hofman, M.A., 1988. *Dev Brain Res* 44, 314–318

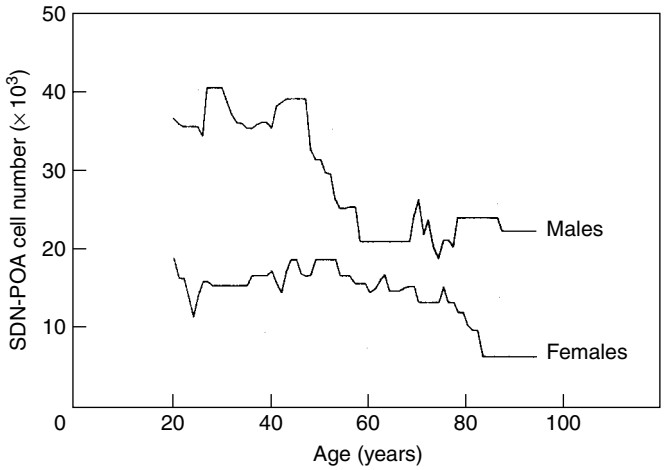


Figure XIV.4 Age-related changes in the total cell number of the SDN-POA in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in men, the SDN cell number declines steeply between the ages of 50 and 70 years, whereas in women, a more gradual cell loss is observed around the age of 80 years. These curves demonstrate that the reduction in cell number in the human SDN in the course of ageing is a nonlinear, sex-dependent process. Reproduced by permission from Hofman, M.A. and Swaab, D.F., 1989. *J Anat* 164, 55–72

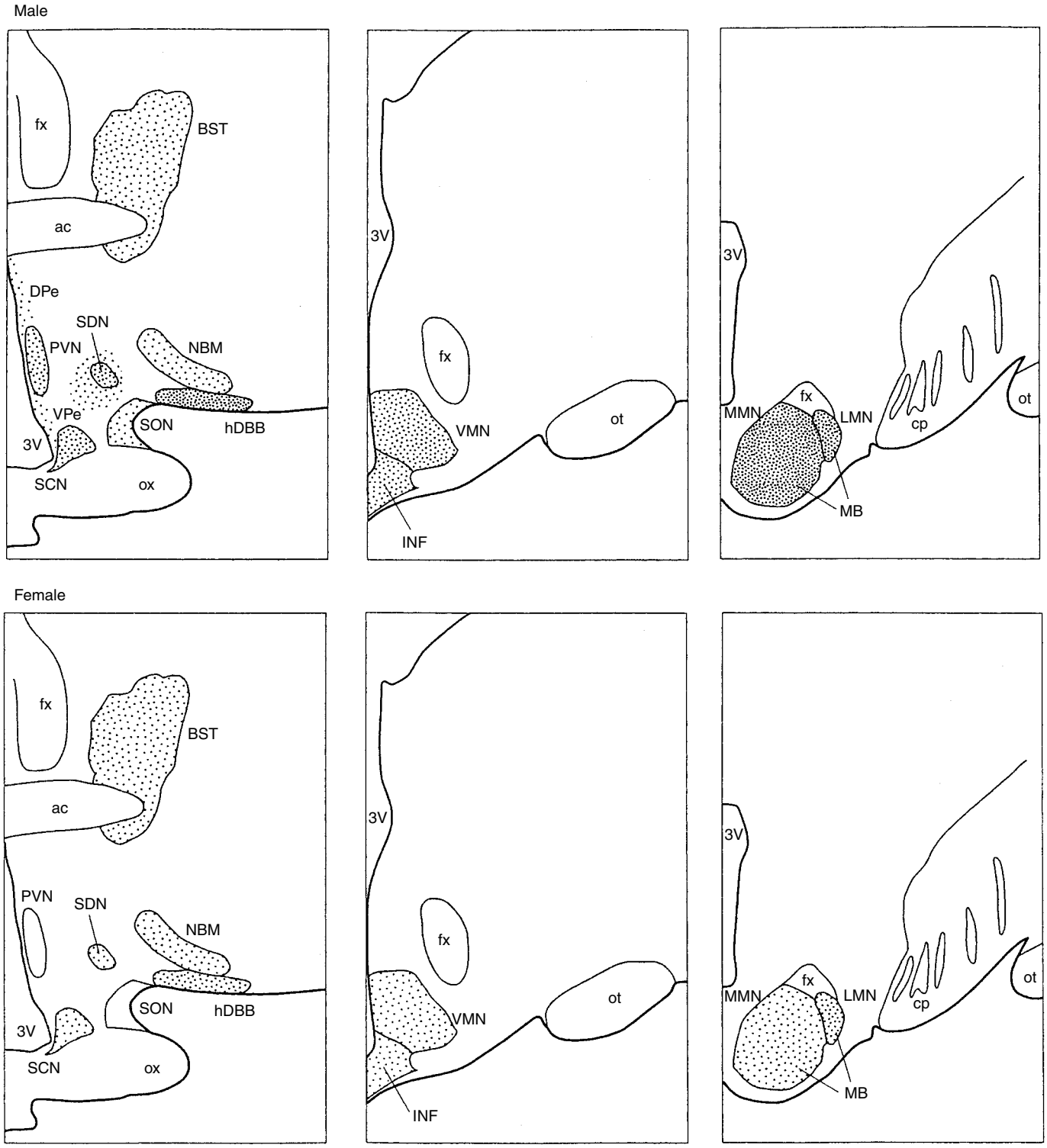


Figure XIV.6 Schematic representation of the sex differences in the intensity of androgen receptor immunoreactivity in the human hypothalamus. The three different sections correspond to plates 22, 27 and 30 of Mai *et al.* (1997) human brain atlas. 3V, third ventricle; ac, anterior commissure; cp, cerebral peduncle; DPe, periventricular nucleus dorsal zone; fx: fornix; hDBB, horizontal limb of the diagonal band of Broca; INF, infundibular nucleus; MB, mammillary body, i.e. MMN (medial mamillary nucleus) + LMN (lateromamillary nucleus); NBM, nucleus basalis of Meynert; OT, optic tract; ox, optic chiasma; VMN, ventromedial hypothalamic nucleus; VPe, periventricular nucleus ventral zone. Reproduced by permission from Fernández-Guasti, A., Kruijver, F.P.M., Fodor, M. and Swaab, D.F., 2000. *J Comp Neurol* 425, 422–435

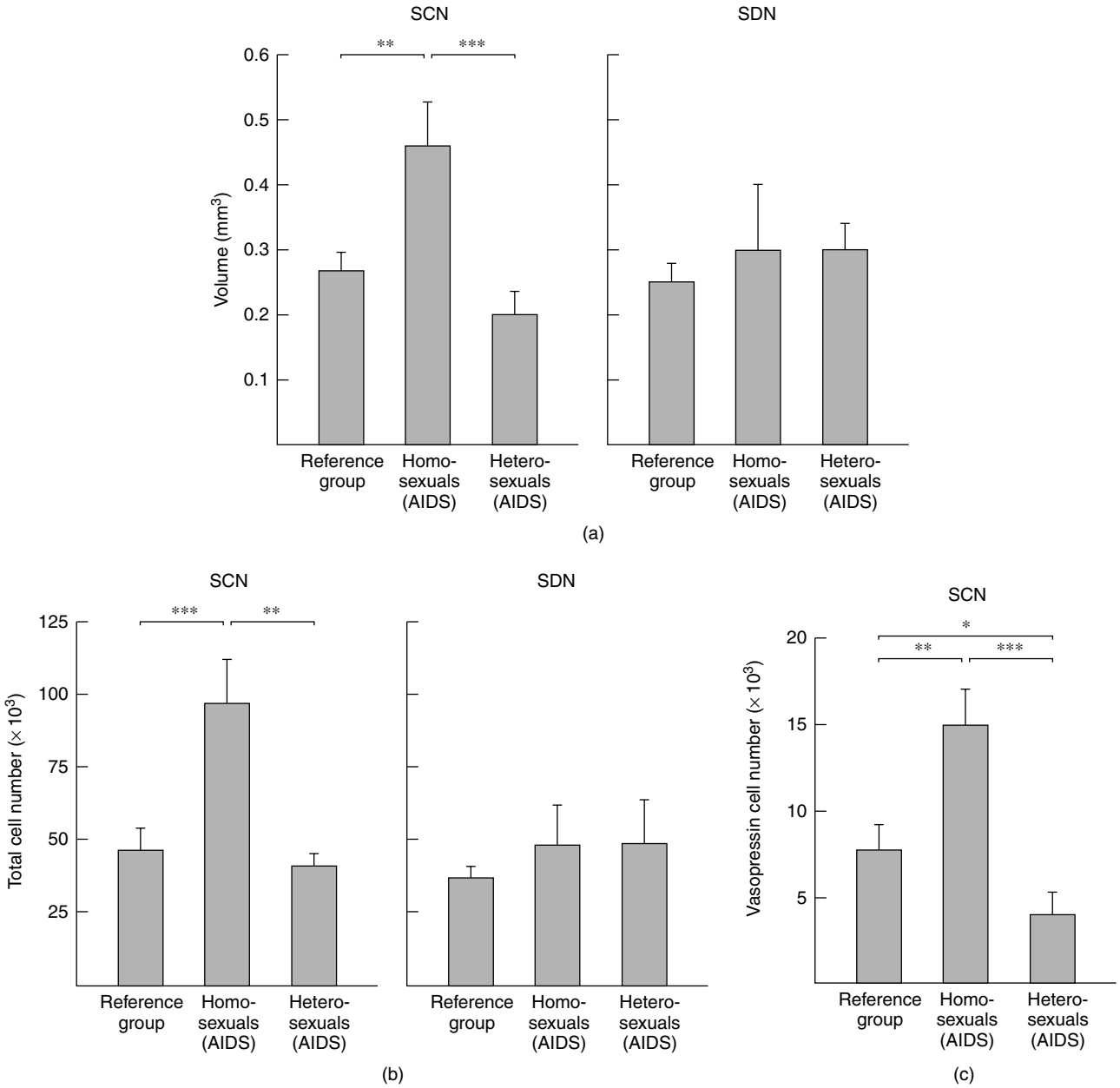


Figure XIV.7 (a) Volume of the human SCN and SDN as measured in three groups of adult subjects: (1) male reference group ($n = 18$); (2) male homosexuals who died of AIDS ($n = 10$); and (3) heterosexuals who died of AIDS ($n = 6$; 4 men, 2 women). The values indicate medians and the standard deviation of the median. The differences in the volume of the SCN between homosexuals and the subjects from both other groups are statistically significant (Kruskal–Wallis multiple comparison test, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Note that none of the parameters measured in the SDN (a,b) showed significant differences among the three groups (P always > 0.4). (b) Total number of cells in the human SCN and SDN. The SCN in homosexual men contains 2.1 times as many cells as the SCN in the reference group of male subjects, and 2.4 times as many cells as the SCN in heterosexual AIDS patients. (c) The number of vasopressin neurons in the human SCN (the SDN does not contain vasopressin-producing cells). The SCN in homosexual men contains, on average, 1.9 times as many vasopressin neurons as the SCN in heterosexual AIDS patients. Notice that the SCN of heterosexual individuals who died of AIDS contains fewer vasopressin cells than the SCN of the subjects from the reference group. Reproduced by permission from Swaab, D.F. and Hofman, M.A., 1990. *Brain Res* 537, 141–148

The Suprachiasmatic Nucleus in Relation to Sex and Sexual Orientation

Sex Differences in Sleep

The sex differences in the shape of the vasopressinergic SCN and in the numbers of VIP-expressing cells (Swaab *et al.*, 1985; Swaab *et al.*, 1994; Zhou *et al.*, 1995b) suggest the possibility of sex differences in circadian patterns. Moreover, in the human SCN, nuclear androgen receptor staining was more apparent in men than in women (Fernández-Guasti *et al.*, 2000), which may be one of the mechanisms causing functional sex differences in this nucleus. Indeed, sex differences have been reported in sleep patterns that may be related to SCN sex differences. Women have

higher percentages of slow-wave sleep and lower percentages of stage 1 sleep than men (Van Hilten *et al.*, 1994). Women have about twice as many sleep spindles as men, and they tend to spend more time sleeping than men in a free-running environment. In addition, middle-aged women display more slow-wave sleep than middle-aged men. The period of free-running circadian rhythm is shorter and the fraction of sleep significantly larger in women than in men (Wever, 1984). In addition, testosterone has relatively specific and discrete effects on sleep and hormonal rhythms in men (Leibenluft *et al.*, 1997). In healthy elderly women and men, differences in entrained circadian temperature rhythms and sleep patterns exist that indicate that ageing may affect the circadian timing system in a sexually dimorphic way (Campbell *et al.*, 1989; Moe *et al.*, 1991). Animal experiments indicate that only part of the sex differences in paradoxical sleep are dependent on circulating hormones (Fang and Fishbein, 1996).

In the rat SCN, there is a sex-specific circadian pattern in VIP mRNA but not in vasopressin mRNA (Krajnak *et al.*, 1998).

The Suprachiasmatic Nucleus, Sexual Behaviour and Reproduction

In addition to its possible involvement in reproduction (see below), the SCN might also play a role in sexual orientation. In fact, the first difference in the human brain in relation to sexual orientation was observed in the SCN. Morphometric analysis of the SCN of ten homosexual men revealed that the volume of this nucleus was 1.7 times larger than that of a reference group of 18 presumed heterosexual men, and that it contained 2.1 times as many cells (Figure XIV.7; Swaab and Hofman, 1990). The same high number of SCN vasopressin neurons as observed in 1–2-year-old children (Swaab *et al.*, 1990) were also found in homosexual men. It seems as if the programmed postnatal cell death, which appears to begin in the SCN between 13 and 16 months after birth, does not occur to the same extent in homosexual men. The increased number of vasopressin-expressing neurons in the SCN of homosexual men appeared to be quite specific for this subgroup of neurons, since the number of VIP-expressing neurons was not changed. However, in both the vasopressin, and the VIP neurons in the SCN, a reduced nuclear diameter was observed in homosexual men, suggesting metabolic alterations in the SCN in relation to sexual orientation (Zhou *et al.*, 1995a).

There are a number of experimental data and observations on human material that indicate that the SCN is involved in aspects of sexual behaviour and reproduction. In the early 1970s, postcoital ultrastructural changes indicating neuronal activation were reported in the SCN of the female rabbit (Clattenburg *et al.*, 1972). Also important is the fact that the activity of SCN neurons increases suddenly around puberty (Anderson, 1981), indicating the addition of a reproductive function to the already mature circadian functions of the SCN. In addition, efferents of the SCN innervate the preoptic area that is involved in reproductive behaviours. Extensive lesioning of the SCN area results in failure of ovulation in the female rat (Brown-Grant and Raisman, 1977). The ovarian reproductive cycle is controlled by the SCN, probably through a direct monosynaptic innervation of luteinizing hormone-releasing hormone (LHRH) neurons by VIP fibres (Van der Beek *et al.*, 1993; Van der Beek *et al.*, 1997). Several morphological sex differences have been reported that support putative reproductive functions. The SCN of male rats contains a larger amount of axospinal synapses, postsynaptic density material and asymmetrical synapses, and their neurons contain more nucleoli than those of female rats (Güldner, 1982; Güldner, 1983). The sex differences in synaptic numbers in the rat SCN depend on androgens in development (le Blond *et al.*, 1982). In gerbils, the volume of the SCN is sexually dimorphic (Holman and Hutchison, 1991), as is the organization of astroglia in the SCN (Collado *et al.*, 1995).

A sex difference was found in the shape of the vasopressin subdivision of the human SCN (Swaab *et al.*, 1985), and a sex difference was observed in the number of VIP-containing neurons in the human SCN. The number of VIP-expressing neurons in the SCN is larger in men aged 10–40 years and women aged 41–65 years (Swaab *et al.*, 1994b; Zhou *et al.*, 1995b). These observations are also consistent with sexually dimorphic functions of the SCN that are still awaiting better definition. It is interesting to note that the pineal hormone 5-methoxytryptophol shows significant sex differences: plasma concentrations increase in boys and decrease in girls after the age of 8 years (Molin-Carballo *et al.*, 1996).

In seasonal breeders, VIP immunoreactivity in the SCN changes in relation to seasonal fluctuations in sexual activity (Lakhdar-Ghazal *et al.*, 1992). The activation of c-fos in the SCN by sexual stimulation also points to a role of the SCN in reproduction (Pfaus *et al.*, 1993). Bakker *et al.* (1993) have found that male rats treated neonatally with the aromatase inhibitor 1,4,6-androstratriene-3,17-dione (ATD) showed a clear sexual partner preference for females when tested in the late dark phase. When tested in the early dark phase, however, they showed a lesser preference for the female, or no preference at all. This is the first indication of the involvement of the clock, i.e. the SCN, in sexual orientation. The number of vasopressin-expressing neurons in the SCNs of these ATD-treated bisexual animals was increased (Swaab *et al.*, 1995), something that was also found in homosexual men (Swaab and Hofman, 1990). This observation supports the possibility that the increased number of vasopressin-expressing neurons in the SCN of adult homosexual men reflects a difference in the early stages of development in the brain.

Other Sexually Dimorphic Hypothalamic Structures

Interstitial Nuclei of the Anterior Hypothalamus-1 and -2

In addition to the sex differences observed in the SCN, the SDN-POA and the BST, Allen *et al.* (1989b) described two other cell groups, the INAH2 and INAH3), that were larger in the male brain than in the female brain (Figure XIV.1). Moreover, LeVay (1991) found that INAH3 was twice as large in heterosexual men as in homosexual men. INAH2 in the human hypothalamus is said to correspond to the anterocentral nucleus and INAH3 to the dorsocentral portion of the anterior hypothalamic nucleus in the rhesus monkey (Byne, 1998). Since nothing is known about their neurotransmitter content, it is unclear which nuclei in the rat or rhesus monkey are homologous to the human INAH2 and INAH3. Recently, we found galanin-containing cells and fibres not only in INAH1 (SDN-POA) but also in INAH2; the question was raised whether INAH1 and INAH2 are indeed two separate nuclei or whether they are both part of a continuous horseshoe-shaped structure. As no chemical marker is known for INAH3, it is not yet clear whether this nucleus has to be considered as a perifornical cell group, islands of the PVN or the BST, or a separate anatomical entity. There is a discrepancy in the literature concerning the sex difference in the size of INAH2 as described by Allen *et al.* (1989b) that could not be confirmed by LeVay (1991) or Byne *et al.* (2000). The fact that LeVay did not observe a smaller INAH2 in women was proposed to be explained by an age-related sex difference in this nucleus. INAH2 shows this sex difference only after the childbearing age, with one exception: a 44-year-old woman who had a hysterectomy with ovarian removal 3 years before her death and who had a small INAH2 (Allen *et al.*, 1989b). The sex difference in INAH2 thus seems to come to expression only after menopause, when circulating oestrogens are absent. This would also explain why LeVay could not confirm the difference in INAH2 in his group of young patients. The sex difference in INAH2 was considered to be the first human example of a sex difference

depending on circulating levels of sex hormones, i.e. a difference based on a lack of activating effects of sex hormones in menopause rather than on organizing effects of sex hormones in development. However, Byne *et al.* (2000) could not confirm the relationship between INAH2 and reproductive status as suggested by the data of Allen *et al.* (1989b). A second example of a functional sex difference can be found in the supraoptic nucleus and PVN (see later). INAH4 is not sexually dimorphic (Allen *et al.*, 1989b).

The Anterior Commissure, Interthalamic Adhesion and Third Ventricle

The anterior commissure appears as early as gestational day 47 (Hori, 1997) and contains about seven million fibres. It connects anteriorly two-thirds of the right and left temporal neocortices and the posterior part of the orbital aspect of the frontal lobes (Demeter *et al.*, 1988). The anterior commissure was investigated in humans with circumscribed hemispheric lesions. The largest contingent of commissural axons appeared, from this study, to originate in the inferior part of the temporal lobe. In addition, axons originating from the inferior part of the occipital lobe, occipital convexity, central fissure and prefrontal convexity were found to cross the anterior commissure (Di Virgilio *et al.*, 1999). There is also a small olfactory part rostrally (Sylvester, 1986).

The anterior commissure was found to be 12% larger in women than in men (Allen and Gorski, 1991). In an earlier study, only a trend towards such a sex difference was noted (Demeter *et al.*, 1988). However, Higley *et al.* (1999) found a greater number of fibres in women than in men. A sex difference in the size of the commissure was also observed in people with Down's syndrome (Sylvester, 1986). These observations point to a greater connectivity between the cerebral hemispheres of women. Allen and Gorski (1992) found that the anterior commissure was larger in homosexual men than in heterosexual men. Sexual dimorphism in the anterior commissure is presumed to underlie sex differences in cognitive skills, developmental language disorders, and functional asymmetries. The anterior commissure mediates interhemispheric transfer of visual information, including the visual recall of dreams, auditory and olfactory information (Allen and Gorski, 1991; Martin, 1985; Botez-Marquard and Botez, 1992; Risse *et al.*, 1978).

In rats, the anterior commissure is larger in females than in males. Prenatal stress, known to disrupt both sexual differentiation and sexual behaviour, causes a disappearance of the sex difference in the size of the anterior commissure (Jones *et al.*, 1997).

Allen and Gorski (1991) found that the interthalamic adhesion, a grey structure that crosses the third ventricle between the two thalami, was present in more women (78%) than men (68%), confirming the old study by Morel (1947). In a comparison between ten control women and eight men, all with a mean age of 79 years, magnetic resonance imaging (MRI) measurements showed that the third ventricle volume was 67% larger in men than in women (Wahlund *et al.*, 1993).

Sex Hormone Receptor Distribution

In most hypothalamic areas that contain androgen receptor staining, nuclear staining in particular is less intense in women than in men (Figure XIV.6). The strongest sex differences were found in the lateral and the medial mammillary nucleus (Fernández-Guasti *et al.*, 2000). The mammillary body complex is known to be involved in several aspects of sexual behaviour. In addition, a sex difference in androgen receptor staining was present in the horizontal diagonal band of Broca, SDN-POA, medial preoptic area, the dorsal and ventral zone of the periventricular nucleus, PVN, supraoptic nucleus, ventromedial hypothalamic nucleus, and the infundibular nucleus. However, no sex differences were observed in androgen receptor staining in the BST, the nucleus basalis of Meynert, or the island of Calleja (Fernández-Guasti *et al.*, 2000). Nuclear androgen receptor activity in the mammillary complex of heterosexual men did not differ from that of homosexual men, but it was significantly stronger than in women. A female-like pattern was found in 26- and 53-year-old castrated men and in intact old men. These data indicate that the amount of nuclear receptor staining in the mammillary complex is dependent on the circulating levels of androgens, rather than on gender identity or sexual orientation. This idea is supported by the finding that a male-like pattern of androgen receptor staining was found in a 36-year-old bisexual non-castrated male-to-female transsexual and a heterosexual virilized woman aged 46 years (Kruijver *et al.*, 2001).

The Bed Nucleus of the Stria Terminalis

The BST is situated at the junction of the hypothalamus, septum and amygdala (Lesur *et al.*, 1989; Walter *et al.*, 1991; Figure XIV.1). It plays an essential part in rodent sexual behaviour (Liu *et al.*, 1997). Oestrogen and androgen receptors have been found in the human BST (Fernández-Guasti *et al.*, 2000; Kruijver *et al.*, 2002, submitted), and it is a major aromatization centre in the developing rat brain, i.e. converting androgens into oestrogens. The BST in rat receives projections mainly from the amygdala, and provides a strong input in the preoptic-hypothalamic region. Reciprocal connections between the hypothalamus, BST and amygdala are well documented in experimental animals (Zhou *et al.*, 1995c; Liu *et al.*, 1997). There is a strong innervation of galanin fibres in the BST, and galanin receptors have also been shown in this structure (Mufson *et al.*, 1998). The BST and centromedial amygdala have common cyto- and chemoarchitectonic characteristics, and these regions are considered to be two components of one distinct neuronal complex. Neurons in the substantia innominata form cellular bridges between the BST and amygdala (Martin *et al.*, 1991; Heimer, 2000; Lesur *et al.*, 1989; Walter *et al.*, 1991). In most mammals, including humans, the extended amygdala presents itself as a ring of neurons encircling the internal capsule and the basal ganglia (Heimer *et al.*, 1997). The BST–amygdala continuum contains, for example, LHRH neurons (Rance *et al.*, 1994).

Five principal sectors have been identified in the BST, including a 'darkly staining posteromedial component (dspm) of the BST' (Allen *et al.*, 1989a; Lesur *et al.*, 1989; Kruijver *et al.*, 2000; Martin *et al.*, 1991). This part of the BST is situated in the dorsolateral zone of the fornix (Figure XIV.1) and is sexually dimorphic (Figure XIV.8). This sex difference does not seem to occur before adulthood. Its chemical composition and relationship to the other four principal BST sectors (see above) is unknown. Although the BST contains nuclear androgen receptors, no sex differences in the staining of this receptor were observed (Fernández-Guasti *et al.*, 2000; Figure XIV.6).

Reversed Sex Differences in the Bed Nucleus of the Stria Terminalis in Transsexuals

The volume of the BST-dspm is 2.5 times larger in men than in women (Allen *et al.*, 1989a). We have found that the central nucleus of the BST (the BSTc), which was defined by its dense VIP innervation (Figure XIV.8), probably originating in the amygdala or by its somatostatin fibres and neurons, is sexually dimorphic. The BSTc is 40% smaller and also contains some 40% fewer somatostatin neurons in women compared with men (Figure XIV.9). No relationship was observed between BSTc volume or somatostatin cell number and sexual orientation: in the heterosexual male reference group and a group of homosexual men, similar BSTc volume and somatostatin cell number were observed. The size and somatostatin cell number of the BSTc were, moreover, not influenced by abnormal hormone levels in adulthood. However, a remarkably small

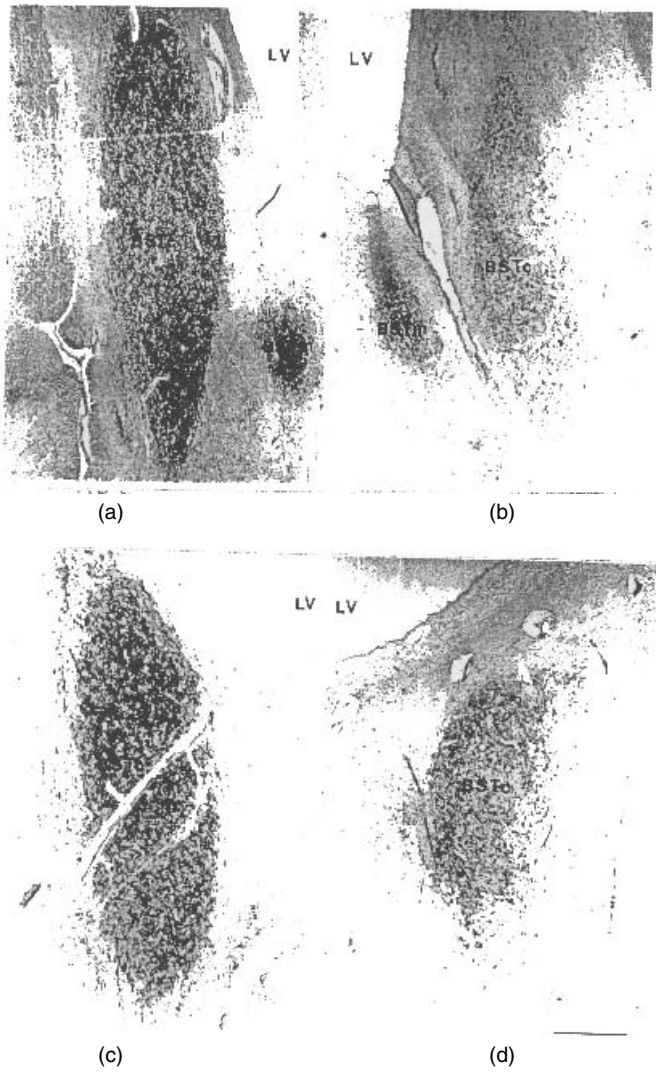


Figure XIV.8 Representative sections of the BSTc innervated by VIP. (a) Heterosexual man, (b) heterosexual woman, (c) homosexual man, (d) male-to-female transsexual. Scale bar, 0.5 mm. Note that there are two parts of the BST in (a) and (b): small medial subdivision (BSTm) and large, oval-sized central subdivision (BSTc). A female volume was observed in genetically male (male-to-female) transsexuals. There was no difference according to sexual orientation. Reproduced by permission from Zhou, J.N., Hofman, M.A., Gooren, L.J.G. and Swaab, D.F., 1995. *Nature* 378, 68–70
LV, lateral ventricle

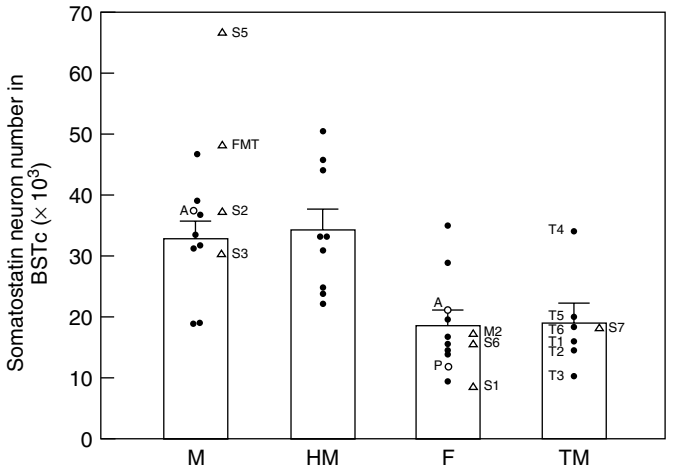


Figure XIV.9 Distribution of the BSTc neuron numbers among the different groups according to sex, sexual orientation and gender identity. M, heterosexual male reference group; HM, homosexual male group; F, female group; TM, male-to-female transsexuals. The sex hormone disorder patients S1, S2, S3, S5, S6 and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. The difference between the M and the TM group ($P < 0.04$) also becomes statistically significant according to the sequential Bonferonni method if S2, S3 and S5 are included in the M group, or if S7 is included in the TM group ($P \leq 0.01$). Note that the number of neurons of the female-to-male transsexual (FMT) is fully in the male range. A, AIDS patient. The BSTc number of neurons in the heterosexual man and woman with AIDS remained well within the corresponding reference group (see Figure XIV.1), so AIDS did not seem to affect the somatostatin neuron numbers in the BSTc. P, postmenopausal woman. S1 (25 years of age): Turner syndrome (45,X0; ovarian hypoplasia). M2 (73 years of age): postmenopausal status. Reproduced by permission from Kruijver, F.P.M., Zhou, J.N., Pool, C.W., Hofman, M.A., Gooren, L.J.G. and Swaab, D.F., 2000. *J Clin Endocrinol Metabol* 85, 2034–2041

BSTc (40% of the male reference volume and somatostatin neuron number) was observed in a group of six male-to-female transsexuals (Figures XIV.8 and XIV.9). These data suggest that the female size of this nucleus in male-to-female transsexuals was established during development, and that the BSTc is part of a network that might be involved in gender, i.e. the feeling of being either a man or a woman (Zhou *et al.*, 1995c; Kruijver *et al.*, 2000).

Sex Differences in the Supraoptic and Paraventricular Nuclei

Although we did not find a sex difference in vasopressin neuron numbers, a sex difference was reported in vasopressin plasma levels. Men have higher vasopressin levels than women (Van Londen *et al.*, 1997; Asplund and Åberg, 1991). In addition, it was found that the posterior lobe of the pituitary is larger in boys than in girls (Takano *et al.*, 1999). This sex difference is explained by the higher activity we found in vasopressin neurons in the SON in young men compared with women, as determined by the size of the Golgi apparatus. In the course of ageing, probably triggered by the decrease in oestrogen levels, the neuronal activity increases gradually in women but remains stable in men. The sex difference in neuronal activity in the SON thus disappears after the age of 50 years (Ishunina *et al.*, 1999). This is thus an example of a hypothalamic system that does not show a structural sex difference but rather a functional sex difference. It is also an example of a sex difference based on the 'activating' (or, in this case, 'inhibiting') effects of sex hormones.

The activation of neurosecretory vasopressin neurons in postmenopausal women was confirmed by measurement of the cell size as a parameter for neuronal activity. The vasopressin neurons in the SON appeared to be larger in young men than in young women. In elderly women (>50 years old), vasopressin cell size considerably exceeded that of young women. In addition, vasopressin cell size correlated positively with age in women but not in men. Sex differences in the size of the PVN vasopressin neurons were pronounced at the left side and absent at the right side, indicating the presence of functional lateralization of this nucleus. No difference was found in any morphometric parameter of oxytocin neurons in the PVN among the four groups studied. These data demonstrate sex differences in the size of the vasopressin neurons, and thus presumably in their function, that are age- and probably also side-dependent, and the absence of such changes in oxytocin neurons

in the PVN (Ishunina and Swaab, 1999). The activation of vasopressin neurons in postmenopausal women is probably mediated by a decrease in the presence of oestrogen receptor β in these neurons and an increase in oestrogen receptor α nuclear staining (Ishunina *et al.*, 2000a).

The low-affinity neurotrophin receptor p75 ($p75^{NTR}$) may be involved in the mechanism of activation of vasopressin neurons in postmenopausal women. This receptor was found to be expressed in the SON neurons of aged individuals, while $p75^{NTR}$ expression was shown to be suppressed by oestrogens in a cell line. We investigated whether $p75^{NTR}$ immunoreactivity in SON neurons was age- and sex-dependent in post-mortem brains of control patients ranging in age from 29 to 94 years old with an anti-$p75^{NTR}$ antibody and determined the area of $p75^{NTR}$ immunoreactivity per neuron profile using an image analysis system. To study whether the $p75^{NTR}$ might also participate in the activation of SON neurons, we related Golgi apparatus size to the amount of $p75^{NTR}$ in the same patients. We found that $p75^{NTR}$ immunoreactivity did indeed correlate significantly with age and Golgi apparatus size, but only in women and not men. These observations suggest that $p75^{NTR}$ modulates the effects of oestrogens on vasopressinergic neurons in the human SON (Ishunina *et al.*, 2000b).

The sex differences in activity of the SON are consistent with other observations showing oestrogen and progesterone interference with renal actions of vasopressin. Women have a higher turnover than men, and the greatest difference is present during the lateral phase of the menstrual cycle (Claybough *et al.*, 2000).

SEXUAL DIFFERENTIATION OF THE BRAIN AND SEXUAL BEHAVIOUR

> We must remember that all our provisional ideas in psychology will one day be explained on the basis of organic substrates. It seems then probable that there are particular chemical substances and processes that produce the effects of sexuality and permit the perpetuation of individual life.
>
> Sigmund Freud, *On Narcissism*

Mechanism of Sexual Differentiation of the Brain

Sexual differentiation of the brain is thought to be 'imprinted' or 'organized' by hormonal signals from the developing male gonads. On the basis of animal experiments, this process is presumed to be induced by androgens during development following conversion to oestrogens by cytochrome P-450 aromatase. Male sexual differentiation of the human brain is thus probably determined in the two first periods during which sexually dimorphic peaks in gonadal hormone levels are found, i.e. during gestation and during the perinatal period, while from puberty onwards, sex hormones alter the function of previously organized neuronal systems ('activating effects') (for references, see Swaab *et al.*, 1992; De Zegher *et al.*, 1992; Forest, 1989; Tucker Halpern *et al.*, 1998). Testosterone is formed in the foetal Leydig cells in the testicle from about 8 weeks of gestation (Hiort, 2000). The possible importance of the male testosterone surge for sexual differentiation of the brain following aromatization to oestrogens agrees with the observation that girls whose mothers were exposed to diethylstilboestrol during pregnancy have an increased chance of developing bi- or homosexuality (Ehrhardt *et al.*, 1985; Meyer-Bahlburg *et al.*, 1995), while girls with congenital adrenal hyperplasia have a greater chance for becoming homosexual or having gender identity problems (Meyer-Bahlburg *et al.*, 1996; Money *et al.*, 1984; Dittmann *et al.*, 1992; Zucker *et al.*, 1996; (Table XIV.2; see also below) oestrogens produced locally by aromatase are thought to participate in numerous biological functions, including sexual differentiation of the brain. Aromatase is found throughout the adult human brain, with the highest levels in the pons, thalamus, hypothalamus and hippocampus. The amount of aromatase mRNA is also highest in the hypothalamus, thalamus and amygdala. No differences were detected between the four men and two women studied (Sasano *et al.*, 1998). Such a study has, however, not been performed in development. The neonatal peak of testosterone is probably induced not by the hypothalamus of the child but by chorionic gonadotrophins from the placenta, since a normal neonatal increase of testosterone was found in a patient with hypogonadism based upon a DAX-1 gene mutation that causes hypogonadotrophic hypogonadism (Takahashi *et al.*, 1997).

In human neonates at 34–41 weeks of gestation, the testosterone level is ten-fold higher in men than in women (De Zegher *et al.*, 1992). Although the peak in serum testosterone in boys at 1–3 months postnatally approaches the levels seen in adult men, most testosterone is bound to globulin. Yet the amount of free testosterone in male infants is about one order of magnitude larger than that in female infants at this time (Bolton *et al.*, 1989). During the adrenarche, i.e. from 7 years of age to the onset of puberty, the adrenals start to produce more androgens, predominantly dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS). After the age of 8 years, testosterone from the adrenals also starts to rise, while a small but significant testicular production of testosterone is also present in prepubertal boys (Forest, 1989). Although the testosterone peak during puberty is generally thought to be involved in activation rather than organization (Forest, 1989), the neuron numbers of the female domestic pig hypothalamus, to our surprise, showed a two-fold increase in a sexually dimorphic hypothalamic nucleus around puberty (Van Eerdenburg and Swaab, 1991), which means that although this phenomenon may have been programmed earlier, late organizational effects cannot, at present, be excluded. Few data are available on the exact period in development when the human brain differentiates according to sex. Brain weight is sexually dimorphic from 2 years postnatally, taking differences in body weight between boys and girls into account (Swaab and Hofman, 1984). As discussed earlier, sexual differentiation of the human SDN-POA becomes apparent between 4 years and puberty (Swaab and Hofman, 1988), and a similar late sexual differentiation was found in the BST (Allen *et al.*, 1989a). Concluding, one might say that the limited evidence that is currently available suggests that sexual differentiation of the human hypothalamus becomes apparent between 2 years of age and puberty, although this may, of course, be based upon processes that were programmed much earlier, e.g. by a peak in sex hormone levels in mid-pregnancy or during the neonatal period (see earlier). On the basis of existing prenatal serum samples from their mothers, it appears that higher androgen exposure in the second trimester of foetal life may masculinize a girl's behaviour (Udry *et al.*, 1995).

Although the process by which oestrogens are derived from testosterone by aromatization is considered to be the major mechanism for androgenization of the brain during development, testosterone itself may also be of importance for sexual differentiation of the brain. The androgen receptor located on the X chromosome at Xq11-12 may be mutated in such a way that the subject has a complete androgen insensitivity syndrome. In spite of normal testis differentiation and androgen biosynthesis, the phenotype has a normal female external and behavioural appearance (Batch *et al.*, 1992). Phenotypic women with complete androgen insensitivity syndrome perceive themselves as highly feminine. They do not have gender problems, and they largely report their sexual attraction, fantasies and experiences as heterosexual women (Wisniewski *et al.*, 2000; Wilson, 1999). This means that for the development of human male gender identity and male heterosexuality, direct androgen action on the brain seems to be of crucial importance, and that the aromatization theory may even be of secondary importance for human sexual differentiation of the brain.

The observation by Macke *et al.* (1993) that DNA sequence variation in the androgen receptor is not a common determinant of sexual orientation seems to be at variance with these data, but it should be noted that the DNA variations in that study did not prevent normal androgenization of the subjects studied, so there was no loss of function of this receptor. This view agrees with the lack of gender problems in a brother and a sister with aromatase deficiency due to a mutation (Morishima *et al.*, 1995). Moreover, a 28-year-old man with oestrogen resistance due to a mutation of the oestrogen receptor gene was described as tall, with continued linear growth in adulthood, incomplete epiphysal closure, and increased oestrogen and gonadotrophin levels. A change in a single base pair in the second exon of the oestrogen receptor gene was found. However, the patient did not report a history of gender identity disorder, he had a strong heterosexual interest, and he had normal male genitalia. The elevated serum oestrogen levels are explained by a possible compensatory increase in aromatase activity in response to oestrogen resistance (Smith *et al.*, 1994). The observations in complete androgen sensitivity syndrome (Wisniewsky *et al.*, 2000) and the male gender heterosexual behaviour of patients with 5-α-reductase-2 or 17β-hydroxy-steroid dehydrogenase-3 deficiency (Imperato-McGinley *et al.*, 1979; Imperato-McGinley *et al.*, 1991; Wilson *et al.*, 1993; Wilson, 1999) indicate that a direct action of testosterone may be more important than dihydrotestosterone for male heterosexual psychosexual development.

Not only sex hormones may affect sexual differentiation of the brain: on the basis of animal experiments, it is expected that all compounds that influence hormone or neurotransmitter metabolism in development may also affect sexual differentiation of the brain (Pilgrim and Reisert, 1992). For example, young adult male mice that were exposed prenatally to alcohol were found to have a decreased preference for female partners and an increased preference for males (Watabe and Endo, 1994). Exposure during development to some drugs (e.g. barbiturates) causes deviations in testosterone levels persisting into adulthood (Ward, 1992). This agrees with the findings of Dessens *et al.* (1999) that children born of mothers who were exposed to anticonvulsants, such as phenobarbital or diphantoin, have an increased probability of transsexuality (see below). Exposure of rats to drugs such as opiates led to behavioural changes despite apparently normal adult gonadal hormone levels (Ward, 1992). Similar observations in human sexual differentiation have not yet been reported.

It is of great interest that there is also recent animal experimental evidence for primary genetic control of sexual differentiation that does not involve sex hormones. Results obtained from cultures of embryonic rat brain indicate that dopaminergic neurons may develop morphological and functional sex differences in the absence of sex steroids (Pilgrim and Reisert, 1992). For such hormone-independent effects, the most likely candidates are those genes located on the non-recombining part of the Y chromosome and believed to be involved in primary sex determination of the organism. Two candidate genes are the two testis-determining factors ZFY and the master switch for differentiation of a testis SRY. Those are putative transcription factors. We have shown that SRY and ZFY are transcribed in the hypothalamus and frontal and temporal cortex of adult men and not in women. It may well be possible that they function as sex-specific, cell-intrinsic signals that are needed for full differentiation of a male human brain, and that continuous expression throughout life may be required to maintain sex-specific structural or functional properties of differentiated male neurons. Sexual differentiation of the human brain may thus be a multifactorial process, although a role of SRY and ZFY in this process still needs to be proved (Mayer *et al.*, 1998). An alternative mechanism could be the actions of an imprinted X-linked locus (Skuse, 1999). Recent clinical studies on human subjects with mutations in genes involved in sexual differentiation also point to the possibility that the interaction between sex hormones and brain development may not be the only mechanism involved in the development of gender and sexual orientation.

The relative contributions of the different sex hormones and other non-hormonal factors on sexual differentiation of the human brain should clearly be a focus for future research.

Sexual Differentiation, the Hypothalamus and the Amygdala

Sex differences in the hypothalamus and other limbic structures are thought to be the basis of sex differences in reproduction (e.g. copulatory behaviour in both sexes, the menstrual cycle in women), gender identity (i.e. the feeling that one is either a man or a woman), gender identity disorders (transsexuality) and sexual orientation (homosexuality, heterosexuality, bisexuality) (Gooren *et al.*, 1990; Swaab *et al.*, 1992; Swaab and Hofman, 1995). The PVN in the rat, in particular its oxytocin neurons, is involved in erectile functions in copula. For penile erections initiated by psychogenic stimuli, the medial amygdala, the PVN and, to a lesser extent, the BST are involved, while medial preoptic lesions in the rat have little, if any, effect on this type of erections (Liu *et al.*, 1997). Sexual arousal and orgasm produce long-lasting alterations in plasma prolactin concentrations in both men and women (Exton *et al.*, 1999), which might be related to postorgasmic loss of arousability (Bancroft, 1999), indicating a role of the medial-basal hypothalamic dopamine neurons.

There is extensive animal experimental literature that shows that the medial preoptic area of the hypothalamus is a key structure for male and female copulatory behaviour (Pilgrim and Reisert, 1992; Yahr *et al.*, 1994; McKenna, 1998) and that the hypothalamus is involved in seminal vesicle contractions at coitus (Cross and Glover, 1958). However, the exact role of its subarea, the SDN-POA, in these functions is not clear, and literature on hypothalamic structures involved in sexual orientation in experimental animals is scarce (Paredes *et al.*, 1998; Kindon *et al.*, 1996). Paredes and Baum (1995) found that lesions of the medial preoptic area/anterior hypothalamus in the male ferret affected not only masculine coital performance but also heterosexual partner preference. Edwards *et al.* (1996) observed a decrease in partner preference in male rats following a lesion of the BST. Perkins *et al.* (1995) showed that testosterone, oestrone and oestradiol plasma concentrations were higher in female-oriented rams than in homosexual rams. In the preoptic area, the aromatase activity was higher in the female-oriented rams than in the male-oriented rams, indicating again the possible importance of the preoptic area in sexual orientation (Resko *et al.*, 1996). The content of oestrogen receptors in the amygdala was found to be similar in both homosexual rams and ewes, but less than the receptor content in heterosexual rams, while the oestrogen receptor content of the hypothalamus, anterior pituitary and preoptic area of these two groups did not differ (Perkins *et al.*, 1995). These data suggest that the amygdala might also play a role in sexual orientation. Indeed, in some Klüver–Bucy syndrome cases due to damage of the temporal lobe, patients were reported to change from heterosexual to homosexual behaviours, indicating that the temporal lobe might be of importance for sexual orientation (Lilly *et al.*, 1983; Marlowe *et al.*, 1975; Terzian and Dalle Ore, 1955). Also, there are a few case histories of changing sexual orientation, from heterosexual to paedophilic or homosexual, on the basis of a lesion in the hypothalamus or the temporal lobe, from which the amygdala has strong connections to the hypothalamus (Miller *et al.*, 1986). There is no information available on the exact nature of the preoptic and amygdala neuronal systems and connections that may be involved in sexual orientation. Data on the hypothalamus in relation to gender identity in animals are, of course, nonexistent.

There are a few studies in the medical literature that implicate the hypothalamus and adjoining structures in various aspects of sexual

behaviour. Direct electrical or chemical stimulation of the septum may induce a sexually motivated state of varying degrees up to penile erection in men and building up to orgasm in both sexes (Heath, 1964). Markedly increased sexual behaviour was observed following the placement of the tip of a ventriculoperitoneal shunt into the septum in two cases (Gorman and Cummings, 1992). Meyers (1961) described a loss of potency following lesion in the septo-fornico-hypothalamic region. Electrical stimulation of the mammillary body in monkeys induced penile erection (MacLean and Ploog, 1962; Poeck and Pilleri, 1965). Precocious puberty and hypersexuality have been reported following lesions in the posterior part of the hypothalamus, and hypogonadism is an early sign of pathology in the anterior part of the hypothalamus (Bauer, 1954; Bauer, 1959; Poeck and Pilleri, 1965). In particular, hamartomas that affect the posterior region of the hypothalamus, i.e. those that are pendulated and attached to the region of the mammillary bodies, may cause precocious puberty (Valdueza *et al.*, 1994). In addition, precocious puberty is found in cases of pineal region tumours that produce gonadotropins.

A German stereotactic psychosurgical study (Müller *et al.*, 1973) reported on 22 mainly paedophilic or ephebophilic (preferring pubertal boys) homosexual men ($n = 14$) and six cases with disturbances of heterosexual behaviour (hypersexuality, exhibitionism or paedophilia). In 12 homosexual patients, a lesion was made in the right ventromedial nucleus of the hypothalamus. In eight patients, homosexual fantasies and impulses disappeared. According to this paper, in six patients a 'vivid desire for full heterosexual contacts' occurred after the operation. In one paedophilic patient, bilateral destruction of the ventromedial nucleus was performed and he lost all interest in sexual activity after the operation. The heterosexual patients reported a significant reduction of their sexual drive. Unilateral ventromedial hypothalamotomy in 14 cases treated for aggressive sexual delinquency caused a decrease in sexual drive (Dieckmann *et al.*, 1988; Albert *et al.*, 1993). Although these studies at first sight appear to suggest that the human hypothalamus is indeed involved in sexual orientation and sexual drive, they are highly controversial from an ethical point of view, and they are methodologically deficient (Heimann, 1979; Schorsch und Schmidt, 1979; Rieber and Sigusch, 1979).

Transsexuality and Other Gender Identity Problems

Transsexuality is a rare condition. The annual incidence of transsexuality in Sweden has been estimated to be 0.17/100 000 inhabitants. The sex ratio (genetic male : female) varies from country to country between 1.4 : 1 and 3 : 1 (Garrels *et al.*, 2000; Landén *et al.*, 1996). There is little information about the factors that may influence gender and cause transsexuality in humans (Table XIV.2). The disparate maternal aunt/uncle ratio in transsexual men, pointing to an X-related heredity, has been hypothesized to be due to genomic imprinting (Green and Keverne, 2000). There are only a few reports that have found chromosomal abnormalities in transsexuals. Six cases of male-to-female transsexuals with 47,XYY chromosome and one female-to-male transsexual with 47,XXX have been reported (Tayfun Turan *et al.*, 2000). Moreover, transsexualism has been reported in a man with Klinefelter's (XXY) syndrome. In addition, pairs of monozygotic female twins have requested sex reassignment, and familial cases of gender identity problems have been reported, suggesting a genetic basis for this disorder (Green, 2000; Sadeghi and Fakhrai, 2000). Although only a minority of the transsexuals has an underlying endocrine abnormality (Meyer *et al.*, 1986), there are some indications of a possible disorder of the hypothalamo-pituitary-gonadal axis in some transsexuals that may have a basis in development, such as a high frequency of polycystic ovaries, oligomenorrhoea and amenorrhoea in female-to-male transsexualism (Sadeghi and Fakhrai, 2000; Futterweit *et al.*, 1986).

Table XIV.2 Factors that influence sexual differentiation of the human brain

Factor	Notes
Gender identity (transsexualism)	
Chromosomal disorders	Rare: 47XYY (male to female), 47XXX (female to male) (Tayfun Turan *et al.*, 2000), monozygotic twin (female; Sadeghi and Fakhrai, 2000), genomic imprinting (Green and Keverne, 2000)
Phenobarbital/diphantoin	Dessens *et al.* (1999)
Hormones	Intersex (Zucker *et al.*, 1987; Reiner, 1996)
	Cloacal extrophy
	5-α-reductase deficiency, 17-β-hydroxy-steroid-dehydrogenase-3 deficiency (Imperato-McGinley *et al.*, 1979; Imperato-McGinley *et al.*, 1991; Wilson, 1999).
	Congenital adrenal hyperplasia girls with gender problems (Meyer-Bahlburg *et al.*, 1996; Zucker *et al.*, 1996)
	More polycystic ovaries, oligomenorrhoea and amenorrhoea are found in transsexuals (Futterweit *et al.*, 1986)
Social factors?	Bradley *et al.* (1998): not effective; John/Joan/John case (Diamond and Sigmundson, 1997)
Sexual orientation	
Genetic factors	Twin studies (Kallmann, 1952; Bailey and Bell, 1993)
	Molecular genetics (Hamer *et al.*, 1993; Hu *et al.*, 1995); but see Rice *et al.* (1999)
Hormones	Congenital adrenal hyperplasia girls (Money *et al.*, 1984; Dittmann *et al.*, 1992; Zucker *et al.*, 1996)
	Diethylstilboestrol (Ehrhardt *et al.*, 1985; Meyer-Bahlburg *et al.*, 1995)
	Male-to-female sex reassignment (Bailey *et al.*, 1999)
	Stress during pregnancy (Ellis *et al.*, 1988; Bailey *et al.*, 1991)
Social factors?	Raising by transsexual or homosexual parents does not affect sexual orientation (Green, 1978; Golombok *et al.*, 1983)

Dessens *et al.* (1999) reported that three children born of a group of 243 women exposed to the anticonvulsants phenobarbital and diphantoin were found to be transsexuals, while in addition there were a few other subjects with gender dysphoria/cross-gender behaviour. Gender problems thus occurred remarkably often in view of the rarity of this disorder. This observation on the effect of compounds that are known to alter steroid levels in animal experiments has to be examined further. In this respect, it is of interest to note that phenobarbital has been used widely as prophylactic treatment in neonatal jaundice and greatly elevated the postnatal rise in testosterone (Forest *et al.*, 1981). In 1996, Meyer-Bahlburg *et al.* reported a gender change from woman to man in four 46,XX individuals with classical congenital adrenal hyperplasia. Congenital adrenal hyperplasia, characterized by high androgen levels during prenatal development, constitutes a risk factor for the development of gender identity problems. Although it should be emphasized that the large majority of women with this disorder do not experience a marked gender identity conflict, the odds ratio that a genetic female with this disease would live as an

adult in the male social role compared with genetic females in the general population was found to be 608 : 1 (Zucker *et al.*, 1996). These observations support the view that intrauterine or perinatal exposure to abnormal levels of sex hormones may permanently affect gender identity.

The concept of sexual neutrality at birth, after which infants differentiate as masculine or feminine as a result of social experiences, was proposed by Money *et al.*, (1955a; Money *et al.*, 1955b). Gender imprinting was presumed to start at the age of 1 year and to be well established by 3–4 years (Money and Erhardt, 1972). Observations on children with male pseudohermaphroditism due to 5-α-reductase-2 deficiency were supposed to support the influence of life experience on psychosexual make-up (Al-Attia, 1996). A classic report that has strongly influenced the opinion that the environment plays a crucial role in gender development was that described by Money of a boy whose penis was accidentally ablated at the age of 8 months during a phemosis repair by cautery and who was subsequently raised as a girl. Orchiectomy followed within a year to facilitate feminization, and further surgery to fashion a full vagina was performed later. Initially, this individual was described as developing into a normally functioning woman. Later, however, it appeared that the patient had rejected the sex of raising and switched at puberty to living as a man again, when he requested male hormone shots, a mastectomy and phalloplasty. At the age of 25, he married a woman and adopted her children. This famous John–Joan–John story, although just one case, illustrates that there is little if any support for the view that individuals are sexually neutral at birth and that normal psychosexual development is dependent on the appearance of the genitals (Diamond and Sigmundson, 1997). In a second case of penile ablation, in which the decision was made to reassign the patient as a girl and raise the baby as a girl, the remainder of the penis and testes were removed at the slightly earlier age of 7 months. Although her sexual orientation was bisexual, and even though she was attracted mainly to women, her gender identity was female. The different outcome as compared with the former case is explained by the authors on the basis of the earlier decision to reassign the sex (Bradley *et al.*, 1998).

Reiner (1996) described a 46,XY child with mixed gonadal dysgenesis, one immature testis, hypoplastic uterus and clitoral hypertrophy, who was raised without stigmatization as a girl but who declared himself male at the age of 14. Following corrective surgery and testosterone substitution, he lived as a boy despite the social factors that were clearly in favour of maintaining the assigned sex. Apparently, the deficient testis had been able to organize the brain during development, even though the hormone levels were prenatally so inadequate that ambiguity of the genitalia was induced.

A child with true hermaphroditism, 45X (13%) 47XYY (87%) sex chromosome mosaic pattern in blood, uterus, fallopian tubes, phallus, testicular tissue and epididymis, was assigned male sex at birth. At 5 weeks, the decision was made to reassign him to female. At 9 months, an operation was performed to make the genitalia female, at 13 months the testicle was removed, and at the age of 5, another operation was carried out to make the genitalia female. The patient was raised as a girl but she had masculine interests, and at about 8 years of age, she declared that 'God had made a mistake' and that she 'should have been a boy'. Apparently, the male sex hormones to which she had been exposed *in utero* had imprinted the male gender, although the authors also presumed postnatal psychosocial factors to have played a role (Zucker *et al.*, 1987).

As described above, we recently found a female-sized central nucleus of the BST in male-to-female transsexuals. These data were confirmed by neuronal counts of somatostatin cells, the major neuron population in the BSTc. Changes in hormone levels could not explain this difference (Zhou *et al.*, 1995c; Kruijver *et al.*, 2000). This supports the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones.

Homosexuality

Xq28–Thanks for the genes, mom

Seen on T-shirts in the USA

Sexual orientation is influenced by a number of genetic and non-genetic factors (Table XIV.2). Genetic factors appear from studies in families and twins, and through molecular genetics (Kallman, 1952; Bailey and Bell, 1993; Hamer *et al.*, 1993; Turner, 1995; Hu *et al.*, 1995; Pillard and Bailey, 1998; Bailey *et al.*, 1999). Hamer and colleagues found linkage between DNA markers on the X chromosome and male sexual orientation. Genetic linkage between the microsatellite markers on the X chromosome, i.e. Xq28, was detected for the families of gay males but not for the families of lesbians (Hamer *et al.*, 1993; Hu *et al.*, 1995). In a follow-up study, Rice *et al.* (1999) studied the sharing of alleles at position Xq28 in 52 Canadian gay male sibling pairs. Allele and haplotype sharing for four markers were not increased more than expected, which did not support the presence of an X-linked gene underlying male homosexuality. In a reaction to this paper, Hamer (1999) stated that (1) the family pedigree data from the Canadian study supported his hypothesis, (2) three other available Xq28 DNA studies found linkage, and (3) the heritability of sexual orientation is supported by substantial evidence independent of the X chromosome data. In a meta-analysis of the four available studies, he found a significant linkage. Rice *et al.* (1999) responded extensively and remained convinced that an X-linked gene could not exist in the population with any sizeable frequency. This controversy will undoubtedly continue.

Sex hormones during development also have an influence on sexual orientation, as appears from the increased proportion of bisexual and homosexual girls with congenital adrenal hyperplasia (Money *et al.*, 1984; Dittmann *et al.*, 1992; Meyer-Bahlberg *et al.*, 1996). Then there is diethylstilboestrol (DES), a compound related to oestrogens that increases the occurrence of bisexuality or homosexuality in girls whose mothers received DES during pregnancy (Ehrhardt *et al.*, 1985; Meyer-Bahlburg *et al.*, 1995) in order to prevent miscarriage (which it does not do). Whether environmental oestrogens from plastics can influence sexual differentiation of the human brain and behaviour is in debate but is certainly not established. In addition, phyto-oestrogens, such as resveratrol, present in grapes and wine and an agonist for the oestrogen receptor, should be considered in this respect (Gehm *et al.*, 1997).

Maternal stress is thought to lead to increased occurrence of homosexuality in boys (Ellis *et al.*, 1988) and girls (Bailey *et al.*, 1991). As interesting case histories of this potentially environmental factor, Weyl (1987) mentions that Marcel Proust's mother was subjected to the overwhelming stress of the Paris commune during the fifth month of her pregnancy in 1871; and Mary, Queen of Scots, the mother of the homosexual King James I of England, towards the end of the fifth month of pregnancy experienced her secretary and special friend Riccio being killed. Although postnatal social factors are generally presumed to be involved in the development of sexual orientation (Byne and Pearson, 1993; Zucker *et al.*, 1996), solid evidence in support of such an effect has not yet been reported. The observation that children raised by lesbian couples or by transsexuals generally have a heterosexual orientation (Green, 1978; Golombok *et al.*, 1983; Kirkpatrick *et al.*, 1981) does not support the possibility of the social environment in which the child is raised as an important factor for determining sexual orientation, nor is there scientific support for the idea that homosexuality has psychoanalytical or other psychological or social learning explanations, or that it would be a 'lifestyle choice' (Ellis, 1996). Various hypothalamic structures are structurally different in relation to sexual orientation, i.e. the suprachiasmatic nucleus, INAH3 and the commissura anterior (see earlier), suggesting that

a difference in hypothalamic neuronal networks that occurs in development may be the basis of differences in sexual orientation.

Disorders of Sexual Behaviour

Hypothalamo-pituitary disorders often interfere with the expression of sexuality. In fact, a decreased sexual desire was significantly present in 72% of adult men with a pituitary tumour. One-third of these patients reported decreased sexual desire, even as the first symptom of the tumour. Decreased sexual drive is the first symptom in 50% of men with pituitary tumours accompanied by hyperprolactinaemia. In addition, sexual desire is absent or decreased in two-thirds of women with hypothalamo-pituitary disorders. This problem was recorded for 84% of women with hyperprolactinaemia and for only 33% of those with normal serum prolactin. Almost all these women had amenorrhoea (Lundberg and Hulter, 1991; Lundberg and Brattberg, 1992). As men age, there is a decrease in total and free testosterone levels and an associated increase in gonadotropin levels, while a decrease in sexual activity is also notable (Davidson *et al.*, 1983). Although the hormonal changes during ageing are generally considered to contribute only to a minor degree to the decline in sexual function in men, there are many studies showing the relationship between testosterone levels and sexual activity. Testosterone does increase the incidence of all types of male sexual activity in hypogonadal men, and the antiandrogen cyproterone acetate inhibits sexual activity (Davidson *et al.*, 1982; Albert *et al.*, 1993; Yates, 2000). There is also clear evidence that testosterone modulates sexual behaviour in women: adrenalectomy decreases sexual activity and testosterone replacement restores it (Albert *et al.*, 1993; Tuiten *et al.*, 2000).

Disturbed sexual behaviour has, moreover, been reported in a number of neurological and psychiatric disorders (Chandler and Brown, 1998), such as rabies (Dutta, 1996), encephalitis lethargica, multiple sclerosis, Wolfram syndrome, Prader–Willi syndrome, anorexia nervosa and bulimia nervosa, Kallmann syndrome, Klinefelter's syndrome, depression, Down's syndrome, Kleine–Levin syndrome and Parkinson's disease. Antipsychotic treatment in schizophrenia results in more sexual disturbances in men than in women (Hummer *et al.*, 1999). In depression, sexual activity *per se* is said not to be reduced during the depressed state. Rather, loss of sexual interest appears to be related to the cognitive set of depressive symptoms, i.e. loss of sexual satisfaction. Nocturnal penile tumescence alterations may be more trait-like than state-like in depression (Nofzinger *et al.*, 1993).

A newly discovered group of neurological and reproductive disturbances is due to mutations in sex hormone receptors or aromatase. Some abnormalities of the androgen receptor result in the syndrome of androgen resistance, which goes together with normal male differentiation. More recently, mutations of the androgen receptor were also described in a form of Kennedy's disease, a motor neuron disease. In Kennedy's disease (X-linked bulbospinal muscular atrophy), a trinucleotide repeat expansion occurs in exon A of the androgen receptor gene (MacLean *et al.*, 1995). People with Kennedy's disease have normal virilization, although progressive gynaecomastia, testicular atrophy and infertility may occur (MacLean *et al.*, 1996). In addition, mutations of the oestrogen receptor may cause oestrogen resistance.

Cases of female pseudohermaphroditism, hypergonadotropic hypogonadism and multicystic ovaries have been reported to be associated with missense mutations in the gene encoding aromatase (P450). In one 14-year-old girl, breast development did not occur, her clitoris was enlarged, and circulating androgens and gonadotrophins were high, causing hyperstimulation of the ovaries (Conte *et al.*, 1994). Female pseudohermaphroditism has also been found to be caused by placental aromatase deficiency. Maternal serum levels of oestrogens were low and those of androgens high in the third trimester, causing maternal virilization and pseudohermaphroditism of the female foetus (Shozu *et al.*, 1991). Another mutation of the human gene encoding for aromatase P450 has been reported in male and female siblings. During both pregnancies, the mother exhibited signs of progressive virilization. The girl had nonadrenal female pseudohermaphroditism at birth, developed progressive virilization at of puberty, and had pubertal failure with no signs of oestrogen action, hypergonadotropic hypogonadism and polycystic ovaries. The girl's brother was 204 cm tall. He was sexually mature and had elevated testosterone levels, low oestrogen levels and high gonadotrophins. Interestingly, the psychosexual orientation of both brother and sister was reported to be appropriate for their phenotypic sex (Morishima *et al.*, 1995). There has also been a publication on two mutations in the CYP19 gene that were responsible for aromatase deficiency in an 18-year-old woman with ambiguous genitalia at birth, primary amenorrhoea, sexual infantilism and polycystic ovaries (Ito *et al.*, 1993). No information is present on the sexual differentiation of the hypothalamus in such subjects.

Stereotactic hypothalamotomy aimed at the ventromedial nucleus has been performed during detention in violent sexual offenders. Sexual drive was reported to be markedly reduced and no relapses occurred. As side effects, a strong feeling of hunger and weight gain were reported (Dieckmann and Hassler, 1977). In one patient, agitation followed by unconsciousness and hemiparesis resulted from a haemorrhage into the third ventricle after removal of the electrode after such an operation. Another patient became unconscious but recovered later (Šramka and Nádvorník, 1975). Stereotactic hypothalamotomy was not only a risky operation but also a controversial one, both from an ethical and scientific point of view.

CONCLUSIONS

Sex differences in the brain and hormone levels are not only of importance for sexual behaviour; they are also thought to be the structural and functional basis of pronounced sex differences in the prevalence of neurological and psychiatric diseases.

There is an increasing amount of literature concerning morphological and functional sex differences in the human brain. Focusing on the hypothalamus, sex differences have been reported in volume and cell numbers of particular areas in neuronal metabolic activity of such areas and in transmitter content. Some of the sex differences seem to be related to age or certain disorders like Alzheimer's disease, others to reproduction, gender identity and sexual orientation. The BSTc, a brain area involved in reproduction, was found to be twice as large in men as in women. In male-to-female transsexuals, this sex difference was found to be reversed. The BSTc of genetically male transsexuals was found to be of female size and neuron number, supporting the idea that this structure may be involved in the feeling of being a man or a woman. The BSTc in transsexuals thus seems to have followed a course of sexual differentiation during development that is opposite to that of the sex organs.

Based on animal experiments, sexual differentiation of the brain is thought to be 'imprinted' or 'organized' by androgens during development, following conversion by aromatase to oestrogens, presumably during gestation or the perinatal period. From puberty onwards, sex hormones alter the function of previously organized neuronal systems. This action is known as 'activating' effects of sex hormones, but such effects may also inhibit neuronal function. However, genetic males with a complete androgen insensitivity syndrome, despite normal testis differentiation and androgen biosynthesis, have a phenotype that is normal female, both externally and behaviourally. They perceive themselves as highly feminine and do not have gender problems. This means that for the development of human male gender identity and male heterosexuality,

direct androgen action on the brain seems to be of crucial importance. The aromatization theory, which emphasizes the importance of oestrogens, may thus be of secondary importance for human sexual differentiation.

Apart from the interactions of hormones and the developing neurons, a number of other factors seem to influence sexual differentiation of the brain and thus influence sexual orientation and gender identity. Chromosomal disorders, genetic factors, stress during pregnancy, and medicines taken by the pregnant woman may play a role. However, postnatal social factors do not seem to be of primary importance for the development of sexual orientation or gender identity.

The interaction between hormones, hypothalamic structures and the amygdala seem to be of the utmost importance for sexual behaviour. Disorders of sexual behaviour may not only go together with decreased hormone levels in elderly people; they may also result from countless neurological, neuroendocrine or psychiatric diseases and therefore demand careful diagnostic attention.

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Part B

CLINICAL SYNDROMES

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XV

Cognitive Disorders

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XV-1

Animal Models of Cognitive Disorders

Thomas Steckler

INTRODUCTION

Animal models of human cognitive function are required for an understanding of the aetiology of human cognitive disorders, e.g. identifying novel therapeutic targets and the mechanism of action of drug effects on cognitive function. Animal models reflect approximations to the situation to be modelled. Although it should not be assumed that attempts to model a certain aspect of a situation (e.g. the generation of a tau transgenic mouse) will lead to all facets of that situation (i.e. a 'demented' mouse), these models will help to understand part of the complexity of the situation (e.g. tau overexpression leading to neuronal loss) and to evaluate related concepts (e.g. the hypothesis that dysfunction of tau can lead to neurodegeneration and cognitive impairments).

These objectives demand validation of the model (Sarter and Bruno, 2001; van der Staay and Steckler, 2001; van der Staay and Steckler, 2002; Willner, 1991). The focus of validation will depend on the type of model (1) *screening* models, (2) more complex or *simulation* models, and (3) *assay* or mechanistic models (Figure XV-1.1) (Ellenbroek and Cools, 2000; Willner, 1991) (see also Chapters III and XVII-1). It should be noted that there is a certain degree of overlap between these categories. Screening models rely strongly on predictive validity, i.e. on whether a manipulation (e.g. a drug treatment) predicts performance in the condition to be modelled (Willner, 1991). Simulation models attempt to incorporate both the neurobiological and behavioural abnormalities underlying a disorder (see Chapter III); they require assessment of how well a measure of a particular process, trait or state reflects theoretical assumptions (construct validity; Willner, 1991). Moreover, simulation models require face validity, i.e. a high degree of phenomenological similarities between the measure of the model and the condition it simulates (Willner, 1991).

In drug development, there can be a third model, the assay or mechanistic model, which allows easy assessment of whether a compound is active on a target, e.g. a receptor. This can be, but does not have to be, a behavioural assay, but it could be any easily and reliably measurable and specific physiological outcome. Assessment of perioral movements or parasympathetic activation, for example, can serve as indices of *in vivo* cholinergic activity (Salamone *et al.*, 1986). Such models allow fast ranking of compounds according to potency and duration of action, and give first indications as to whether a drug penetrates the brain. Of note, these models are not necessarily related to the desired therapeutic effect of a drug, which needs to be assessed separately in screening and simulation models. Therefore, the mechanistic model will not be further discussed here.

This does not mean that one model category is better or worse. The important point is to use the appropriate model to answer a particular question. Often, conclusions are drawn from mechanistic or screening models prematurely as these types of models lack certain validity aspects. For example, a screening model can give an indication for activity of a compound, but often data are overinterpreted. Conclusions that drug *x* is a cognitive enhancer and will be useful for treatment of dementia cannot be drawn from a screening model, as it poses the risk of many false positives later on in the clinic. It is important to acknowledge the limitations of the model used, and it is advisable to use more than one model to enhance the robustness of the result.

In principle, these model categories can be divided further into *causative* and *symptomatic* models (Figure XV-1.1). A causative model uses an approach that is linked directly to the disease process, such as exposure to the noxious agent known to cause the neuropsychiatric disease itself. For example, chronic ethanol treatment results in both cognitive and structural changes in the brains of rats (Arendt *et al.*, 1988; Savage *et al.*, 2000) and is thought to mimic the effects of alcohol abuse. Likewise, the brain damage and behavioural deficits caused by ischaemia induced by occlusion of the middle cerebral artery (Bederson *et al.*, 1986; Markgraf *et al.*, 1992; Tamura *et al.*, 1985; Van der Staay *et al.*, 1996; Yamamoto *et al.*, 1988) constitute a causative model of stroke.

Alternatively, drugs or lesions known to modulate brain systems hypothesized to be involved in the aetiology of neuropsychiatric diseases, but not necessarily being a key factor in the underlying aetiology, can be used. Pyrithiamine-induced thiamine deficiency, for example, has been used as a model of Wernicke–Korsakoff syndrome, which is related to alcohol abuse and is due to the thiamine deficiency seen in alcohol abusers. Treatment with pyrithiamine produces a range of neuropathological and behavioural abnormalities in rodents, which model the cognitive deficits seen in Wernicke–Korsakoff syndrome (Irle and Markowitsch, 1983; Langlais *et al.*, 1992; Mumby *et al.*, 1999; Savage *et al.*, 1999), although it is other dietary issues, not pyrithiamine, that cause the thiamine deficiency in alcohol-dependent patients.

Another example derives from the field of psychosis: chronic phencyclidine (PCP) administration has been proposed to model psychosis, although the tool itself is not aetiologically relevant (most schizophrenic patients do not take PCP, although *N*-methyl-D-aspartate (NMDA) antagonists can worsen their symptoms; Lahti *et al.*, 2001). Both pyrithiamine and PCP models have to be considered as symptomatic models. At times of course, there is overlap, and a symptomatic model can turn into a causative model and vice versa, depending on whether a process can be linked aetiologically to a disease.

Note that the fact that the tool is not related causally does not necessarily reduce the validity of the model. For example, NMDA antagonists such as PCP or ketamine produce a range of schizophrenia-like positive and negative symptoms in humans (Krystal *et al.*, 2000; Lahti *et al.*, 2001). Long-term exposure to PCP produces prefrontal cortical cognitive and dopaminergic dysfunction in rats and monkeys, even after discontinuation of drug treatment (Jentsch *et al.*, 1997a; Jentsch *et al.*, 1997b; Jentsch *et al.*, 1999; Jentsch and Taylor, 2001), and can lead to neurodegeneration in

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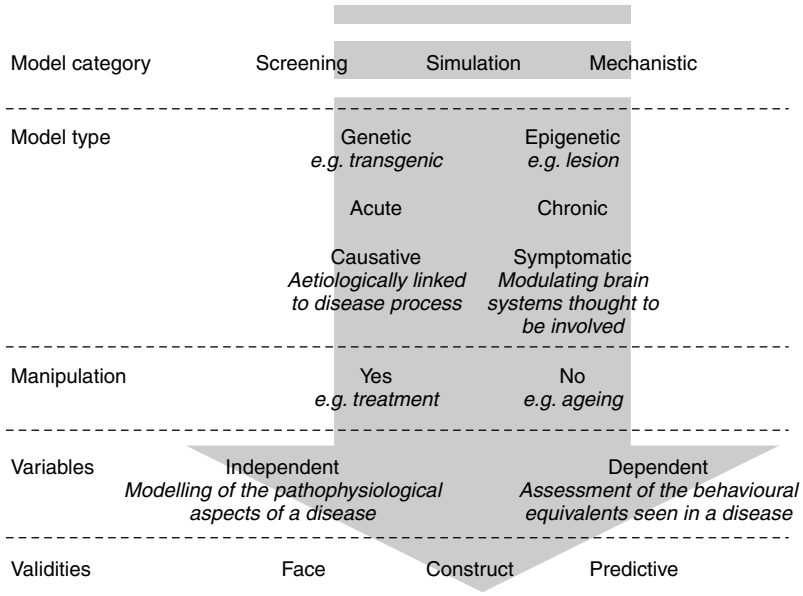

Figure XV-1.1 Classification of animal models of human cognitive dysfunction

certain brain areas, including various cortical areas (Ellison, 1995). These symptomatic similarities give this model high face validity, and the dopaminergic changes indicate good construct validity. Moreover, the cognitive impairments induced by subchronic PCP administration in rats can be reversed by antipsychotic drugs such as risperidone (Schroeder *et al.*, 2000). This suggests that (sub)chronic PCP administration may also be a model with good predictive validity for treatment of cognitive symptoms in psychosis.

Within these model categories, we can further define independent and dependent variables (Figure XV-1.1). First, different aspects of a condition (e.g. a disease state) can be modelled, such as genetic (e.g. tau mutations) and epigenetic (e.g. ageing, pharmacological challenge) phenomena. Models may be based on naturally occurring features (e.g. old animals) or on experimental manipulation (e.g. transgenic mice and brain-specific lesions), or they can consist of combinations thereof. These aspects of a model, by definition, constitute independent variables. Second, the independent variables have both neurobiological and behavioural consequences, which make up the dependent variables. Both variables will be viewed separately in this chapter, as the number of possible combinations are enormous.

This does not mean, however, that independent and dependent variables have no bearing on each other. Ultimately, the validity of a model will depend on both the independent variables (i.e. the physiological manipulation) and the dependent variables (i.e. the outcome measure, such as performance in a cognitive task), and the model has to be judged in total. Failure to take all these aspects into account poses the risk to generate invalid results.

Neuropathological features of Alzheimer's disease, for example, include widespread cerebral atrophy, neuronal loss, deposition of β-amyloid (Aβ) peptide in extracellular neuritic plaques (derived by endoproteolysis from amyloid precursor protein (APP)), and intracellular neurofibrillary tangles (comprised mainly of hyperphosphorylated forms of the microtubule-associated protein tau; Selkoe, 1991). Furthermore, a wide range of neurotransmitter abnormalities occurs in Alzheimer's disease, involving catecholamines and neuropeptides. Among these neurotransmitter alterations, possibly the most significant changes can be seen in the cortical projection from the nucleus basalis of Meynert, which undergoes extensive degeneration, leading to cholinergic hypofunction (Perry, 1986; Perry *et al.*, 1978). Behaviourally, patients suffering from Alzheimer's disease do not have a non-specific memory decline but are particularly impaired in certain aspects of mnemonic function, while other parts are relatively spared. They seem to have particular difficulties with encoding and acquisition, leading to declarative memory impairments, including recall and recognition, and a rapid rate of forgetting, while procedural memory is preserved (Brandt and Rich, 1995). An animal model of Alzheimer's disease should aim to model the neuropathological/neurochemical, molecular and behavioural/cognitive features.

From the examples given so far, it is already obvious that not only neurodegenerative disorders, such as Alzheimer's disease, but also other psychiatric disorders, such as attention deficit hyperactivity disorder (ADHD), schizophrenia and depression, stroke and drug abuse, and the normal ageing process are associated with significant cognitive dysfunction, and that amelioration of this dysfunction would be beneficial for long-term outcome in all of these disorders. Thus, talking about animal models of human cognitive function inevitably will cover a wide range of different human psychiatric and neurological diseases, although the focus is probably on animal models of dementia. It cannot be the aim of this chapter to review all these models in detail; rather, I will give some relevant examples, which can then be used to illustrate general principles and which hopefully can be extrapolated to other, newly developed models in the future. Although the cognitive deficits seen in the various neuropsychiatric disorders are specific (see the example of Alzheimer's disease, above), it is also of note that there is overlap between the disorders. For example, executive functions, such as concept formation, problem solving and set shifting, such as on the Wisconsin Card Sorting Test (WCST), are impaired in Alzheimer's disease (Whitehouse *et al.*, 1993). WCST performance is also impaired in schizophrenic patients (Goldberg and Gold, 1995). Thus, a behavioural model assessing executive functions in an animal, and using a task comparable to the WCST, would be useful as a model for both human disorders, i.e. there is also overlap between models. However, an Alzheimer model may approach the issue by using a manipulation of amyloid deposition, while a psychosis model may focus on chronic PCP treatment, for example.

MAKING THE MODEL: A SURVEY OF THE INDEPENDENT VARIABLES

Looking at the independent variables of an animal model of cognitive dysfunction, we can distinguish epigenetic and genetic, and unmanipulated and experimentally manipulated models. The wide range of different approaches used to model a particular disease can be illustrated, for example, in the field of Alzheimer's disease. Here, animal models used in the past included unmanipulated adult animals, old rodents or non-human primates (epigenetic, unmanipulated), selectively bred strains, such as senescent-accelerated prone (SAMP) mice (genetic, unmanipulated), acute pharmacological systemic or local challenge (e.g. with scopolamine or hemicholinium-3; epigenetic, acute symptomatic manipulation), basal forebrain lesions using various toxins (epigenetic, chronic symptomatic manipulation), Aβ infusions (epigenetic, chronic causative manipulation), and APP, tau or other transgenics (genetic, chronic causative manipulation), to mention just a few. Advantages and disadvantages of these models will be addressed in the following sections.

Epigenetic Models of Dementia with Naturally Occurring Features

The Unmanipulated Adult Laboratory Rodent

Seemingly, one of the most simple and straightforward approaches to investigating the effects of manipulations on cognitive function is the use of normal, not further manipulated animals. This is standard practice, for example, when looking at the side-effect profile of a drug in a screen for drug-induced cognitive deficits. This approach has also been employed to look explicitly at cognitive enhancement, but here it makes the assumption that animals function at a suboptimal level. It is well known, however, that animals can learn to respond with high accuracy in a wide variety of cognitive tasks. Therefore, the chances of failing to see drug-induced changes due to ceiling effects, e.g. in spatial learning tasks, are high, although there are exceptions (see below).

These models primarily fulfil the characteristics of screening models as they may have predictive validity for cognitive effects of a manipulation in humans (e.g. as part of a side-effect profile of a drug), but most of them have neither face nor construct validity for human disease states associated with cognitive impairment.

A modification of this approach has been to use the naturally occurring variation amongst rodents, to segregate individuals into poor and good performers, and to then use poor performers as a model for cognitive dysfunction, e.g. to model ADHD (Puumala *et al.*, 1996; Puumala *et al.*, 1997). This approach, however, is also flawed, as it assumes either that the cognitive process under investigation is also intact in the human condition and just reflects the extreme of a normal variation, or that some laboratory rats exhibit some uncontrolled pathological features. In the absence of a clear neurobiological correlate, the construct and face validities of the independent variable of such a model must be considered poor, even if behavioural assessment is done in highly relevant paradigms with high validity of the dependent variable. Similar dissociations between good and poor performers have also been carried out in old animals, but here the deficits are also accompanied with age-related pathology (see below).

Possible exceptions to these restrictions are the latent inhibition and the prepulse inhibition (PPI) models of schizophrenia, which are also based on unmanipulated animals (see Chapter XVII-1 in this book for extensive discussions of these models). Latent inhibition is a cognitive process that refers to the detrimental effects of prior stimulus pre-exposure on subsequent conditioning to that stimulus (Lubow, 1989). PPI is a measure of sensorimotor gating and is related to the startle response, characterized by a reduction in startle amplitude following prior presentation of another stimulus of lower intensity, which is unable to elicit a response on its own (Graham, 1975). Both latent inhibition and PPI are highly conserved across species, the brain circuits are similar in animals and humans, both phenomena can be assessed in animals and humans with highly comparable methods, and both phenomena are impaired in schizophrenia (Koch, 1999; Lubow, 1989; Moser *et al.*, 2000; Swerdlow and Geyer, 1998; Weiner and Feldon, 1997).

Both phenomena may also tax comparable processes, as both latent inhibition and sensorimotor gating may serve to allocate attentional resources to relevant stimuli. Altered information processing has been viewed as a core cognitive symptom in schizophrenia (Cadenhead and Braff, 2000; Callaway and Naghdy, 1982), and this may form the basis of the latent inhibition and PPI impairments in these patients. Moreover, both latent inhibition and PPI can be potentiated in animals by antipsychotic drugs and have been claimed to have good predictive validity for antipsychotic activity in patients (Moser *et al.*, 2000; Swerdlow and Geyer, 1998; but see Depoortere *et al.*, 1997a and Depoortere *et al.*, 1997b for a different view on the predictive validity of PPI). Because of these points, both latent inhibition and PPI have also been suggested to model schizophrenia with high face and construct validity. However, this applies for the dependent variable only (i.e. for the behaviour under investigation). Assessment of latent inhibition and PPI in the otherwise not manipulated animal is of limited construct and face validity with respect to the independent variable. To model a disease process with high face and/or construct validity, it would be necessary to introduce at least some of the changes that may form the pathophysiological basis underlying the (cognitive) symptoms of that disease.

The Unmanipulated Aged Laboratory Rodent

The chances of detecting cognitive improvement following intervention are better in animals with predetermined cognitive dysfunction, i.e. in deficiency models, such as old, lesioned, transgenic or otherwise compromised animals.

Old animals have been suggested to represent good models for human aging (Barnes, 1990; Decker, 1995; Gallagher and Pelleymounter, 1988). Amongst rodents, rats have been the model of choice, but there is an increasing interest in mouse models of age-related neurobiological and behavioural function (Ingram and Jucker, 1999). Importantly, ageing is not just a function of time; there is also a clear effect of genotype interacting with age: strains differ in the vulnerability to ageing, develop different degrees of age-related behavioural, neurochemical and anatomical impairments, and show pronounced differences in longevity (Ingram and Jucker, 1999; Wyss *et al.*, 2000). This can also be seen at the behavioural level, as strains can adopt different strategies to solve a behavioural paradigm at different ages (Wyss *et al.*, 2000), which may hamper comparability of data across studies.

Often, there are individual differences within aged animal populations in both behavioural and neurochemical measures (Lee *et al.*, 1994), and it may be advisable to separate old good from poor performers as drug effects may require a deficient group (Caprioli *et al.*, 1995; Quirion *et al.*, 1995). As a caveat, however, it needs to be stressed that some of the age-related effects may be due to poorly controlled unspecific age-related confounds, such as cataracts, which impair visual acuity, cardiovascular insufficiency, thermoregulation and stress reactivity (Andrews, 1996; Lindner and Gribkoff, 1991). In particular, when it comes to drug studies, age-related changes in pharmacokinetic and pharmacodynamic parameters must be taken into consideration (Mares *et al.*, 1994; Yang *et al.*, 1984). Motivational factors have also been reported to change with age (Blokland and Raaijmakers, 1993). Moreover, aged rodents often have a very different history of housing compared with young controls, which could in principle interfere with performance. These factors need strict control as they will compromise the validity of the model.

In general, however, rodent spatial memory deficits have been suggested to constitute a good model for age-related human cognitive decline (Gallagher and Nicolle, 1993; Ingram *et al.*, 1994), and age-related impairments in spatial performance have been correlated with measures of central nervous system activity, such as cholinergic function (Dunbar *et al.*, 1993; Fischer *et al.*, 1989). Aged rodents have also been used as models of dementia, and results obtained with potential cognitive enhancers have been extrapolated rather liberally to the demented patient in the past. At face validity, it is normal ageing that is modelled in aged rats and mice. However, aged rodents do not mimic the neuropathological features of dementia. Unless one assumes a continuum between ageing and neurodegenerative disorders, such as Alzheimer's disease, then their value for the understanding of these disorders is limited to the part of the disease contributed by the aging process *per se*. This suggests that studies using aged rodents as animal models of dementia use a suboptimal model. Following this logic, it may not come as a big surprise that the literature is repleted with drugs showing cognitive enhancement in the aged animal but failing in the patient.

The aged monkey may represent a better model for dementia. In contrast to the rodent, aged non-human primates develop not only cognitive impairment but also neuropathological changes more comparable to those seen in dementia, including neuronal loss, cholinergic impairment, amyloidosis, senile plaques and tau-immunoreactive neurofibrillary tangles (Cork *et al.*, 1990; Price *et al.*, 1991; Struble *et al.*, 1982; Struble *et al.*, 1985; Walker *et al.*, 1987; Walker *et al.*, 1990). They represent a model with better face validity for dementia. However, their availability is limited for both practical (low reproductive rate, long generation time, etc.) and ethical reasons (Price *et al.*, 1998a).

Genetic Models of Dementia with Naturally Occurring Features

Breeding of selected lines of rodents has led to animals with an accelerated ageing process. Although selection of extreme phenotypes can already be considered an experimental manipulation, it is done on the basis of naturally occurring alleles, and the neurobiological and behavioural changes seen in these lines can be considered a naturally occurring feature, equivalent to a comparison between strains of rats or mice. The SAMP mouse, for example, shows an earlier and faster ageing process and earlier onset of cognitive deficits than its normal counterpart, the senescent-accelerated resistant mouse (SAMR), which ages at normal rates (Miyamoto *et al.*, 1986; Takeda *et al.*, 1981; Takeda, 1999). At least 14 SAMP inbred strains and four SAMR inbred strains have been generated on the basis of longevity, and incidence and degree of age-associated dysfunction through selective breeding from the AKR/J inbred mouse strain (Takeda, 1999). In particular, sublines SAMP-8 and SAMP-10 have been reported to show early-onset learning and memory impairments, and SAMP-10 also exhibits age-related cerebral atrophy, hippocampal cholinergic dysfunction, altered intracellular signalling (a reduction in hippocampal protein kinase C level) and hippocampal overexpression of APP (Flood and Morley, 1992; Jeong *et al.*, 1997; Markowska *et al.*, 1998; Miyamoto *et al.*, 1992; Okuyama *et al.*, 2000; Shimada, 1999; Shimada *et al.*, 1994; Takeda *et al.*, 1997). However, SAMP-8 mice also suffer from age-related changes in blood pressure (Han *et al.*, 1998) and altered emotionality (Markowska *et al.*, 1998; Miyamoto *et al.*, 1992), both of which could confound cognitive performance. SAMP mice bear the same restrictions as normally aged animals: primarily, they model ageing, not neurodegenerative disease processes, although the finding of amyloidosis in some sublines favours this model over simply aged rodents if used as a model of dementia.

Experimentally Induced Epigenetic Models of Dementia

Acute Manipulations

Drugs acting at neurotransmitter systems known or at least suggested to play an important role in neuropsychiatric disorders have been employed in a wide range of studies to mimic the neurochemical alterations seen in these disorders. Several drugs are known to impair cognitive performance in both animals and humans, and a prominent role has been attributed to the effects of cholinergic blockade.

The cholinergic hypothesis of dementia predicts that many of the cognitive deficits seen in patients are due to degeneration and consequent hypofunction of the cholinergic basal forebrain (Perry *et al.*, 1978; Perry, 1986). One of the most frequently employed drugs to mimic this hypofunction is the muscarinic antagonist scopolamine, alone or in combination with other treatments, such as nicotinic blockade or serotonergic or noradrenergic manipulations (Dawson *et al.*, 1992; Decker *et al.*, 1990; Levin *et al.*, 1991; Steckler and Sahgal, 1995). The scopolamine model has been proposed to resemble the cholinergic dysfunction seen in Alzheimer's disease (Bartus, 1978).

However, scopolamine treatment produces only some of the cognitive deficits seen in Alzheimer's disease if tested in healthy volunteers (Beatty *et al.*, 1986; Ebert and Kirch, 1998; Litvan *et al.*, 1995), and, in fact, the model may mimic the cognitive changes seen in normal ageing more closely than those seen in dementia. This is possibly not surprising, given that the neuropathological and neurochemical alterations seen in dementia encompass much more than just a cholinergic deficit. Although this can be taken into consideration by combining the scopolamine model with manipulations in other neurotransmitter systems (Decker *et al.*, 1990; Levin *et al.*, 1991; Steckler and Sahgal, 1995), there are additional limitations to the scopolamine model: for example, it can be criticized for its acute effects, while dementia is a chronically progressing disorder, which allows for compensation and other secondary changes to occur. Moreover, systemic injections of scopolamine, as is most frequently done, will affect all cholinergic systems, both centrally and peripherally. Therefore, scopolamine causes a wide range of non-specific deficits in animals, which can easily overshadow any specific effect on cognitive function.

One possibility to overcoming these non-specific effects would be to infuse scopolamine directly into selected brain areas, such as the hippocampus (e.g. Dunnett *et al.*, 1990; Ragozzino and Kesner, 1998). That way, it could be argued, one has a model at hand that, at face validity, mimics one specific aspect of dementia, namely cholinergic dysfunction. However, construct validity must be considered low as the induction of this deficit (acute, systemic, postsynaptic effects) and its mode of action (muscarinic receptor blockade) is very different from that seen in dementia (chronic, brain-specific degeneration of certain presynaptic cholinergic projections, especially those of the basal forebrain). Likewise, predictive validity is limited: numerous studies investigating potential cognitive enhancers in the scopolamine model reported stunning beneficial effects of these compounds to reverse scopolamine-induced behavioural deficits in animals, but almost all of these compounds failed at later stages of drug development (see Sarter *et al.*, 1992a and Sarter *et al.*, 1992b for overview). Part of the poor predictive validity could be the use of inappropriate behavioural tests, as will be discussed below. However, part of it might also be the limitations of the scopolamine model *per se*, which may be best suited to predicting beneficial effects of drugs developed within a cholinergic programme. Indeed, all drugs currently marketed for the treatment of Alzheimer's disease (galantamine, donepezil, rivastigmine, tacrine, idebenone) are cholinomimetics. They block acetylcholinesterase and thereby inhibit the breakdown of acetylcholine. All these drugs also show

good and possibly most consistent activity in the scopolamine model (Ballard and McAllister, 1999; Bejar *et al.*, 1999; Bores *et al.*, 1996; Chopin and Briley, 1992; Harder and Kelly, 1997; Kirkby *et al.*, 1996; Ogura *et al.*, 2000; Wang *et al.*, 2000; Yamazaki *et al.*, 1989), but this also applies to antagonism of the non-cognitive effects of scopolamine (Baraka and Harik, 1977; Dokla and Rydelek-Fitzgerald, 1991; Shannon and Peters, 1990). Of note, all these drugs represent only symptomatic improvement, and they are able to only partially improve memory and slow down further deterioration in dementia, but none of them leads to complete restitution or stops progression of the illness.

Thus, it could be argued that the scopolamine model may be a good model for hypocholinergic disease states, including ageing and dementia, but it must be realized that it does not necessarily represent a good model for the other aspects of dementia. Notwithstanding these shortcomings, the scopolamine model still enjoys popularity for evaluation of potential cognition-enhancing therapies.

The hemicholinium model also targets the cholinergic system, but it has been suggested to be of higher construct validity than the scopolamine model because hemicholinium-3 (HC-3) primarily affects presynaptic cholinergic function (Salamone, 1991). HC-3 blocks the high-affinity choline uptake into the presynaptic neuron. Compared with the ease with which many other drugs can be administered peripherally, the model is limited by the fact that it has to be administered intracerebroventricularly (ICV) or directly into the brain as it is unable to pass the blood–brain barrier. Naturally, however, ICV HC-3 also avoids the problems inherent in the peripheral administration of cholinergic antagonists. ICV HC-3 has been reported to impair attention (Muir *et al.*, 1992) and spatial learning (Hagan *et al.*, 1989, 1990), and to disrupt delayed (non)matching to position performance in rats (Andrews *et al.*, 1994; Steckler *et al.*, 1995). At least some of these deficits can be reversed with cholinomimetics (Hagan *et al.*, 1989; Hagan *et al.*, 1990). Although the hemicholinium model has a number of advantages over the scopolamine model, it will still target all cholinergic brain systems, including those that are relatively unaffected in disease states such as dementia.

Taken together, a common theme of both the scopolamine and the hemicholinium models is their attempt to acutely shift the activity in brain systems that have been suggested to be related causally to the cognitive abnormalities seen in the disease state. As such, these models already present with better face and construct validity than the naturally occurring models for neuropsychiatric disorders. However, limitations of these models are also obvious. These limitations can be reduced by refinements of the model, which improve face and construct validity further (compare peripheral versus central administration of scopolamine, or the scopolamine versus the HC-3 models). Despite these improvements, however, there is no clear evidence that these models have better predictive validity over models using unmanipulated animals.

Chronic Manipulations

Animal models of human cognitive dysfunction using chronic manipulations are, in principle, covered by two main approaches: chronic drug administration and, in particular, the huge field of lesion studies.

Probably the most frequently used chronic symptomatic model in the field of Alzheimer's disease is the cholinergic basal forebrain lesion model, especially lesions of the nucleus basalis magnocellularis (NBM), the animal homologue of the human nucleus basalis of Meynert, which lead to cholinergic cortical deafferentiation. Compared with the scopolamine and HC-3 models, these lesion models have the advantage that they are chronic, leading to region-specific degeneration of presynaptic cholinergic projections arising in the basal forebrain.

Original approaches employed electrolytic lesions (LoConte *et al.*, 1982), excitotoxic lesions with kainic acid, quisqualic acid, ibotenic acid, NMDA or α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid (AMPA) (Dunnett, 1985; Etherington *et al.*, 1987; Lerer *et al.*, 1985; Wenk *et al.*, 1984), or lesions induced with ethylcholine mustard aziridinium ion (AF64A) (Fisher and Hanin, 1986). However, all these approaches suffer substantial non-specific toxic damage to different degrees (Dunnett and Barth, 1991; Fibiger, 1991; McGurk *et al.*, 1987).

Lesion specificity, however, constitutes an important factor for the validity of an animal model of cholinergic dysfunction. This can be illustrated by findings that quisqualic acid lesions or lesions with AMPA, which produce more cholinergic but less non-cholinergic damage than ibotenic acid, have fewer effects on mnemonic performance than ibotenic acid in some tasks (Dunnett *et al.*, 1991; Etherington *et al.*, 1987). This indicates that there is a substantial, but unintended, non-cholinergic component that contributes to the lesion-induced deficit following administration of excitotoxins such as ibotenic acid. However, comparable behavioural effects can also be induced by the different excitotoxins under certain circumstances, despite different non-cholinergic damage. For example, Robbins *et al.* (1989) showed that ibotenic acid- and quisqualic acid-induced lesions of the NBM produced comparable effects in an attentional paradigm, which suggests that the deficit seen in this task was mediated cholinergically. This in turn suggests that the cholinergic deficit seen in dementia contributes to the attentional impairment of these patients, and that cholinergic basal forebrain lesions may have particularly good face validity as animal models of dementia in combination with attentional paradigms, but that their value can be less in combination with other types of behaviour.

More recently, the immunotoxins 192 IgG-saporin for rodents and ME 20.4 IgG-saporin for primates have been developed, which bind to the low-affinity p75 nerve growth factor receptor expressed by cholinergic basal forebrain neurons and lead to selective damage of the cholinergic basal forebrain if administered locally (Book *et al.*, 1994; Fine *et al.*, 1997; Heckers *et al.*, 1994; Wiley, 1992; Wiley *et al.*, 1991). Both immunotoxins can also be administered ICV, but this will lead to additional cerebellar damage, as Purkinje cells also express the p75 receptor (Berger-Sweeney *et al.*, 1994; Heckers *et al.*, 1994). In agreement with the findings from the more selective excitotoxic lesion studies with quisqualic acid or AMPA, the effects of 192 IgG-saporin-induced cholinergic lesions in rats are relatively subtle despite substantial cholinergic damage, but involvement of cholinergic basal forebrain projections in cognitive processes, including attentional functions, mnemonic functions and conditional learning, were clearly seen after such lesions (Fine *et al.*, 1997; McGaughy *et al.*, 1996; McGaughy *et al.*, 2000; Steckler *et al.*, 1995; Torres *et al.*, 1994; Wrenn and Wiley, 1998).

Interestingly, an upregulation of APP expression has also been reported in the cortex and hippocampus following 192 IgG-saporin lesions (Leanza, 1998; Lin *et al.*, 1998). As mentioned before, overexpression and aberrant processing of APP are key features of Alzheimer's disease. This not only adds to the face validity of the model, but also suggests that the cholinergic cortical and hippocampal projections from the basal forebrain may be required for maintenance of physiological APP levels.

The immunotoxin models have an advantage over pharmacological or other lesion models in that they selectively target those parts of the cholinergic system that also undergo degeneration in dementia. As a caveat, however, it has been noted that the cholinergic projection from the basal forebrain to the amygdala is spared following 192 IgG-saporin lesions (Heckers *et al.*, 1994), a cholinergic projection clearly affected in Alzheimer's disease (Fishman *et al.*, 1986; Shinotoh *et al.*, 2000). Moreover, some methodological issues

can contribute to variability of results, such as exact stereotactic coordinates, volume and concentration of the toxin, and hence the exact site and the degree of cholinergic depletion (Wrenn and Wiley, 1998).

In any case, the lesion models must be considered symptomatic. Do chronic causal models of Alzheimer's disease exist? An early approach trying to model the pathophysiology of Alzheimer's dementia more closely was the aluminium toxicity model. In the original study, administration of aluminium salts was shown to lead to delayed neurofibrillary degeneration in rabbits (Klatzo *et al.*, 1965), although comparable effects in rats were lacking (King *et al.*, 1975). The aluminium model was based on the hypothesis that exposure to aluminium could serve as a risk factor for the development of dementia (Rifat and Eastwood, 1994). However, the aluminium-induced tangles in animals are structurally different from those seen in Alzheimer's patients (Selkoe *et al.*, 1979), and there is accumulating evidence questioning a role for aluminium in the aetiology of dementia (Forster *et al.*, 1995; Makjanic *et al.*, 1998; Reusche *et al.*, 2001; Rifat and Eastwood, 1994). Given this more recent evidence, the aluminium model must be considered to be of low face and construct validity.

A possibly more promising approach for the development of an animal model of dementia is the infusion of β-amyloid peptides directly into the brain. The principal component of these amyloid deposits is Aβ, which is derived from proteolytic processing of APP. Two major C-terminal variants of Aβ exist (Aβ40 and Aβ42), which differ in length. Aβ42 is the principal component in amyloid plaques (Iwatsubo *et al.*, 1994). In the rat brain, intracerebral injection with Aβ40 and Aβ42 led to the formation of aggregates, with Aβ40 aggregates containing fibrillar structures similar to the amyloid fibrils seen in Alzheimer's disease (Shin *et al.*, 1997). Thus, current evidence suggests that this model has good face and construct validity. Moreover, chronic ICV infusions with Aβ peptides led to a reduction in cortical and hippocampal cholinergic activity and impaired mnemonic function (Nakamura *et al.*, 2001; Yamada *et al.*, 1998), adding further evidence in favour of this model. Alternative techniques that allow investigation of the effects of Aβ or APP in the brain include the overexpression of these peptides by adenovirus-mediated gene transfer (Masamura *et al.*, 2000; Nishimura *et al.*, 1998) or transgenesis (e.g. Holcomb *et al.*, 1998; Hsiao *et al.*, 1996; Nalbantoglu *et al.*, 1997), which leads us to the experimentally induced genetic models. In contrast, ICV administration of secreted forms of APP (APPs751 and APPs695) has been reported to enhance performance of mice tested on visual discrimination or lever-press learning tasks (Meziane *et al.*, 1998).

Experimentally Induced Genetic Models of Dementia

Experimentally induced genetic models can comprise transgenic animals, which have an extra gene added to their genome, or knockout animals, which have a selective inactivation or deletion of specific genes. Knockdown models, on the other hand, have a downregulation of the gene product following infusion of antisense oligodeoxynucleotides (ODNs) complementary to a specific sequence of mRNA, which interfere with translational processes. Moreover, genes can be transferred into brain cells using viral vectors. These models can give new insights into the pathophysiological processes underlying neurodegenerative diseases, such as Alzheimer's disease, or other disorders associated with cognitive dysfunction (Duff, 1997; Higgins and Cornell, 1995; Price *et al.*, 1998b.)

Genetic models of Aβ amyloidosis include transgenic mice that express wild-type APP, fragments of APP, mutated familiar Alzheimer APP variants or Aβ (Bronfman *et al.*, 2000; Buxbaum *et al.*, 1993; Games *et al.*, 1995; Irizarry *et al.*, 1997; Holcomb *et al.*, 1998; Hsiao *et al.*, 1996; Kammesheidt *et al.*, 1992; LaFerla *et al.*, 1995; Moran *et al.*, 1995; Nalbantoglu *et al.*, 1997; Riekkinen *et al.*, 1998). Most of these studies have focused on transgenes linked to familiar early-onset Alzheimer's disease rather than sporadic cases (Seabrook and Rosahl, 1999). The 'Swedish' mutation K670N/M671L shows primarily overproduction of Aβ40, while the mutations V717F and V717I, and the 'London' mutation V642I, result in predominance of Aβ42 (Maruyama *et al.*, 1996; Moechars *et al.*, 1999; Thinakaran *et al.*, 1996).

These models show amyloidosis and plaque formation to varying degrees. Early models failed to produce plaques, but plaque formation was achieved by using models with high enough expression of Aβ, using appropriate promoters (Games *et al.*, 1995; Hsiao *et al.*, 1996). The neuropathological features such as amyloid deposition and plaque formation develop with age, but they can be speeded up by combination with other transgenes (e.g. Holcomb *et al.*, 1998). The development of progressive neuropathology adds strongly to the face validity of these models.

APP transgenics have been studied on their own and in combination with other mutations, e.g. in the presenilin 1 (PS1) gene. Mutations of the PS1 gene have been suggested to cause up to 50% of all early-onset familiar Alzheimer's disease cases (Alzheimer's Disease Collaborative Group, 1995; Sherrington *et al.*, 1995). Several mutations that have been reported to cause Alzheimer's disease are located in the related PS2 gene (Li *et al.*, 1995; Rogaev *et al.*, 1995). Mutations in the PS1 and PS2 genes have been linked to strong deposits of Aβ42 in human brain and periphery (Lemere *et al.*, 1996; Scheuner *et al.*, 1996). Transgenic mice expressing mutated PS1 show increases in the Aβ42 : Aβ40 ratio, suggesting increased concentrations of Aβ42 (Citron *et al.*, 1997; Duff *et al.*, 1996), but they do not develop plaques. However, the amyloid deposition is enhanced in mice coexpressing PS1 and mutated APP together with dystrophic neuritis and astrocytosis (Borchelt *et al.*, 1997). Despite this strong amyloid deposition, even PS1/APP double-transgenic mice fail to show significant neurodegeneration in hippocampus or cortical areas (Takeuchi *et al.*, 2000).

Colocalization of amyloid plaques with acetylcholinesterase and dystrophic cholinergic neurites has been reported in APP transgenics (Stürchler-Pierrat *et al.*, 1997), and a reduction in size of cortical and hippocampal cholinergic boutons was observed in double-transgenic mice overexpressing APP and PS1 (Wong *et al.*, 1999). More recently, association of APP overexpression with cholinergic deafferentiation and cholinergic cell shrinkage has been shown in the 'London' mutation (Bronfman *et al.*, 2000). This indicates that these transgenic models show not only the amyloidosis associated with Alzheimer's disease but also the cholinergic dysfunction. Together with findings that APP expression is upregulated following basal forebrain lesions (Leanza, 1998; Lin *et al.*, 1998), one could speculate about a self-perpetuating circle in which amyloidosis leads to cholinergic damage, which enhances amyloidosis further.

Although a number of different Aβ amyloidosis and PS1 transgenics have been developed, cognitive testing has centred around a handful of paradigms, most frequently the water-maze place-navigation task. The limited number of behavioural tests is one of the major drawbacks of the transgenic approach compared with the rat experiments. In general, the deficits seen in these mutants have been reported to be age related (Arendash *et al.*, 2001; Berger-Sweeney *et al.*, 1999; Chapman *et al.*, 1999; Moran *et al.*, 1995; Morgan *et al.*, 2000), thus having the advantage over previously discussed models that cognitive alterations also develop gradually. Some studies have linked the memory deficits seen in these mice with the accumulation of amyloid in the brain (Arendash *et al.*, 2001; Dodard *et al.*, 2000; Gordon *et al.*, 2001; Hsiao *et al.*, 1996), while others have described performance deficits in memory tasks without the occurrence of amyloid deposition (Dodard *et al.*, 1999; D'Hooge *et al.*, 1996; Holcomb *et al.*, 1999; Kumar-Singh *et al.*, 2000; Moechars *et al.*, 1999; Yamaguchi *et al.*, 1991). A

few studies have also used more extensive test batteries and were able to dissociate the deficit seen in these mutants further. For example, Arendash *et al.* (2001) reported impaired circular or radial water-maze acquisition, but spared water-maze retention, circular platform performance, and Y-maze alternation in transgenic animals overexpressing APP and PS1. However, impairments in sensorimotor performance (Arendash *et al.*, 2001; Tremml *et al.*, 1998), increased stress responsivity and hypoglycaemia (Pedersen *et al.*, 1999), altered aggression, neophobia (Moechars *et al.*, 1998), and reduced exploratory activity (Tremml *et al.*, 1998) have also been reported in some APP or PS1/APP double-transgenic lines. This indicates the need to clearly dissociate mnemonic and non-mnemonic alterations in APP transgenics, a goal that can be difficult to achieve using some of the standard murine paradigms for testing cognitive function, as will be discussed below.

Moreover, APP transgenic mice do not develop tau filaments, another hallmark of Alzheimer's disease (Games *et al.*, 1995). Although no mutation has been identified in the tau gene in Alzheimer's disease, mutations of this gene in frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) have shown that dysfunction of tau can also lead to dementia and neurodegeneration (Hutton *et al.*, 1998). Transgenic mice expressing different human tau isoforms or tau mutations have been developed (Brion *et al.*, 1999; Götz *et al.*, 1995; Ishihara *et al.*, 1999; Lewis *et al.*, 2000; Probst *et al.*, 2000; Spittaels *et al.*, 1999). Mice carrying tau mutations develop neurofibrillary tangles (Lewis *et al.*, 2000; Lewis *et al.*, 2001) but instead lack other features of Alzheimer's disease, such as amyloidosis. Therefore, Aβ amyloidosis/tau double-transgenics represent an interesting extension of this approach. More recently, such mice were generated and indeed show both features of Alzheimer's disease (Lewis *et al.*, 2001). However, overexpression of tau results in motor deficits, peripheral neuropathy and muscle atrophy in transgenic mice (Ishihara *et al.*, 1999; Lewis *et al.*, 2000; Probst *et al.*, 2000; Spittaels *et al.*, 1999), which renders cognitive testing difficult.

An important genetic risk factor for Alzheimer's disease is the ε4 allele of apolipoprotein E (ApoE) (Corder *et al.*, 1993; Strittmatter *et al.*, 1993). Evidence from ApoE-expressing transgenic and ApoE-lacking knockout mice suggests that ApoE directly influences Aβ deposition in the brain and facilitates the formation of neuritic and cerebrovascular plaques (Bales *et al.*, 1997; Holtzman *et al.*, 1999; Holtzman *et al.*, 2000). Hartman *et al.* (2001) reported that transgenic mice expressing the ApoE4 isoform display deficits in a working-memory radial-arm maze task, while no deficits were seen in water-maze place navigation. Moreover, these mice were described as being 'emotionally more reactive'. Some ApoE4 transgenics have also been reported to develop motor problems, which can be accompanied by muscle wasting, loss of body weight, and premature death (Huber *et al.*, 2000; Tesseur *et al.*, 2000). This again suggests that potential non-specific confounding factors must be controlled strictly when cognitive function is assessed in these mutants.

Despite these shortcomings, mouse mutants with amyloidosis, tau or ApoE4 overexpression must be considered the animal models of dementia with the highest face and construct validities. At present, not enough data have been generated to draw conclusions about the predictive validity of these models, but recent reports are encouraging, showing that immunization with Aβ not only causes a marked reduction in brain amyloid deposits, but also reduces learning deficits in APP transgenic mice (Janus *et al.*, 2000; Morgan *et al.*, 2000). However, it appears feasible to improve the validity of this model even more by using behavioural paradigms that allow a more rigorous control of the different factors that govern performance in cognitive tasks, and that more readily tax those cognitive processes that are affected in dementia (Steckler, 1999; see discussion below).

A number of general issues remain to be addressed when it comes to experimentally induced genetic models, such as the genetic background and flanking genes (Gerlai, 1996; Gerlai, 2001). For example, some mouse strains are characterized by poor visual abilities, poor cognitive function or hypoactivity. Using such strains as background strains to generate a mutation that is expected to cause cognitive impairment asks for problems, as the features of the background strain can interfere with performance on the cognitive task. This has certainly been neglected in some studies in the past. Some of the behavioural alterations seen in conventionally generated mutant mice may be due to peripheral rather than central effects of the transgene. Using brain-specific promoters to generate conditional mutants will reduce this problem. Finally, compensatory processes may take place that could also interact with the effects of a genetic manipulation, although this may be less of a problem for the Alzheimer's disease models discussed above, as here many features develop gradually with age.

Models of Psychosis and Depression Associated with Cognitive Dysfunction

As discussed already, there is evidence for good predictive validity in the latent inhibition and PPI models of schizophrenia using unmanipulated animals, but this is a quality of the dependent, not independent, variables. The next question to address is whether models based on experimental manipulations are any better. Acute drug manipulations have been used to model the latent inhibition deficits seen in schizophrenia (see Chapter XVII-1 of this book for more extensive discussion). Latent inhibition, for example, is impaired by amphetamine; this impairment can be attenuated by antipsychotic drugs (Moran *et al.*, 1996; Moser *et al.*, 1996; Moser *et al.*, 2000; Warburton *et al.*, 1994; Weiner *et al.*, 1994; Weiner *et al.*, 1995; Weiner *et al.*, 1996). Apomorphine- or PCP-induced sensorimotor gating deficits in the prepulse inhibition model of schizophrenia have also been attenuated by antipsychotics (Geyer, 1998; Martinez *et al.*, 2000; Swerdlow *et al.*, 1996). All these models target neurotransmitter systems that might play important roles in schizophrenia (dopamine and glutamate). Hence, the amphetamine, apomorphine and PCP models of latent inhibition impairment are clearly of higher face validity than the latent inhibition models employing normal animals, although predictive validity may be comparable.

The neonatal hippocampus-lesioned rat represents a lesion model of schizophrenia that has gained increasing interest over recent years (Lipska *et al.*, 1993). Neonatal hippocampal lesions not only lead to PPI impairment (Le Pen *et al.*, 2000; Lipska *et al.*, 1995), but also impair avoidance performance and spatial learning and memory when animals are tested as adults (Chambers *et al.*, 1996; Le Pen *et al.*, 2000).

The Flinders sensitive (FSL) rat has been proposed as a model of depression on the basis of its reduced locomotor activity, passive coping strategies in forced swimming, reduced body weight, increased REM sleep, and passive avoidance deficits (Overstreet, 1986; Shiromani *et al.*, 1988; see Chapter XVIII-1 in this book for an extensive discussion of animal models of depression). FSL rats have been generated by genetic selection. Two rat lines were bred to be either sensitive or resistant to pharmacological manipulation of cholinergic activity (Overstreet *et al.*, 1986), with the FSL rats being hypersensitive to cholinergic agonists. However, changes in hypothalamic-pituitary-adrenal (HPA) axis regulation seen in the FSL animals differ markedly from the alterations seen in human depression (Holsboer, 1999), which questions the face validity of this model. Interestingly, no accuracy differences were seen between FSL and their control line, the Flinders resistant (FRL) rats, in an operant delayed matching-to-position/visual discrimination task or in a radial-arm maze paradigm, although

FSL rats responded more slowly than the other rats during acquisition of both tasks. Abnormal performance has been reported in comparable human paradigms in depressed patients, in particular with increasing age (Abas *et al.*, 1990; Deldin *et al.*, 2001; Freedman, 1994; Purcell *et al.*, 1997; Sweeney *et al.*, 2000). Cognitive performance of FSL rats also failed to show altered sensitivity to cholinergic blockade (Bushnell *et al.*, 1995), which is surprising given that these animals were bred explicitly for different sensitivity to cholinergic manipulation and that cholinergic blockade has clear effects on mnemonic function. These limited data sets suggest that the FSL rat is not a good model for the cognitive changes seen in depressed patients.

Furthermore, some transgenic mice have been suggested to resemble changes seen in depression. Functional abnormality of the HPA axis is one of the most prominent features of depression, including impaired mineralocorticoid receptor function, restrained glucocorticoid receptor feedback, raised plasma cortisol level, and increased corticotropin-releasing hormone (CRH) activity. Normalization of the neuroendocrine abnormalities seems to be a prerequisite for stable remission in depressed patients (Steckler *et al.*, 1999a). One possibility to model these changes is the use of mouse mutants with altered HPA axis activity, which show a range of neuroendocrine and behavioural alterations comparable to those seen in depression.

Mice overproducing CRH have been reported to be impaired in forced choice alternation and water-maze place-navigation learning. The place-navigation deficit was attenuated by the benzodiazepine chlordiazepoxide (CDP). However, since CDP has both amnesic and anxiolytic actions, it can be suggested that the place-navigation deficit is possibly confounded by heightened anxiety or overarousal (Heinrichs *et al.*, 1996).

Mutants expressing a glucocorticoid receptor antisense construct show an abnormal response in the dexamethasone suppression test (Barden *et al.*, 1997) and altered stress responses that can be normalized with antidepressant treatment (Montkowski *et al.*, 1995; Steckler *et al.*, 1999a). Spatial allocentric, but not egocentric, learning is impaired in these mice (Rousse *et al.*, 1997; Steckler *et al.*, 1999b; 2001). Moreover, glucocorticoid receptor antisense transgenics exhibited altered impulsive behaviour besides an accuracy deficit in an operant two-choice visual discrimination task (Steckler *et al.*, 2000). However, these animals also display increased dopaminergic system activity compared with controls (Cyr *et al.*, 2001; Sillaber *et al.*, 1998), which might be a major contributor to the performance deficits seen in these mice.

Clearly, many other mutants exist that have been proposed as animal models of human psychiatric and neurological disorders and that show cognitive alterations (Campbell and Gold, 1996; Gold, 1996; Picciotto and Wickman, 1998; Steckler, 2001b). A discussion of all these models is beyond the scope of this chapter.

Another approach that needs to be addressed and that differs fundamentally from the models discussed so far is the use of early developmental manipulations. Here, the animals are manipulated early in life, leading to a number of long-lasting molecular changes that persist into adulthood (Meaney *et al.*, 1995; Steckler, 2001b). These early-life stress models have been used to model both depression and psychosis (the importance of the models for schizophrenia or depression has been suggested to depend at least in part on the exact timing of stress exposure within the early development of the animal). Prenatal or post-natal stress (e.g. maternal deprivation) or social isolation shortly after weaning have been shown to lead to a range of cognitive deficits (Cilia *et al.*, 2001; Ellenbroek and Cools, 2000; Geyer *et al.*, 1993; Jones *et al.*, 1991; Lapiz *et al.*, 2001; Lehmann *et al.*, 1999; Matthews *et al.*, 1996; Oitzl *et al.*, 2000; Rao *et al.*, 1999; Stevens *et al.*, 1997).

These models deserve acknowledgement as they show important cognitive abnormalities that may be used to model the cognitive impairments seen in depression and psychosis.

Models of Attention Deficit Hyperactivity Disorder

A genetic model of ADHD, originating from selective breeding, is the spontaneously hypertensive rat (SHR) (Sagvolden *et al.*, 1992). ADHD is a disorder characterized by inattention, hyperactivity and heightened impulsivity (Sagvolden and Sergeant, 1998). SHR rats were established in 1963 from the Wistar–Kyoto (WKY) outbred strain, but the original selection process focused on hypertension rather than an animal model for ADHD (Okamoto, 1969). Only later was it discovered that SHR rats display hyperactivity, may show attentional deficits, and may be more sensitive to immediate reinforcement, which can be improved with methylphenidate, a common treatment of ADHD (Sagvolden *et al.*, 1992).

ADHD has been suggested to result from dysfunction in reinforcement mechanisms, which may involve mesolimbic catecholaminergic projections (Sagvolden and Archer, 1989; Sagvolden *et al.*, 1998), and allelic polymorphisms for the genes encoding for the dopamine transporter and the dopamine D_4 receptor have been reported in ADHD (Amara, 1993; Swanson *et al.*, 2000). Indeed, changes in dopaminergic activity have also been reported in the SHR rat. Dopaminergic activity is decreased in the frontal cortex of SHR rats when compared with normotensive WKY control rats (Myers *et al.*, 1981; Russell *et al.*, 1998; Russell *et al.*, 2000), while noradrenergic activity is increased (Russell *et al.*, 2000). However, these changes in dopaminergic activity seem to be unrelated to dopamine transporter function (Russell *et al.*, 1998). Moreover, in *in vitro* slice preparations, methylphenidate, which is a standard treatment in children with ADHD, is less potent in inducing dopamine release in prefrontal cortex, accumbens and caudate-putamen from SHR rats compared with slices from WKY rats, while amphetamine is more potent (Russell *et al.*, 1998), i.e. different rank orders in potency were seen with the two drugs in SHR and WKY rats. Since both methylphenidate and amphetamine are used to treat ADHD (Solanto, 1998), both drugs should affect dopaminergic activity in similar directions and to a comparable degree in the SHR model. In addition, amphetamine induces hyperactivity in SHR rats, which is even more pronounced than in WKY rats (McCarthy *et al.*, 1980; Van den Buuse and De Jong, 1989), although amphetamine decreases activity in children with ADHD. These findings led Russell *et al.* (1998) to conclude that the SHR rat 'may not be a perfect model for ADHD'. Adding to this view, Bull *et al.* (2000) also failed to see impaired performance in SHR rats responding on a task of impulsivity, the differential low rate (DRL) 60s schedule. The failure of SHR rats to be more impaired on this task than WKY rats again suggests that the SHR rat is limited as a model for ADHD. Moreover, part of the cognitive deficits seen in SHR rats can be attenuated by antihypertensive treatment (Wyss *et al.*, 1992), indicating that secondary changes induced by increased blood pressure contribute to the effects seen in this model — physiological changes that are not described in ADHD.

Other genetic ADHD models with naturally occurring features are the WKHA rat, an inbred strain developed in the 1970s from the SHR rat, which is normotensive and differs in some neurochemical and behavioural features. Importantly, there is evidence for increased, not reduced, mesocortical dopaminergic activity at the frontal cortex level, at least in female WKHA rats (Hendley, 2000) and behavioural differences include better habituation of the hyperactive response following repeated exposure to an environment in WKHA than in SHR rats (Hendley, 2000). The opposite response would be predicted from a model of ADHD.

The Naples high excitability rat has also been proposed as an ADHD model (Gonzalez-Lima and Sadile, 2000). This rat originates from selective breeding for high activity on spatial tasks. Furthermore, the acallosal inbred mouse strain I/LnJ, which shows a complete penetrance of corpus callosum agenesis, has been considered to be a model for ADHD (Magara *et al.*, 2000). In particular,

Table XV-1.1 Validity of some animal models of cognitive dysfunction focusing on the independent variable

Disease/process	Model	Validity			Comment
		Face	Construct	Predictive	
	Unmanipulated adult rodent	—	—	+	Screen for cognitive side effects
Ageing	Old rodent	++	++	(+)	
	Old monkey	++	++	(+)	
	SAMP mouse	++	++	(+)	
Dementia	Old rodent	(+)	(+)	(+)	
	Old monkey	+	+	(+)	
	SAMP mouse	+	(+)	(+)	
	Scopolamine, systemic	+	+	(+)	
	Scopolamine, local	++	+	(+)	e.g. intrahippocampal
	HC-3	++	++	(+)	
	BF lesions, excitotoxic	++	++	(+)	
	BF lesions, immunotoxic	+++	++	?	
	Aluminium	(+)	—	?	
	Aβ(40/42) infusion	+++	+++	?	
	APP transgenics	++++	++++	?	Very limited evidence suggests good predictive validity
	PS1 transgenics	+++	++++	?	
	Tau transgenics	+++	+++	?	
	ApoE4 transgenics	+++	+++	?	
	APP/PS1 double-transgenics	++++	++++	?	
	APP/tau double-transgenics	+++++	+++++	?	
ADHD	Poor-performing adult rodent	—	—	?	
	SHR rat	(+)	(+)	(+)	
	WKHA rat	(+)	(+)	?	
	Naples rat	?	?	?	
	I/LnJ mouse	?	?	?	
Psychosis	Unmanipulated adult rodent	—	—	+	In latent inhibition and PPI models
	Amphetamine, acute	+	+	+	In latent inhibition and PPI models
	Apomorphine, acute	+	+	+	In latent inhibition and PPI models
	PCP, acute	+	+	+	In latent inhibition and PPI models
	PCP, chronic	+++	++	+	
	Hippocampal lesion, neonatal	++	+	(+)	
	Prenatal stress	++	++	?	
	Maternal separation	++	++	?	
Depression	FSL	(+)	(+)	?	
	Prenatal stress	++	++	?	
	Maternal separation	++	++	?	

—, (+), +, ++, +++, grades of validity of the model with respect to disease. Note that the predictive validity focuses on cognitive performance only; this will be influenced at least in part by the behavioural paradigm used. ?, no, or no sufficient, data. BF, basal forebrain; PS, presenilin.

acallosal mice have been suggested to model the defective interhemispheric cross-talk that may play a role in ADHD. They show limited evidence for impaired learning, impulsivity and hyperactivity (Magara *et al.*, 2000). However, data are too limited at the moment, and WKHA rats, Naples rats and I/LnJ mice warrant further investigations to prove their utility as animal models of ADHD.

Overview of Animal Models of Cognitive Disorders

Table XV-1.1 provides a summary of the models discussed so far. It is evident that these models differ primarily in their face and construct validity. Predictive validity in general must be considered relatively poor for various reasons. Some models have been tested with a limited range of standard drugs only, where the desired effect was seen but it is too early to conclude from the effects of newer compounds with activity in these models whether these drugs are also active in the clinic. Some models show a high number of false positive results (see the scopolamine model of dementia), while others (e.g. Aβ infusions) have not been tested sufficiently. It is also obvious that there are a number of models at hand with high face and construct validity.

MEASURING IN THE MODEL: A DISCUSSION OF THE DEPENDENT VARIABLES

Whatever the principle of a model, it must be realized that an animal model of human cognitive function can only be as good as it is possible to assess cognition in the animal or to predict cognitive effects in humans from the animal model. Therefore, it is of utmost importance to define a given psychological process and to break down these criteria into components testable in animals (van der Staay and Steckler, 2002).

Animal Cognition is not a Unitary Process

First, it must be acknowledged that different types of cognitive function can be distinguished not only in humans but also in animals. These cognitive processes may entail all processes involved in animal learning, such as discrimination, attention, executive functions, association, encoding, storage, rehearsal, retrieval and response selection. Although intelligence and logic can be subsumed under the heading cognition, this is not part of the definition as used here, as these processes are very difficult to measure in

animals (Steckler, 2001a). Thus, we are faced with processes that may in part run sequentially (e.g. encoding, storage/consolidation, retrieval), in parallel, or possibly in reverberating loops. There is a qualitative component, and different forms of, for example, attention and memory can be distinguished, some of them involving very different brain circuits. There is also a quantitative component, and tasks of different complexity will demand different degrees of processing capacity, and hence will be differentially sensitive to manipulation. Furthermore, these processes are dynamic and will change over time as a result of experience, and more extensive training may result into a shift from more effortful processing to more automatic processing of information (Hasher and Zacks, 1979; Sarter, 1990), both of which will again show different sensitivity to manipulation.

These processes are influenced by non-specific factors, such as stimulus processing, reward-related properties and/or motor output, and it is important for data interpretation to be able to distinguish clearly these non-specific factors from the specific cognitive processes under investigation (Steckler, 1999; Steckler, 2001a).

Measurement of Disease-Relevant Types of Behaviour

Mnemonic Abilities

One frequently employed distinction of mnemonic processes is that between declarative (effortful) and procedural (automatic) processes. In humans, declarative memory includes higher-order processes, such as memory for facts (semantic memory) and memory for events (episodic memory), while procedural memory includes habituation, sensitization and simple classical conditioning. As already pointed out, patients suffering from Alzheimer's disease do not have a non-specific memory decline, but exhibit pronounced declarative memory impairments, while procedural memory is preserved (Brandt and Rich, 1995). Consequently, behavioural paradigms used to test cognitive function in animal models of dementia should also tax declarative-like, not procedural-like, memory in the animal. Concurrent discrimination, for example, is impaired in temporal lobe and diencephalic amnesia (Zola-Morgan and Squire, 1989), and comparable processes can be studied in rats, in which animals are required to learn a number of discriminations simultaneously (Aggleton *et al.*, 1991; Bussey *et al.*, 1994; Rothblat *et al.*, 1993; Wible *et al.*, 1992).

Another frequently employed type of paradigm in humans as well as in rats comprises delayed (non-)matching paradigms, which assess recognition or recency memory (Steckler *et al.*, 1998). In general, subjects have to remember a sample over a certain retention interval, followed by a choice between two or more choice stimuli, one being identical with the previous sample, the other being novel or less recent. A correct matching response constitutes a response to the choice stimulus that matches the sample; a correct non-matching response would be a response to the other stimulus. Two major variants of these tasks can be distinguished: delayed response and delayed comparison, taxing different types of memory and being differentially susceptible to mediating strategies (Pontecorvo *et al.*, 1996; Steckler *et al.*, 1998). In delayed-response tasks, all the information the animal needs to perform correctly is available before the onset of the retention interval. In delayed-comparison paradigms, the information of where to respond is available to the animal only after the delay is over and the comparison stimuli are presented. Therefore, the risk of mediating strategies is higher in delayed-response tasks. Rats have been shown frequently to master both delayed-response and delayed-comparison procedures, and more recently, comparable maze-based and operant procedures have been described for mice (Beracochea and Jaffard, 1999; Cho and Jaffard, 1994; Estape and Steckler, 2001; Steckler *et al.*, 1999b; Ward *et al.*, 2001), which could be of great potential for testing transgenic mouse models of Alzheimer's disease. Moreover, these methods are ideally suited for further analysis using methods such as the mathematical methods of signal detection theory, which allow a distinction between accuracy and bias (Marston, 1996; Pontecorvo *et al.*, 1996; Sahgal, 1987; Steckler, 2001a). These tasks also allow us to study retention of information over time, i.e. a forgetting curve. The slope of this forgetting curve provides information about the mnemonic ability of the subject. Moreover, the tight control of stimulus and response contingencies enables fine-tuning of the procedural parameters, which can help to dissociate further the cognitive processes that may be affected in the model (Steckler *et al.*, 1998; Van Hest and Steckler, 1996).

The Morris water maze is also frequently employed to study the spatial orientation and memory deficit seen in Alzheimer's disease. In particular, for the assessment of mouse mutants, this task has become the gold standard (see the discussion on transgenic models of Alzheimer's disease, above). In this task, a rodent has to swim in a water tank and find an escape platform submerged under the surface of the water. The platform always remains in a stable spatial position relative to extramaze cues surrounding the maze (place navigation). To control for non-specific effects, the most frequently employed control situation is a so-called cue-navigation task, in which the animal can directly see the platform, and which therefore should not require spatial navigation. However, it has been pointed out that not only mnemonic, but also motor, motivational, perceptual and other cognitive factors, such as altered arousal or attention, can influence water-maze performance, and that a deficit in the place-navigation task in combination with intact cue navigation does not allow us to draw conclusions about spatial memory (Steckler, 1999). For example, the latency to find the hidden platform can be confounded easily by motor deficits (Huerta *et al.*, 1996). A motor deficit may not necessarily become apparent in the cued-navigation task, as animals can find the visual platform more easily than the hidden one, therefore they swim less and may be less exhausted. Place navigation involves not only a spatial but also a non-spatial learning component (knowing that there is a hidden platform). Moreover, often superimposed on spatial navigation are search strategies that contribute to performance (Hodges, 1996). Therefore, the so-called probe trial, where the platform is removed and the animal explores the maze freely in search for the platform, is considered by some authors to be more sensitive. A preference for the previous platform location is taken as indication for spatial learning. However, an impairment during the probe trial does not exclude the possibility that this impairment is due to non-specific factors that interfered with performance during the acquisition stage. For example, it has been shown that lesions of the dorsal noradrenergic bundle alter maze performance if rats are tested in cold water, but leave performance unaffected if tested in warm water (Selden *et al.*, 1990), suggesting that anxiogenic and stressful features of the test can influence maze performance. Clearly, animals that are impaired during acquisition due to altered stress responses are likely to be impaired during the probe trial as well. All these factors must be considered when using the water maze (or any other learning and memory paradigm) if the dependent variable of the model should remain valid. It can be concluded that the water maze is a useful paradigm for testing spatial learning and memory in rodents, especially mice, but unfortunately the potential confounding factors often seem to be neglected.

An alternative water-maze paradigm that has been used in both rats and mice is a two-choice spatial-discrimination task, in which animals have to make a choice between two visible platforms. Neither platform can be distinguished on the basis of visual features, but must be discriminated on the basis of spatial cues. One platform is stable, while the other sinks if the animal tries to climb on to it (Arns *et al.*, 1999; Morris *et al.*, 1986; Steckler *et al.*, 1999b). This task allows a dissociation between errors of commission (choice of sinking platform) and errors of omission (no choice of platform at all). This distinction gains relevance from the fact that maze

navigation may be impaired by non-specific factors. Introduction of a choice reduces the influence of these confounds because a probability serves as the critical variable (Arns *et al.*, 1999).

The need to pay attention to investigating mnemonic processes analogous to the cognitive symptoms observed in dementia is often forgotten too easily, which can be illustrated with the example of the passive avoidance paradigm. In passive avoidance, the attempt of an animal to move from one place to another in a test chamber is punished by foot shock. Upon re-exposure to the test chamber without shock, the animal will refrain from executing that movement, given that an association between the place or the movement and the shock had been formed. The time interval from beginning of re-exposure to reinstatement of the response is generally taken as an indicator of memory retention. Many non-specific factors will confound performance on this task, such as pain threshold, motor function and emotionality. Moreover, the type of memory involved in performing such a task may be fundamentally different from the memory impaired in dementia (Steckler and Muir, 1996). Despite these shortcomings, passive avoidance has been, and is being, used rather uncritically as one of the most popular tasks to study rodent animal models of dementia, as validity aspects were widely neglected (Sarter *et al.*, 1992b).

Clearly, when it comes to face and construct validity, paradigms such as delayed (non-)matching paradigms or concurrent discrimination paradigms are more advanced. However, whether they show superior predictive validity remains to be proven, given that all we have to treat Alzheimer's disease patients are cholinomimetics such as galantamine, donepezil, rivastigmine, tacrine and idebenone.

Attentional Function

Another feature of Alzheimer's disease is an attentional and executive dysfunction, in particular in divided attention but also in selective attention (Coslett and Saffran, 1996; Morris, 1996). Different types of attention, such as vigilance/sustained attention, divided attention and selective attention, can also be modelled in the rat (Bushnell, 1998; Muir, 1996; Robbins, 1998; Sarter and Bushnell, 1995; Steckler and Muir, 1996). Tasks bearing good face validity with human tasks of divided attention have been described for rats (McGaughy *et al.*, 1994). In the paradigm used by McGaughy *et al.*, animals were tested in a conditional discrimination paradigm, where they had to discriminate between constant/flashing lights or constant/pulsing tones, the to-be-attended modality being unpredictable for a given trial. Animals were required to respond to one of two levers if a constant light or a pulsing tone was presented, and to respond to the other lever if a flashing light or a constant tone occurred.

Related to the ability to divide attention to stimuli from different modalities is the ability to shift attention either within dimensions (intradimensional shift) or between dimensions (extradimensional shift). Initial studies reported intra- and extradimensional set shifting in rats (Schwartz *et al.*, 1971; Shepp and Eimas, 1964), but only recently was a dissociation between rule-shifting and true shifting of an attentional set in rats achieved (Birrell and Brown, 2000). Here, rats were trained to dig for reward in bowls, which had to be selected on the basis of odour, filling medium, or the texture that covered its surface. Rats had to perform a series of discriminations, including reversals, intradimensional shifts and extradimensional shifts. For intradimensional set shifting, they had to learn a new discrimination within a sensory dimension (e.g. olfaction); for extradimensional set shifting, they were also required to shift between sensory dimensions (e.g. from odour to filling medium). This task bears resemblance to the Wisconsin Card Sorting Test, and could also be useful for the assessment of cognitive functions in animal models of both dementia and schizophrenia.

Selective attention paradigms have also been described for rats (Carli *et al.*, 1983; Cole and Robbins, 1987; Muir *et al.*, 1992) using paradigms that resemble those used in humans (Wilkinson, 1963). In these paradigms, at least two stimuli are employed, with one stimulus serving as a distractor that should be ignored (e.g. a burst of white noise), while the animal has to attend to the relevant stimulus signalling reinforcement. Selective attention has been shown to be impaired following manipulation of the noradrenergic system (Carli *et al.*, 1983; Cole and Robbins, 1987).

Clearly, tasks such as latent inhibition can also be subsumed under this category, as a deficit to attend selectively to the relevant stimulus during the initial stage of the task will interfere with learning during the final stages of the task. However, it can be difficult to dissociate an attentional impairment from other factors, e.g. impaired learning, in this paradigm.

Moreover, tasks measuring sustained attention have been developed for rats and, more recently, mice (Carli *et al.*, 1983; Humby *et al.*, 1999; Marston *et al.*, 2001; McGaughy and Sarter, 1995; McGaughy *et al.*, 1996; Muir *et al.*, 1992; Robbins *et al.*, 1989; Sahgal and Steckler, 1994), some of which also bear strong resemblance to human paradigms of sustained attention (Sahakian *et al.*, 1993). In the five-choice serial reaction time task, rats or mice have to monitor an arc of five holes, one of which is illuminated by a short-lasting light stimulus of less than one second; the animal has to nose poke into that hole in order to get reward (Humby *et al.*, 1999; Marston *et al.*, 2001; Muir *et al.*, 1992; Robbins *et al.*, 1989). This task has been shown, for example, to be highly sensitive to cholinergic drugs and lesions, and both impairment and enhancement of performance have been demonstrated (Grottick and Higgins, 2000; Humby *et al.*, 1999; Mirza and Bright, 2001; Mirza and Stolerman, 1998; Muir *et al.*, 1992; Robbins *et al.*, 1989).

Other Cognitive Functions

Besides mnemonic and attentional functions, there is a range of other cognitive types of behaviour that have been described to undergo changes in certain forms of dementia, such as impulsivity (Lindau *et al.*, 1998) or timing behaviour (Cummings, 1986); these types of behaviour can also be modelled in the animal (Bizot and Thiebot, 1996; Bull *et al.*, 2000; Evenden, 1999a; Evenden, 1999b; Harrison *et al.*, 1997; Meck, 1996; Meck and Church, 1987; Paule *et al.*, 1999; Puumala *et al.*, 1996). In fact, assessing these types of behaviour may be even more relevant in animal models of other psychiatric disorders, such as ADHD, where increased impulsivity is a key symptom (American Psychiatric Association, 1994; Sagvolden and Sergeant, 1998).

Testing Cognitive Function

The final point to address is how to best test cognitive function in an animal. It has been argued that most information may be gained if animals are tested in species-typical societies in natural environments (Gerlai and Clayton, 1999; Würbel, 2002). Although this idea has its merits, the argument is flawed as it remains unclear what constitutes a naturalistic environment for a laboratory animal, e.g. for a transgenic mouse with altered genetic information. Therefore, a natural environment must be considered to be as artificial as every other environmental setting. Moreover, it has been argued that such 'natural' environments are defined less precisely and hence are less amenable to standardization and comparison (Van der Staay and Steckler, 2002). To have a valid animal model (of cognitive disorders), however, it is of utmost relevance to have tasks at hand that, irrespective of the degree of ecological relevance, allow a clear-cut dissociation between the behaviour of interest (e.g. cognitive function) and non-specific factors. It is a basic requirement to be able to differentiate between different types and stages of cognitive function (Steckler, 1999). Only then can we address the relevant questions, using models that tax disease-relevant types of cognitive function and that are comparable

between humans and animals. These are essential prerequisites to improving face and construct and, possibly, the predictive validity of animal models of cognitive disorders.

ANIMAL MODELS OF COGNITIVE DISORDERS: SUMMARY

Over recent years, there has been tremendous development in the field of animal models of cognitive dysfunction, which is probably most advanced in the field of Alzheimer's disease. Models in this area have developed from rather unspecific approaches, such as old animals, progressing to more theory-driven models with improved, though not sufficient, face and construct validity, such as basal forebrain-lesioned animals, and advancing to transgenic models.

Clearly, recent developments, such as the generation of double-transgenic mice, indicate that we have not reached the end of this process, and further improvements are possible. At the same time, there are a number of good behavioural paradigms available to model the cognitive impairment seen in various human disorders. Those are based primarily on work with rats, but murine equivalents are emerging. Importantly, it must be realized that any animal simulation model of cognitive dysfunction is only as good as the cognitive processes measured in the animal. If this does not reflect human cognitive processes accurately, then the value of the data is negligible. This situation changes if one considers screening or mechanistic models, but the ultimate proof will be in models of the simulation type. It is this combination between models with high validity at the level of the independent variable and high validity at the behavioural level that can be expected to advance this field most dramatically and to improve predictive validity and the development of novel treatment strategies for neuropsychiatric disorders associated with cognitive deficits.

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XV-2

Aminergic Transmitter Systems in Cognitive Disorders

John P. Bruno and Martin Sarter

INTRODUCTION

The major categories of cognitive disorders defined in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) include various types of dementias, deliriums, and amnestic disorders (American Psychiatric Association, 2000). The goal of this chapter is to present a thorough, but certainly not exhaustive, summary of the evidence for dysregulations in aminergic neurotransmitter systems in several representative cognitive disorders. Aminergic transmitter systems include the biogenic amine acetylcholine (ACh), the catecholamines dopamine, noradrenaline and adrenaline, and the indoleamine serotonin (5-hydroxytryptamine; 5-HT). For a more detailed discussion of the neuropharmacology and chemoanatomy of aminergic transmitter systems, the reader is referred to Chapter IV of this book.

Not surprisingly, there is considerable variation in the extent of the literature on the neurochemical dysregulations accompanying delirium, dementia and amnestic disorders. The scope of our review will be limited to several syndromes for which there is considerable evidence implicating specific aminergic transmitter systems to these cognitive disorders. Thus, the discussion of the dementias will be limited to dementia of the Alzheimer's type (DAT) and AIDS-associated dementia (AAD). The discussion of amnestic disorders will be limited to those that accompany chronic alcohol consumption (Korsakoff's syndrome) or administration of the psychostimulant 3,4-Methylenedioxymethamphetamine (MDMA; ecstasy). Finally, the discussion of delirium will focus on the ability of several drugs or toxins to impair aminergic function. This chapter will focus exclusively on clinical populations. However, valuable corroborating evidence for links between certain cognitive dysfunctions and specific aminergic populations can be found in various animal models of these cognitive disorders. Several of these models are discussed in other chapters within this book.

An important issue that permeates any summary and interpretation of the neurochemical dysfunctions accompanying neuropsychiatric disorders is the considerable variation in the methods used to collect such data, and the relative strength of inferences supported by these various methods. A review of the literature reveals neurochemical data collected from post-mortem tissue, biopsy tissue, analysis of cerebrospinal fluid (CSF), neuroimaging techniques with transmitter-selective markers, and finally a 'reverse deduction' on the basis of drug effects (therapeutic or abused). There are obvious limitations to constructing hypotheses about the neurochemical bases of complex cognitive disorders on the basis of each of these types of data. Several of these methods lack the spatial resolution for highlighting regional contributions within distributed neural systems and, more importantly, rarely capture the dynamics and subtleties of chemotransmission likely to be at the heart of many cognitive disorders. As such, the overinterpretation of negative data (i.e., seemingly intact neurotransmitter systems in neuropsychiatric populations) is particularly problematic. In this regard, the use of animal models with more precise neurochemical methods can be invaluable in formulating and testing hypotheses about the neurochemical bases of cognitive disorders (see Chapter III in this book). Throughout this chapter, we will point out the source of the neurochemical evidence supporting the relationship between dysregulation in the aminergic system and the cognitive disorder, along with any concerns surrounding the interpretation of such evidence.

Of course, simply documenting a specific neurochemical impairment in a patient diagnosed with a cognitive disorder is not sufficient evidence to conclude a causal relationship between the neurochemical deficit and the cognitive construct. Extending these correlations into the realm of causality is a difficult task, particularly when it comes to clinical populations. The primary strategy for probing the causal relationship between two variables is to manipulate levels of one of the variables and look for systematic changes in the other variable. While this can be done readily in experimental animals, and in fact should be a critical component of any animal model of neuropsychiatric condition, this is often difficult to achieve in clinical populations. Certainly one can examine, either between subjects or, ideally, within subjects, whether the cognitive impairments worsen as markers of the neurochemical pathology become more severe (i.e., with time since onset in a neurodegenerative condition). A second strategy, and one that is often used in studies of the biological bases of disease, is a type of *post hoc* logic in which the causal relationship between neurochemical pathology and symptom is inferred on the basis of the effectiveness of pharmacotherapeutics. This, however, can be a risky strategy and may lead to both false positives (i.e., the drug does not interact directly with neurochemical systems that are necessary and sufficient for the disease but rather modulates the activity of these systems) and false negatives (i.e., the drug affects the critical neurochemical systems but does not 'replicate', in sufficient physiological fashion, signal processing within this damaged system). We will identify in this chapter those few situations in which such strong relationships exist between the degree of neurochemical dysfunction and the cognitive symptoms.

DEMENTIAS

The DSM-IV defines dementia as a syndrome of multiple cognitive deficits that include memory impairments and at least one of the following: aphasia, apraxia, agnosia and disturbances in executive functions (American Psychiatric Association, 2000). The memory impairment is a necessary feature of the diagnosis, progressing from an early presentation of difficulty in learning new material to eventually an inability to remember previously learned material (memory). The impairments in executive function are related to disorders of the frontal lobe and involve the ability to think abstractly and to plan, initiate, sequence, monitor and inhibit

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complex behaviour. These cognitive deficits must represent a marked decline from a previous level of functioning, and must also be severe enough to cause significant impairment in social or occupational functioning. There are a number of dementia syndromes, including DAT, frontal lobe dementia (DFL), vascular dementia, dementia of the Lewy Body type (LBD), AAD, dementia associated with Huntington's disease, dementia associated with Pick's disease, dementia due to other general medical conditions, and substance-induced persisting dementia. The two dementias that have received the most attention with respect to possible roles of aminergic transmitter systems are DAT and AAD. Each of these syndromes will be discussed below.

Dementia of the Alzheimer's Type

The search for a neurochemical basis of DAT began in earnest almost 40 years ago with the hope that such an identification would lead to the development of effective pharmacotherapies. This exploration was fuelled by the identification of a dopaminergic component to Parkinson's disease and the ensuing success of L-Dopa-based 'replacement pharmacotherapy'. Over the ensuing decades, a great deal of evidence has revealed aminergic dysfunctions in DAT (summarized below). Unfortunately, the development of efficacious pharmacotherapies for DAT has not met with the same degree of success as seen in the treatment of Parkinson's disease. This probably reflects the fact that a simple replacement strategy designed to elevate tonic levels of deficient neurotransmitters (e.g., ACh), borrowed from our experience with Parkinson's disease, is inconsistent with the normal functions of such systems in information processing (see Sarter and Bruno, 1997, Sarter and Bruno, 1998 and Sarter and Bruno, 1999 for a discussion of this issue).

Most of the attention on the neurochemical bases of dementia has focused on DAT. This is the result of two observations. First, while certainly not homogeneous in nature, DAT reveals the most consistent profile of neurochemical dysregulations of any of the dementias. Second, the cognitive deficits in DAT correlate best with the reduction in various markers of cholinergic transmission (Francis *et al.*, 1985; Lehericy *et al.*, 1993), and the prominent focus on pharmacotherapeutics has occupied considerable attention (Lawrence and Sahakian, 1998; Rogers and Friedhoff, 1996; Sahakian *et al.*, 1989; Sahakian *et al.*, 1993). In addition to deficits in cortical cholinergic transmission, the literature reveals clear and consistent reductions in markers of adrenergic, serotonergic and, to a lesser degree, dopaminergic transmission in DAT. An overview of these findings appears below.

Cholinergic Systems in Alzheimer's Dementia

The earliest and most consistent neurochemical abnormalities to appear in the brains of DAT occur in the cholinergic system. The decline in cholinergic transmission is not uniform throughout the brain. There are significant reductions in the basal forebrain cholinergic system, where there is estimated to be a 30–90% loss of neurons with no loss in other cholinergic-rich areas, particularly the brainstem (Mesulam, 1996). This loss of neurons manifests itself in a reduction in the biosynthetic enzyme choline acetyltransferase (ChAT) in temporal lobes and more moderate reductions in frontal lobes. Biopsy studies suggest that the decline in cortical ChAT activity can occur within 1 year of the onset of clinical signs (Bowen *et al.*, 1983). Consistent with the loss of basal forebrain neurons is an accompanying reduction in the density of (presumably presynaptic) nicotinic receptors in the cortex and parahippocampal gyrus, as measured post-mortem (Perry *et al.*, 1995) and *in vivo* with positron emission tomography (PET) (Nordberg, 1996).

A recent study investigated the relationship between declines in nicotinic receptor (nAChR) binding and vesicular ACh transport sites (vesamicol binding), using autoradiographic methods, in medial temporal cortex in DAT (Sihver *et al.*, 1999). In age-matched controls, binding was particularly high in layers I, III and V. Autopsied tissue analysis revealed that binding was reduced in all layers in DAT by 40–55%. While the loss of nAChR binding correlated well with the reduction in ChAT activity, vesamicol binding was reduced by only 25%, suggesting some compensatory activity within residual cholinergic terminals.

The basis for the selective vulnerability of basal forebrain cholinergic neurons, as opposed to other populations of cholinergic neurons, remains unclear. Autoradiographic studies demonstrate the presence of high-affinity ^{125}I-NGF binding sites (presumably corresponding to tyrosine kinase A, TrkA) in the nucleus basalis of meynert (nMB) and striatum but not in the pedunculopontine nucleus. Immunohistochemical studies have revealed TrkA expression on nBM and the cholinergic interneurons of the striatum. This supports the hypothesis that there is a relationship between the dependence upon NGF and the neuronal vulnerability in DAT. In DAT, the number of neurons expressing TrkA was decreased in nBM, likely as a result of cholinergic neuronal loss (Boissiere *et al.*, 1997). While neither NGF levels or mRNA are altered in DAT, there may still be more subtle problems with receptor function/transduction.

As with any other neurodegenerative disease, early detection is an important goal. The ability to develop an *in vivo* imaging protocol for revealing cholinergic denervation is viewed as paramount in this quest (Volkow *et al.*, 2001). ChAT, the most specific marker expressed by cholinergic neurons, does not have an available tracer for imaging studies. Recently, however, a tracer, C-11-labelled *N*-methyl-4-piperidyl-acetate (C-11-MP4A), has been developed for the catabolic enzyme acetylcholinesterase (AChE). The use of this tracer has revealed that AChE activity is reduced in DAT (Herholz *et al.*, 2000; Kuhl *et al.*, 1999; Geuia and Mesulam, 1996), and that this reduction correlates well with reductions in regional cerebral blood flow or glucose metabolism, particularly in temporolateral regions of the cortex (Herholz *et al.*, 2000).

Finally, it should be stated that the functional consequences of a loss of cortical cholinergic innervation need not be limited to direct changes in interneuronal communication. A double immunocytochemical study for ChAT and reduced nicotinic adenine diphosphate (NADPH) (as a marker for nitric oxide) revealed inputs to cortical microcirculation (Vaucher and Hamel, 1995), raising the possibility that cholinergic denervation will also result in inadequate cortical perfusion. In fact, cortical microvascular abnormalities appear to be related intimately to the pathophysiology of the disease. Regional cholinergic denervation and NADPH have been reported in cortical microvessels in DAT. Moreover, the deficits in cortical perfusion parallel the regional differences in cortical cholinergic denervation (i.e., temporal regions are more affected than primary motor and somatosensory regions). These changes raise the interesting possibility that the cholinergic denervation may compromise the ability to adapt enhanced cortical perfusion to neuronal activity associated with tasks related to arousal and attention (Tong and Hamel, 1999).

Noradrenergic/Adrenergic Systems in Alzheimer's Dementia

There are consistent changes in noradrenergic and, to a lesser extent, adrenergic systems in DAT. The progressive loss of noradrenergic neurons in the locus coeruleus has been documented using post-mortem as well as biopsy tissue samples (see Mann, 1988 for a review). In contrast to the loss of cortical cholinergic inputs discussed above, there is little relationship between the extent of loss of noradrenaline in the locus coeruleus and the cognitive deficits seen in DAT (Palmer *et al.*, 1987a; Palmer *et al.*, 1987b; Palmer and DeKosky, 1993). These losses in noradrenaline may, however, contribute to important noncognitive behavioural

impairments that accompany the syndrome, such as depression and wandering (Zubenko and Moossy, 1988). These noncognitive components represent a disturbing set of complications that contribute to patient suffering, care giver stress and health costs, particularly as these complications may expedite the institutionalization of victims of DAT.

The loss of noradrenergic neurons in the locus coeruleus results in reduced noradrenergic innervation of the cortex. Noradrenaline levels and high-affinity [^{3}H]noradrenaline uptake are consistently reduced in the temporal and parietal cortex. Interestingly, these changes in noradrenaline uptake are seen in patients that have displayed clinical symptoms for less than 2 years, suggesting that such dysfunctions occur rather early in the course of the disease (Palmer *et al.*, 1987a).

The mechanisms underlying the loss of noradrenergic neurons in the locus coeruleus in DAT are not well understood. Recently, it has been speculated that this loss reflects the accumulation of an endogenous neurotoxic compound in DAT. In this regard, a recent study compared levels of the neurotoxic monoamine oxidase A (MAO-A) metabolite of noradrenaline, 3,4-dihydroxyphenylglycolalderhyde (DOPEGAL), in post-mortem tissue samples from DAT victims and age-matched controls (Burke *et al.*, 1999). The authors reported a 2.8–3.6-fold increase, relative to that seen in controls, in DOPEGAL levels in the locus coeruleus from DAT tissue.

As mentioned above for cortical ACh, there is some speculation that compensatory increases in noradrenergic transmission might accompany the loss of noradrenergic neurons in the locus coereleus in DAT. There are higher levels of noradrenaline in the CSF in ageing and in DAT (Raskind *et al.*, 1999). A priori, such increases could reflect increased release of noradrenaline and/or decreased metabolism and clearance. This issue was addressed by comparing the ability of the α_2 antagonist yohimbine to increase noradrenaline release levels in the CSF of young adult controls and DAT patients. Administration of yohimbine led to greater levels of noradrenaline in the CSF in DAT patients than in young controls, consistent with the capacity for greater synthesis/release of noradrenaline following the neuronal loss accompanying the dementia (Raskind *et al.*, 1999).

Post-mortem analyses of adrenergic receptors reveal changes in both number and affinity in DAT. Receptor binding of α_1 (postsynaptic), using [^{3}H]prazosin, revealed a 50% reduction in B_{max} and affinity in the hippocampus with no change in the frontal cortex or nBM. The loss of α_1 sites probably reflects a loss of receptors on glutamatergic neurons. In contrast, receptor binding of α_2 (presynaptic), using [^{3}H]yohimbine, revealed a 50% reduction in B_{max} and a 66% reduction in affinity in nBM, with no changes in the hippocampus or frontal cortex (Shimohama *et al.*, 1986). The loss of α_2 sites may represent the loss of noradrenergic inputs from the locus coeruleus to the basal forebrain. Theoretically, declines in this input could contribute to the cognitive deficits seen in DAT as α_2 receptor activity may positively modulate ACh release in the hippocampus and cortex (Tellez *et al.*, 1999).

There is also evidence indicating changes in brainstem adrenergic systems in DAT. The biosynthetic enzyme phenylethanolamine-*N*-methyl transferase (PNMT) exhibits a progressive loss (50–88%) in the locus coeruleus, frontal cortex, hippocampus and amygdala (all areas with significant neuronal degeneration) but not in the cerebellum or motor cortex (areas with little neuronal degeneration). This pattern has led to the hypothesis that the loss of adrenaline-containing neurons is secondary to some initial degeneration of target neurons and the consequent loss of growth factors that might be critical for the induction of synthetic enzymes within the afferent neurons (Burke *et al.*, 1988). Interestingly, the loss of locus coereleus neurons correlated positively ($r = 0.71$) with the loss of PNMT activity. There was an accompanying loss of mitogenic activity, as measured by brain-derived growth factor (BDGF), consistent with the hypothesized loss of growth factors. Moreover, while there was a significant reduction in PNMT staining in afferents adjacent to the degenerating neurons, there was no change in PNMT staining in projections adjacent to intact blood vessels.

Serotonergic Systems and Alzheimer's Dementia

Although somewhat more variable than the effects seen in adrenergic systems, there are several reports indicating widespread serotonergic dysfunctions in DAT. There is also some suggestion that these impairments may occur relatively early in the disease (Bowen *et al.*, 1983; Palmer *et al.*, 1987c). As is the case with decreases in adrenergic transmission, however, there is little evidence that changes in serotonergic transmission contribute significantly to the cognitive deficits seen in DAT. Rather, dysfunctions in serotonergic transmission are likely to contribute to the affective disorders that accompany DAT (Court and Perry, 1991). Post-mortem analyses reveal significant reductions in 5-HT levels in the frontal cortex (Arai *et al.*, 1984; D'Amato *et al.*, 1987; Palmer *et al.*, 1987b), temporal cortex (Palmer *et al.*, 1987c), hippocampus (Cross *et al.*, 1984), hypothalamus (Sparks *et al.*, 1988), nucleus basalis Meynert (Sparks *et al.*, 1992) and basal ganglia (Sparks *et al.*, 1988). Reductions in the levels of 5-hydroxyindoleacetic acid (5-HIAA), the principal metabolite of 5-HT metabolism, and the number of 5-HT reuptake sites in the cortex (Palmer *et al.*, 1987a) are consistent with the interpretation that there is a loss of serotonergic innervation to the cortex. These autopsy studies have been corroborated by ante-mortem data showing reduced concentrations of 5-HT and 5-HIAA, as well as diminished 5-HT uptake (Palmer *et al.*, 1987b). This decline in presynaptic indices of serotonergic transmission is paralleled by a reduction (30–40%) in the number of 5-HT-positive neurons in the median and dorsal raphe nuclei (Chen *et al.*, 2000).

The mechanisms underlying these reductions in markers of serotonergic transmission are unclear. In this regard, several studies have investigated the relationship between the decline in these markers and more traditional neuropathologies seen in DAT. There is a negative correlation between the number of tangles and 5-HT or 5-HIAA content in the cortex from biopsy tissue samples (Palmer *et al.*, 1987a). Neurofibrillary tangles also appear within the dorsal and medial raphe, but the magnitude of the loss of raphe neurons does not correlate with the density of tangles (Chen *et al.*, 2000).

As is the case with the noradrenergic systems described above, strong evidence supporting a relationship between declines in serotonergic transmission and the cognitive impairments seen in DAT is not available. Declines in cortical and basal forebrain 5-HT content could impact on cortical cholinergic function and, hence, certain cognitive functions. There are clear indications in the animal literature for a 5-HT_2 (Zhelyazkova-Savova *et al.*, 1997) and a 5-HT_4 (Consolo *et al.*, 1994) receptor-mediated release of cortical ACh. On these grounds, the impact of drugs that affect serotonergic transmission on cognitive function in DAT merits additional study.

A role for the deficits in 5-HT function in the agitation syndrome seen in DAT has been substantiated. A recent study examined the relationship between levels of 5-HT activity and the degree of agitation in a group of DAT patients (Mintzer *et al.*, 1998). 5-HT activity was assessed using fenfluramine (an indirect 5-HT agonist) -induced serum prolactin. DAT patients with and without accompanying agitation were challenged with an oral dose of fenfluramine (60 mg). Increases in serum prolactin were larger in the agitated than in the non-agitated group. Moreover, within the agitated group, there was a positive correlation between the degree of agitation and the increase in serum prolactin.

Dopaminergic Systems in Alzheimer's Disease

Changes in the dopaminergic systems in Alzheimer's disease differ from those seen in the cholinergic and noradrenergic systems

in two important ways. First, changes in the dopaminergic systems are more variable than those reported for the other two classes of aminergic transmitter systems. Second, to the extent that there are dysfunctions, changes in the dopaminergic systems appear to be more localized to changes in dopaminergic receptors rather than dopaminergic innervation to critical target regions. Post-mortem concentrations of dopamine have been found repeatedly to be unaltered in the cerebral cortex of patients with DAT (Palmer and DeKosky, 1993). This result has been corroborated with ante-mortem diagnostic craniotomies for dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels (Palmer *et al.*, 1987b), although there is a report of reduced levels of HVA and HVA/dopamine ratios from the CSF in DAT (Reinikainen *et al.*, 1990).

Much of the focus on the role of the mesolimbic and mesocortical dopaminergic systems and DAT has been fuelled by the observation that up to 80% of patients with Parkinson's disease manifest some signs of dementia (Brown and Marsden, 1984). Likewise, extrapyramidal motor dysfunctions have been reported in 20–80% of DAT patients (Merello *et al.*, 1994). Caution must be exercised not to overinterpret these data to suggest that the involvement of the striatal dopaminergic system is similar in the two diseases. While one PET study using ^{18}F-fluorodopa revealed no evidence for dysfunction of dopaminergic terminals in DAT patients with parkinsonian features, others have reported markers of degeneration of nigrostriatal systems (see Leverenz and Sumi, 1986). A recent single-photon positron emission tomography (SPET) study compared the specific striatal uptake of the D2 receptor ligand [I-123]*S*-3-iodo-*N*-(1-ethyl-2-pyrolidinyl)methyl-2-hydroxy-6-methoxybenzamide ([^{123}I]-IBZM) in DAT patients without extrapyramidal symptoms with age-matched controls to test the hypothesis that alterations in striatal dopamine may actually be part of a profile in DAT independent of any parkinsonian symptomatology (Pizzolato *et al.*, 1996). The DAT patients exhibited a decrease in D2 receptor ligand binding relative to the controls. The source of this reduced binding cannot be determined from this particular study; reductions could be at the level of cholinergic interneurons, corticostriatal terminals and/or striatopallidal projections.

Joyce and colleagues (1993) have conducted a series of autoradiographic studies on the density and pattern of D2 receptors in post-mortem tissue from subjects with DAT using the highly selective ligand [^{125}I]epidepride. These studies focused on two structures within the medial temporal lobe: the hippocampus (molecular layer of the dentate gyrus) and the amygdala (basolateral area). The binding studies revealed a modular organization of D2 receptors in rostral and mid-levels of temporal cortex (i.e., higher-order association cortex) with high densities in the dentate gyrus, CA3 and subiculum. There is a loss of these modules in tissue from DAT subjects (Joyce *et al.*, 1998). Interestingly, those areas that are affected most by the formation of more traditional neuropathologies (i.e., plaques and tangles) are the regions that appear to be losing the D2 receptor binding. Those regions exhibiting diminished D2 receptor binding also show a reduction in the number of pyramidal neurons staining for D2 mRNA. There was no evidence for a reduced dopaminergic innervation to the hippocampus as measured by changes in the expression of tyrosine hydroxylase.

Dysfunctions in D2 receptor activity in DAT have prompted investigations of a possible relationship between the D2 receptor gene (DRD2), the A1 allele and Alzheimer's disease (Pizzolato *et al.*, 1996; Small *et al.*, 1997). These studies have addressed the hypothesis that the A1 allele, like the APOE4 allele, is a major risk factor in late-onset DAT. The A1 allele is associated with reduced DRD2 binding sites as well as reduced visuospatial function and prolonged P300 latencies, characteristics of DAT. While still an intriguing hypothesis, the initial study found that the A1 allele does not contribute to risk for DAT either alone or in combination with the APOE4 allele. The study also demonstrated that the A1 allele that contains the gene that codes for the DRD2 decreases significantly with ageing in both DAT patients and in controls (Small *et al.*, 1997). Thus, we must be careful to include age-matched controls in future studies exploring relationships between the A1 allele and risk for DAT.

AIDS-Associated Dementia

The pandemic of HIV-1 infection has evolved into a worldwide problem with a preferential affliction of younger adults and children. It has been estimated conservatively that roughly 7–14% of adult HIV-infected people will eventually experience a syndrome of moderate to severe cognitive deficits known as AAD. This dementia presents with attentional impairments, memory deficits and reduced alertness (Sarter and Podell, 2000). In addition, AAD has all the hallmarks of a subcortical dementia, in that there is little evidence for cortical disconnection syndromes (and the attendant apraxias, agnosias, aphasias). It is accompanied, on the other hand, by psychomotor slowing, bradykinesia, and altered posture and gait, mimicking advanced Parkinson's disease (Berger and Arendt, 2000).

The pathophysiology of AAD has remained somewhat elusive. The specification of the underlying neuropathologies, which are undoubtedly complex and multifactorial, is made difficult by the fact that neurons are not infected directly by HIV; rather, it is thought that infected non-neuronal cells secrete diffusable toxic substances (Sarter and Podell, 2000). Two brain regions that appear to be affected preferentially in AAD are the frontal lobes and the basal ganglia (Hall *et al.*, 1996). Radiological data support correlations between tissue loss in the basal ganglia and whether HIV-positive patients are presenting with or without dementia. Children that present with AAD show extensive calcification within the basal ganglia (Berger and Nath, 1997). There is also diminished regional cerebral blood flow (rCBF) in the basal ganglia in patients presenting with AAD (Berger and Arendt, 2000).

The involvement of the dopaminergic system in AAD was suggested early on by the observation that patients were extremely sensitive to the extrapyramidal side effects of dopamine receptor blockers (Berger and Arendt, 2000; Nath *et al.*, 2000). This heightened sensitivity is suggestive of decreased dopamine receptor activity, a scenario similar to that seen in Parkinson's disease. In this regard, stereological studies reveal decreased (25%) neuronal density in SN_{pc}, but without the presence of Lewy bodies as is typically seen in Parkinson's disease. This reduction in neuronal density may be driven by the viral nuclear regulatory protein *Tat*. Preclinical studies in rats demonstrate that intraventricular injections of *Tat* result in oxidative stress in the striatum (Aksenov *et al.*, 2001) and ultimately produce apoptotic cells predominantly in basal ganglia (see Berger and Nath, 1997). Moreover, dopamine-related drugs of abuse (i.e., amphetamine and cocaine) may synergize with viral proteins such as *Tat* to produce enhanced neurotoxicity (Nath *et al.*, 2000). While there is a trend towards lowered concentrations of dopamine and HVA from the caudate at autopsy in AIDS patients relative to controls, these differences become more pronounced and statistically significant if one focuses exclusively on those AIDS patients who manifested dementia (Sardar *et al.*, 1996). These data are also supported by less direct measures of diminished CSF HVA levels in seropositive subjects and preliminary observations suggesting a trend towards a correlation between the decrease in HVA levels and cognitive deficits (Sardar *et al.*, 1996).

MEMORY DISORDERS

The disorders contained within the 'Amnestic disorders' category of the DSM-IV (American Psychiatric Association, 2000) are

characterized by a disturbance in memory that is due to either the direct physiological effects of a general medical condition or the persisting effects of a substance (e.g., a medication, a drug of abuse, or exposure to a toxin). Individuals with amnestic disorders are impaired in their ability to learn new information or are unable to recall previously learned information or past events. These deficits are seen most readily on tasks that require spontaneous recall; they may also become apparent when the person is required to recall stimuli presented at some earlier time. Amnestic disorders may be preceded by an evolving clinical picture that includes disorientation and confusion, and thus the differential diagnosis between delirium and an emerging amnestic disorder merits caution (American Psychiatric Association, 2000).

In this section, we will examine two substance-induced memory disorders. First, we will discuss alcohol-induced persisting amnestic disorder (Korsakoff's syndrome) that is apparently due to the vitamin deficiency associated with prolonged, heavy ingestion of alcohol (Fadda and Rossetti, 1998). While the neuropathology accompanying this syndrome is complex, there is significant evidence for impaired noradrenergic transmission in this amnestic disorder. Second, we will review evidence for a significant and long-lasting memory deficit associated with repeated administration of the illicit recreational drug MDMA (ecstasy). The preclinical and, to a lesser extent, clinical literature indicates that this drug is neurotoxic for serotonergic nerve terminals and collectively raises the possibility that this amnestic syndrome is related to decreases in this aminergic system.

Korsakoff's Syndrome

Korsakoff's syndrome (or disease or psychosis) represents the chronic amnesic phase of the Wernicke–Korsakoff syndrome. The outstanding clinical feature of this amnesia is a selective and permanent anterograde memory loss (McEntee and Mair, 1990; Squire and McKee, 1993) with accompanying impairments in learning. Neuropsychometric data reveal that as a group, Korsakoff patients have average intellectual capabilities (as measured by Wechsler Adult Intelligence Scale (WAIS) IQ) but severe memory impairments, as revealed by the WAIS and Wechsler Memory Scale (WMS). Korsakoff's syndrome is associated with a thiamine deficiency that is secondary to the nutritional deficiencies of alcoholics. Long-lasting alcohol abuse is not a necessary condition, as Korsakoff's syndrome has also been reported in nutritionally depleted non-alcoholics (McEntee and Mair, 1990; Victor *et al.*, 1989).

The syndrome presents with a very characteristic midline diencephalic and brainstem neuropathology. Post-mortem studies reveal small punctate lesions in the periventricular region (along the walls and floor of the third and fourth ventricles) and the periaqueductual regions of the brainstem and diencephalon. There is often, but not always, damage to the mediodorsal nucleus (MDN) of the thalamus and the mammillary bodies (see McEntee and Mair, 1990 for a review). There is a significant amount of controversy surrounding the necessary and sufficient neuropathologies for the amnesic syndrome. Mammillary body lesions are present in most cases, and the frequency of this pathology has led to the speculation that it is a critical condition for the amnesic syndrome. However, there are several cases of such lesions without an accompanying amnesia, suggesting that damage to mammillary bodies may not be sufficient for the syndrome (see McEntee and Mair, 1990 for a discussion of this issue). Others (e.g., Victor *et al.*, 1989) have suggested that lesions of the MDN are crucial for the memory impairments (Markowitsch, 1988). However, there are reported cases of Korsakoff patients with amnesia yet no MDN lesions (although these patients had significant lesions of the mammillary bodies). Thus, it may be that damage to either the MDN or the mammillary bodies is necessary for the memory deficits.

Three observations have prompted a great deal of speculation about the neurochemical basis of Korsakoff's syndrome. First, a number of aminergic systems course through or near the sites of these brain lesions (Fadda and Rossetti, 1998). Second, there is a long history of animal research demonstrating memory impairments following lesions or pharmacological manipulations that disrupt aminergic systems (see Mair and McEntee, 1983 for a discussion of this literature). Finally, there are preclinical neurochemical data demonstrating that conditions of thiamine deficiency can result in altered precursor transport, synthesis and turnover in several aminergic systems (see Witt, 1985, for a review).

Adrenergic Transmission and Korsakoff's Syndrome

The greatest focus on the aminergic components of Korsakoff's amnesia has been directed toward the brainstem locus coeruleus system and noradrenergic projections to the telencephalon. While several reports indicate diminished noradrenergic transmission in Korsakoff's syndrome, the data are by no means consistent. CSF levels of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) were reported to be reduced (by 41%) in a large group of Korsakoff's syndrome patients, even when corrected for plasma MHPG concentrations. Importantly, the reductions in MHPG from the CSF correlated with the degree of memory impairments (McEntee *et al.*, 1984). The nucleolar volume of the locus coeruleus (a measure of activity within locus coeruleus neurons) was found to be reduced in the locus coereleus, and post-mortem levels of noradrenaline were diminished in the locus coeruleus and target regions, such as the supraoptic and paraventricular nuclei (Mayes *et al.*, 1988). As was the case with CSF levels of MHPG, the reductions in nucleolar volume in the locus coeruleus were greatest in those patients who were most amnestic.

There are, however, several studies that are not consistent with these findings. Martin *et al.* (1984) did not observe any change in CSF MHPG levels, nor did they find a correlation between MHPG levels and memory function when a larger sample size was studied. More recently, Halliday *et al.* (1992) conducted a quantitative study of locus coeruleus integrity comparing four 'uncomplicated' alcoholics with nine Korsakoff's syndrome patients. The authors report no group differences in the number, morphology or distribution of pigmented locus coeruleus neurons. Moreover, the analysis of locus coeruleus neuronal number reveals a rather marked variability within the control group (standard deviation 20%) and a significant ageing-related decline in neuronal counts. Thus, it may be premature to attribute the source of reductions of presynaptic markers of noradrenergic transmission to a loss of neuronal number within the locus coeruleus. While it would appear that cell loss within the locus coeruleus does not account for the amnesia seen in Korsakoff's syndrome, it still remains possible that other, more subtle changes in adrenergic transmission are critical to the symptoms of this disease. The sources of variation among these collective studies are considerable and may contribute to the inconsistency of findings. First, the various neuroanatomical and neurochemical measures utilized in these studies (nucleolar volume of locus coeruleus, number of locus coeruleus neurons, morphology of locus coeruleus neurons, CSF metabolite levels, post-mortem noradrenaline tissue levels) differ in their capacity to reveal physiologically meaningful alterations in noradrenergic transmission. Second, there is significant variation in the sample sizes in these studies. Given the inherent variability of neuropathology in human disease, studies with considerable differences in sample size will be expected to have very different statistical powers for revealing effects. Finally, the discussion above regarding the profile and distribution of lesions in Korsakoff's syndrome does not support a uniform neuropathology, thus there may be multiple variants of this syndrome that are reflected in differential contributions of particular aminergic systems.

Cholinergic Transmission and Korsakoff's Syndrome

The potential role of dysfunction in cholinergic transmission in Korsakoff's amnesia has received significant attention and is justified by several observations. First, cholinergic blockade in healthy subjects produces memory impairments (Kopelman and Corn, 1988). Second, as described earlier in this chapter, there is a significant correlation between the well-established memory deficits and various markers of impaired cholinergic transmission in Alzheimer's dementia (Francis *et al.*, 1985). This raises the possibility that other syndromes characterized by memory deficits are mediated by dysregulations in cholinergic transmission. Third, basal forebrain lesions accompanying certain vascular insults are accompanied by memory impairments (Damasio *et al.*, 1985). Finally, biochemical data suggest that thiamine deficiency can eventually result in diminished levels of acetyl Cheyney A (acetyl CoA) and reduced turnover of ACh (Barclay *et al.*, 1981; Witt, 1985).

A more direct link between the basal forebrain cholinergic system and Korsakoff's syndrome has been suggested by the observation of a significant reduction (47%) of the number of magnocellular neurons (presumably cholinergic) within the nucleus basalis of Meynert in autopsied brains from three patients (Arendt *et al.*, 1983). However, reductions in basal forebrain neurons in Korsakoff's disease are not reported consistently. Mayes *et al.* (1988) conducted a series of post-mortem morphometric measurements on basal forebrain cholinergic nuclei from patients diagnosed with Korsakoff's before death. They report no reduction in number or nucleolar volume of basalis neurons, although the authors comment on the fact that many of these neurons were shrunken and exhibited a loss of Nissl substance. A more recent and complete analysis compared basal forebrain magnocellular neurons among controls, alcoholics, Wernicke's encephalopathy patients who did not present with amnesia, and Wernicke's patients who later developed a classical Korsakoff's amnesia (Cullen *et al.*, 1997). Cell number (Ch4 cholinergic neurons) did not differ significantly between non-alcoholic controls and alcoholics. Ch4 cell number in the Wernicke's encephalopathy group was modestly but significantly reduced (24%) below control levels. However, cell number was comparably reduced (21%) in the Wernicke's group that developed Korsakoff's syndrome. The authors conclude that nucleus basalis neurons are lost in thiamine-deficient alcoholic patients, but that cell loss is relatively minor and does not account for the profound memory disorder seen in Korsakoff's syndrome. Furthermore, they raise the interesting possibility that this loss in Ch4 cell number might contribute to attentional dysfunctions seen in both Wernicke and Korsakoff patients.

Serotonergic Transmission and Korsakoff's Syndrome

An early study on serotonergic transmission in Korsakoff's syndrome reported abnormally low levels of the 5-HT metabolite 5-HIAA (21% decrease) in some but not all patients (McEntee *et al.*, 1984). Halliday *et al.* (1992) utilized more direct immunohistochemical techniques to compare the number of 5-HT-positive raphe neurons in alcoholics that manifested Korsakoff's syndrome with those that did not. While they report a significant (50%) reduction in the number of 5-HT-positive neurons, this value was similar in both groups of alcoholics. The relative contribution of alcohol toxicity versus thiamine deficiency on serotonergic neurons in the median raphe nucleus was addressed in a more recent report from their laboratory (Baker *et al.*, 1996). The authors report no difference between the number of 5-HT neurons in alcoholics without Korsakoff's syndrome and age-matched controls. However, there was a substantial loss (nearly 70%) of serotonergic neurons in brains from previously diagnosed Korsakoff's patients. These data suggest that the cell loss in the raphe reflects the thiamine deficiency rather than the effects of alcohol *per se*.

Dopaminergic Transmission and Korsakoff's Syndrome

The roles of mesocortical and mesolimbic dopaminergic systems in the memory disorders associated with Korsakoff's syndrome have received little attention. This is somewhat surprising given the observation that chronic alcohol consumption is associated with reductions in rCBF and glucose metabolism in medial frontal lobe (Adams *et al.*, 1993), and that dopaminergic systems exert important modulatory influences on cognitive activity mediated by frontocortical neurons (Dolan *et al.*, 1995). One study reported significant reductions (23%) in CSF levels of the dopamine metabolite HVA in Korsakoff's patients (McEntee *et al.*, 1984). However, these results were not seen consistently in all patients and, unlike several of the reports with the noradrenaline metabolite MHPG, did not correlate strongly with memory impairments.

As discussed earlier, the therapeutic efficacy of drugs with relatively selective actions on specific transmitter systems can provide insights into the contributions, in this case, of aminergic systems to the memory disorders associated with Korsakoff's syndrome. The therapeutic agents studied most extensively for the treatment of Korsakoff's amnesia have been drugs that affect the monoamines, noradrenaline, dopamine and serotonin. McEntee and Mair (1990) compared the effects of twice-daily oral administrations of clonidine (an α_2 noradrenaline agonist), amphetamine (an indirect monoaminergic agonist), L-dopa (an indirect monoaminergic agonist) and adrenaline (an α noradrenaline agonist, with weak amphetamine-like actions) on memory performance in a small population of Korsakoff's syndrome patients. Clonidine treatment produced significant improvement, compared with placebo, on several items on the Wechsler memory scale (memory passages, visual reproduction). A single photon emission computerized tomography (SPECT) imaging study tested the hypothesis that frontal lobe function would be increased by clonidine-induced adrenoreceptor stimulation, and that the ability of clonidine to increase metabolic activity would correlate with the drug's ability to enhance memory function (Moffoot *et al.*, 1994). The acute administration of clonidine increased performance on a verbal fluency task, although this effect was variable and not always distinguishable from the effect of placebo. Nevertheless, the increase in neuropsychological performance was correlated with the increased metabolic activity in the left dorsolateral frontal cortex and, to a lesser extent, in the posterior cingulate cortex and thalamus. However, a clinical trial with a larger group of subjects was unable to demonstrate a therapeutic effect of clonidine (O'Carroll *et al.*, 1993). McEntee and colleagues report smaller, less consistent effects with amphetamine (McEntee and Mair, 1990) and ephedrine (Mair and McEntee, 1986). Similar therapeutic effects on a verbal recall test were seen in six Korsakoff's syndrome patients following a week-long administration of the indirect adrenergic agonist methylphenidate (O'Donnell *et al.*, 1986). The effects of these adrenergic agonists must be viewed cautiously, however, as it is possible that drug-induced changes in attentional processing might account for some of the antiamnesic effects of the drugs (McEntee and Mair, 1990). Finally, a possible role for dysfunctions in serotonergic transmission in Korsakoff's amnesia is supported by a study demonstrating that the 5-HT uptake inhibitor fluvoxamine enhanced memory in a small group of patients. Interestingly, the reported improvements of fluvoxamine correlated with the reductions in the CSF levels of the 5-HT metabolite 5-HIAA (Martin *et al.*, 1989).

MDMA-Induced Memory Deficits

Recreational usage of the illicit drug MDMA first became highly visible in the mid-1980s. Its consumption has evolved worldwide so dramatically since then that it poses serious health risks. Demonstrations of MDMA-induced selective impairment in memory coupled

with suggestions of neurotoxicological damage (particularly to brain serotonin systems) suggest that repeated consumption of MDMA promises to be seen increasingly as a diagnostic group in the future.

Consumption of MDMA is associated with profound effects on mood. Users generally report feelings of elation, energy, confidence and enhanced sociability while on the drug. The period of withdrawal is associated with feelings of depression, lethargy and irritability (Curran and Travill, 1997). A number of studies indicate that the psychological effects of MDMA extend beyond changes in mood to selective impairments in memory. Tests designed to evaluate general information-processing speed (simple reaction time, choice reaction time, Sternberg task response time) and sustained attention reveal no significant differences between MDMA users and various control groups (Krystal *et al.*, 1992; Parrott *et al.*, 1998). However, a number of studies indicate significant impairments in a variety of tasks that assess memory function. MDMA users exhibit mild to moderate decrements when compared with alcohol users (Curran and Travill, 1997) in working memory and the initial and delayed paragraph test of the Wechsler Memory Scale (Krystal *et al.*, 1992), and show deficits in delayed and non-delayed word recall (Morgan, 1999; Parrott and Lasky, 1998).

One difficulty associated with ascribing performance deficits in these laboratory tasks to deficits in memory is that MDMA users rarely report memory impairments. There have been several surveys of recreational MDMA users, and they have not revealed subjective evidence for memory deficits (see Krystal *et al.*, 1992). In this regard, Parrott *et al.* (1998) raise the interesting possibility that perhaps these apparent memory deficits reflect a change in cognitive strategy rather than memory deficits *per se*. After all, MDMA users report a change to a more phenomenological, less verbal style not only when on the drug but for some time thereafter. Thus, future studies on neuropsychological function should be expanded to include a range of non-verbal as well as verbal memory tasks and other measures of cognitive strategy.

The preclinical and clinical neurotoxicological literature suggests serious grounds for concern over repeated use of MDMA. Animal studies reveal damage to several markers of 5-HT-containing neurons (decreased 5-HT and 5-HIAA levels, diminished 5-HT uptake), particularly in the hippocampus (Frederick and Paule, 1997; Steele *et al.*, 1994). The reported impact on the hippocampus is particularly important, given the vast literature implicating its role in memory processes (see Nadel and Bobbot, 2001 for a review). The human neurotoxicology literature, although less extensive, is entirely consistent with the animal studies. Levels of 5-HIAA in the CSF are reduced in subjects who have a history of repeated MDMA use (McCann *et al.*, 1994; Ricaurte *et al.*, 1990). These decreases in CSF 5-HIAA correlate with the extent of memory impairments in abstinent MDMA users (Bolla *et al.*, 1998). A PET imaging study found ecstasy users to have reduced 5-HT transporter binding in a number of brain regions, including the frontal and temporal cortices (Szabo *et al.*, 1997). Little is known about the effects of MDMA usage on postsynaptic 5-HT receptor function. A recent study (Reneman *et al.*, 2000) used SPECT imaging and the 5-HT_{2A} receptor ligand [^{123}I]-5-I-R91150 to assess the density of 5-HT_{2A} receptors and, importantly, to correlate changes in the density of this receptor with performance on a memory recall test. Although the sample sizes are small, there was a significant increase in the density of 5-HT_{2A} binding in the occipital cortex in repeated MDMA users (cumulative consumption of at least 50 tabs) relative to controls. There was a strong negative correlation ($r = -0.98$) between performance on the word recall test and binding in the MDMA group and no significant correlation in the control group. It is important to point out that the MDMA users in this study had been drug abstinent for 2–11 months (mean 4.6 months) before this study.

The demonstration of memory deficits in drug-abstinent MDMA users raises the important issue of the permanency of these neuropsychological deficits. In addition to the SPECT study cited above, the study by Parrott and Lasky (1998) was conducted 2 and 7 days after drug consumption, whereas the study by Krystal *et al.* (1992) tested MDMA users who had not taken the drug for an average of 66 days. Collectively, these investigations suggest that the memory deficits induced by repeated MDMA usage may be long-lasting or even permanent. If so, this leads to the chilling conclusion that the mild to moderate deficits reported in these studies represent only the tip of the iceberg, and that many of these users may remain at high risk for more severe ageing-related memory deficits as they experience an age-related loss of 5-HT-containing neurons.

Future research in the area of MDMA-induced amnestic disorders will have to confront several methodological and interpretational challenges. First, as is the case with any study on an illicit drug, the illegal status of MDMA and its potential neurotoxic effects constrain experiments and preclude the use of more traditional double-blind, placebo-controlled designs. Second, it is often difficult to get predrug baseline performance data on cognitive tasks in the MDMA group (but see Parrott and Lasky, 1998 for an example of baseline performance in the drug group). Third, future studies should identify more carefully appropriate control groups against which to compare the performance of the MDMA group. MDMA users tend to be poly-drug users and, thus, it is important to include control groups that have a history of use of several drugs (e.g., alcohol, cigarettes, cannabis, LSD, cocaine, inhalants) but not of MDMA. The comparison of MDMA users with a poly-drug user control group was accomplished in Morgan (1998). Fourth, it will become important to determine how much MDMA needs to be consumed before the emergence of these memory deficits. This is a difficult issue to address. Parrott and Lasky (1998) state that their 'regular users were comparatively more impaired than the novice users'; however, it is not clear how these two groups were defined. In a different study, Parrott *et al.* (1998) found no differences in memory deficits between users who had consumed more than ten tabs of MDMA versus those that had consumed fewer than ten tabs. Finally, an issue confounding the specification of both the dose sufficient to produce memory deficits and the attribution of MDMA to such syndromes is the fact that MDMA tablets often contain impurities (e.g., ketamine) that affect dose and also impair neuropsychological function in their own right (Parrott *et al.*, 1998).

DELIRIUM

The DSM-IV defines delirium as a general disturbance in consciousness that gives rise to changes in cognition that are not related to an existing or evolving dementia (American Psychiatric Association, 2000). Cognitive changes include disorientation (to time/space), reductions in awareness of the environment, and impairments in the ability to focus, sustain or shift attention. Memory (particularly of recent events) may also be impaired temporarily. The delirious state can also be associated with a number of other disorders, including disturbances in sleep–wake cycles, psychomotor activity and emotion (anxiety, fear, depression, irritability).

Delirium typically develops over a short period of time (hours/days), with a fluctuating presence and severity. The two primary aetiological conditions associated with delirium are general medical conditions (head trauma, vascular diseases, metabolic disturbances) and substance-induced (medications, drugs, toxins). The discussion below will focus on the contributions of aminergic transmitter systems to delirium in elderly patient populations (50% of elderly patients may become delirious upon admission to hospitals) and following various medications (several substances that affect aminergic systems can induce delirium).

Cholinergic Transmission and Delirium

The link between cholinergic transmission and delirium has been fostered by three observations. First, it is generally believed that ACh plays an important role in the global cognitive disruption characteristic of delirium (Perry and Perry, 1995), including decreases in wakefulness (Baghdoyan, 1997) and impairments in attentional processes (Sarter and Bruno, 1997). Second, medications with anticholinergic properties often precipitate episodes of delirium (Trzepacz *et al.*, 1996; also see below). Finally, physostigmine, an indirect cholinergic agonist, has been shown to be effective in the treatment of delirium (Granacher *et al.*, 1976).

A recent empirical study by Han *et al.* (2001) evaluated the longitudinal association between the use of cholinergic medications and the severity of delirium in a large cohort of elderly medical inpatients with diagnosed delirium. They also determined whether this association was modified by the presence of dementia in delirious patients. The results indicated an increase in the severity of delirium associated with several measures of cholinergic medication, but a pre-existing condition of dementia did not increase this association. However, this latter point requires additional study as most of these patients were only mildly demented and, thus, cholinergic deficits may have been less evident in these patients.

Another linkage between cholinergic deficiency and delirium has been revealed using a functional competitive binding assay for serum anticholinergic activity developed by Tune and Coyle (1981). This assay measures the ability of the patient's serum to block central muscarinic receptors. An association between delirium and elevated serum anticholinergic activity has been demonstrated in two studies of elderly medical patients (Flacker *et al.*, 1998). The basis of the serum anticholinergic activity is not known, but it is generally thought to reflect residual levels of medications or their metabolites. It should be pointed out, however, that there is a significant amount of overlap in the serum anticholinergic activity in delirious and nondelirious patients, suggesting the importance of other contributing factors.

In spite of these supporting observations, there are certainly some exceptions to the suggested relationship between cholinergic transmission and delirium (see Han *et al.*, 2001 for a discussion of this issue). There are at least three factors that could account for such discrepant findings. First, different studies utilize different measures of ACh medication exposure (serum ACh level, number and dose of cholinergic medications, aggregate risk score of ACh potency), and the relationship between these measures has not been specified clearly. Second, the effects of cholinergic medications on delirium may be confounded by other risk factors, such as age, dementia and a variety of comorbid conditions. Collectively, the evidence suggests that delirium is viewed adequately as a disorder based on multiple transmitter abnormalities. Additionally, various risk factors interact with neuropathological processes to produce heterogeneous symptoms of delirium (Flacker and Lipsitz, 1999).

Serotonergic Transmission and Delirium

A postulated role for 5-HT in delirium is not surprising, given the evidence from both the animal and human literature linking serotonergic transmission with several of the functions affected by delirium, such as wakefulness, mood and cognition (Meneses, 1999). Interestingly, there are suggestions that delirium can be associated with either excessive activation or reduction in serotonergic transmission. Elevated levels of the 5-HT metabolite 5-HIAA have been reported in ill, delirious, non-demented patients when compared with control populations of healthy subjects as well as when compared with groups of nondelirious alcoholics and patients on antipsychotics (Banki and Vojnik, 1978). Consistent with these CSF findings, the literature contains reports of a 'serotonin syndrome' characterized by confusion, restlessness and tremor. This syndrome can be precipitated by medications that act as 5-HT agonists, such as L-tryptophan, MAO inhibitors, and fluoxetine (Steiner and Fontaine, 1986).

Several reports have linked the occurrence of delirium with reduced serotonergic transmission, or at least with reduced levels of tryptophan. Plasma tryptophan to large neutral amino acid ratios were significantly lower post-surgery in a group of delirious patients (van der Mast *et al.*, 1996). Moreover, a retrospective study demonstrated that patients with early symptoms of delirium tremens exhibited improved mini-mental status exam scores, sleep/wake cycle, and reduced tranquilizer use following daily treatments with L-tryptophan (Hebenstreit *et al.*, 1989).

Dopaminergic Transmission and Delirium

Finally, abnormalities in dopaminergic transmission have also been linked to delirium and acute confusion. Dopamine agonists such as L-Dopa (Birkmayer, 1978), pergolide (Cummings, 1991) and buproprion (Golden *et al.*, 1985) have been reported to lead to delirium. In some cases, dopaminergic antagonists such as haldol are effective in relieving the symptoms of delirium.

CONCLUSIONS

A review of the available clinical literature clearly reveals multiple dysregulations in aminergic transmission accompanying a wide range of cognitive disorders. However, there is considerable variation in the consistency and magnitude of some of these linkages. These discrepancies no doubt reflect at least two interacting variables. First, the individual cognitive disorders are indeed syndromes with multiple neuropathologies and aetiologies. Thus, it is not surprising that transmitter profiles vary within heterogeneous and often small groups of patients. Second, a wide range of methodologies is used to characterize the integrity of aminergic systems accompanying these cognitive disorders. We are constrained when assessing neurochemistry in clinical populations, and care should be taken in comparing data obtained by such discrepant and often indirect measures. Nevertheless, there is little doubt that deficits in adrenergic transmission correlate well with neuropsychological performance in a number of cognitive disorders. The challenge facing us now is to understand better the relationships between damage to a particular transmitter system within a brain region and deficits in the various psychological functions that characterize these cognitive disorders.

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XV-3

Disturbances in the Amino Acid Transmitter Systems in Cognitive Disorders Classified and Diagnosed According to DSM-IV

Theodora Duka

The dementia classification according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association, 2000) gives four main groups of dementias. This classification does not reflect the separated neuronal mechanisms underlying the dementias, nor does it correspond to a prominent different symptomatology seen in the different dementias. It does, however, allow a categorization according to the most obvious cause (see Table XV-3.1). It is widely accepted that the most common dementia is Alzheimer's dementia, followed by alcohol dementia including Korsakoff's syndrome. The dementia that is most common in young people and children is human immunodeficiency virus (HIV)-related dementia. We will refer mostly to these three types of dementia.

Amino acid neurotransmitters, especially γ-aminobutyric acid (GABA) and glutamate, are thought to play an important role in the cognitive function of the brain. Therefore, it is not surprising that most research efforts to understand how dementias develop have been concentrated on investigating the way that these amino acids influence cognition. GABA represents the main inhibitory amino acid neurotransmitter and glutamate the main excitatory amino acid neurotransmitter in the brain. There are two ways by which these amino acids are implicated in the dementias: firstly, by their direct participation in learning and memory processes, and secondly, by their involvement in neuronal degeneration. More recently, other amino acid neurotransmitters, such as quinolate, glycine and kynurenic acid, have also been implicated in the development of some dementias, although the role of these latter amino acids in normal cognitive function is less clear. In this chapter, the role of GABA receptors and glutamate receptors in the normal cognitive state will be discussed, followed by their role in the different types of dementias. Finally, we will discuss the complex issue of what underlies the function of a damaged brain.

THE ROLE OF AMINO ACID NEUROTRANSMITTERS IN COGNITION

GABA and Cognition

The amino neurotransmitter GABA mediates fast synaptic inhibition in the brain and directly activates chloride-permeable ion channels. GABA ionotropic receptors are divided into GABA-A and GABA-C. The GABA-A receptors are important as they are the target of benzodiazepine and barbiturate drugs. The GABA-A protein subunits can be divided into seven families with multiple isoforms: α (six isoforms), β (three isoforms) and γ (three isoforms), and the newer δ, ε, θ, and π (Mehta and Ticku, 1999; Neelands *et al.*, 1999; Loh and Ball, 2000; Pirker *et al.*, 2000). The receptors lacking the γ-subunit are insensitive to benzodiazepines and have higher agonist affinities.

Table XV-3.1 Classification of cognitive disorders according to DSM-IV

Type of dementia	Main cause
1. Alzheimer's dementia	Neurodegenerative disorder
2. Alcohol-induced persisting dementia	Long-term alcohol use • *Wernicke's encephalopathy*: acute symptoms related to motor balance • *Korsakoff's syndrome*: chronic condition characterized by poor memory
3. Vascular dementia (multi-infarct dementia)	Ischaemia (neurodegeneration)
4. Dementia related to other general medical conditions	HIV (infection) Head trauma (injury) Parkinson's disease (neurogenerative disorder) Huntington's disease (neurogenerative disorder) Pick's disease (neurogenerative disorder) Creutzfeld–Jacob disease (infection)

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Most information on the role of GABAergic activity on cognition is derived from reports on the effects of benzodiazepine ligands on memory. The discovery of betacarbolines, the first ligands at the benzodiazepine site of the GABAergic complex with inverse agonist activities, led to a breakthrough in our understanding of the role that GABA may play in memory. The inverse agonists bind at the benzodiazepine site and are negative modulators of GABAergic activity. They bring about their effects by reducing the frequency of the chloride-channel openings, preventing the entrance of chloride ions into the cell. The spectrum of the behavioural effects of these inverse agonists were the mirror image of the spectrum of effects of the classical agonists, benzodiazepines, and induced anxiety (Dorow *et al.*, 1983) and convulsions (Stephens and Sarter, 1988). Ligands with selective or partial inverse agonist properties at the benzodiazepine binding sites were developed from the betacarbolines and benzodiazepines. They were characterized in several behavioural tests, and it was revealed that they produced the opposite effects of benzodiazepines and betacarbolines with full or partial agonist properties at the benzodiazepine sites (Stephens and Sarter, 1988). Thus, the partial or full inverse agonists demonstrated promnestic effects (Venault *et al.*, 1986), and furthermore, they were able to counteract the disruptive effects of scopolamine on acquisition (Sarter *et al.*, 1988a).

During the late 1980s, Sarter *et al.* (1988b) put forward a hypothesis to explain how the GABAergic system may affect memory, and what its role may be in the development of dementia. Several pharmacological studies at that time had provided evidence that increased GABAergic activity in the basal forebrain would decrease the acetylcholine release in the cortex (Casamenti *et al.*, 1986). In addition, anatomical studies had provided evidence that GABAergic afferent projections from the nucleus accumbens project to cholinergic cell bodies in the basal forebrain (Zaborszky *et al.*, 1986). Thus, on the basis of the pharmacological and anatomical data, it could be suggested that the activity of cortical cholinergic neurons derived from the basal forebrain are under the control of GABAergic input. Based on this evidence, Sarter *et al.* (1988b) hypothesized that in senile dementia of the Alzheimer's type, degeneration of the cholinergic neurons from the basal forebrain to the cortex leads to a reduction of acetylcholine availability in the cortex. Reduced acetylcholine availability is facilitated further by GABAergic inhibitory control, which remains unchanged, on the remaining intact cholinergic cells in the basal forebrain and on the nerve endings in the cortex. Sarter *et al.* (1988b) further suggested the cognition-enhancing potential of compounds with antagonist or partial inverse agonist properties. Such compounds would have a disinhibitory control of the GABAergic input, and would induce an enhancement in acetylcholine release.

Data from recent studies provide further evidence that GABAergic projections to the basal forebrain control the excitability of basal forebrain cholinergic neurons (Moore *et al.*, 1995; Zaborszky *et al.*, 1991). The increase of cortical acetylcholine would mediate the processing of stimuli and associations and the allocation of processing resources for these attentional functions, and would influence memory via these mechanisms (Muir *et al.*, 1992; Robbins *et al.*, 1997). This result, however, may depend on the degree of neuronal loss due to the neuronal degeneration, and indeed experiments using inverse agonists to improve cognitive effects in animals have shown that the effects on cognition appear to be related to the loss of cholinergic input into the cortex; in animals with 50–60% loss, but not 90% loss, treatment with the benzodiazepine inverse agonist alleviated the lesion-induced impairment in measures of attention (Sarter and Bruno, 1997).

This initial hypothesis on the role of GABA in senile dementia provided a reasonable theoretical approach to the development of a new generation of drugs for the treatment of some symptoms of dementia, which has been followed up since by many pharmaceutical companies.

Duka and colleagues (Duka *et al.*, 1988; Duka *et al.*, 1996) were the first to test the idea in humans that a ligand at the benzodiazepine receptor with partial inverse agonist properties at the GABA–benzodiazepine receptor complex would have the opposite effects of a full agonist on memory, i.e. enhance the abilities of the subject to retain memories. Partial agonist properties of such a compound are essential in order to circumvent other unwanted effects, such as anxiety induction and convulsant activity. Such a compound, a β-carboline antagonist with weak partial inverse agonist properties at the benzodiazepine binding site, ZK 93426, was developed in the late 1970s and tested in several preclinical and clinical studies in the 1980s and 1990s. ZK 93426 was never tested in subjects suffering from any cognitive disorder because of its poor kinetics (very low bioavailability), which required administration as an intravenous infusion. The development of a new compound characterized preclinically as a successor of ZK 93426, showing a similar spectrum of effects in preclinical testing and a good oral bioavailability, was introduced in phase I and was administered to healthy participants and subsequently to Alzheimer's patients. However, its weak cognitive-enhancing properties were accompanied by many psychotogenic effects, and the compound was not developed further.

Recent studies with transgenic mice have highlighted the role of GABA–benzodiazepine receptor complex subunits in the different actions of benzodiazepines, including memory. Rudolph *et al.* (1999), using the technique of homologous recombination, engineered mice with the a1 protein subunit mutated (arginine was replaced in the 101 position by histidine), so that there was no $\alpha 1$ benzodiazepine binding site. The otherwise healthy mutants have GABA-A receptors containing only the $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits for the benzodiazepines to bind. When the mice were given diazepam, the anxiolytic effects, but not the sedating or amnesic effects, of diazepam were seen. Based on these data, it can be suggested that the $\alpha 1$ subunit is the one involved in the mediation of the benzodiazepine effect on memory. However, the tasks used to test the amnestic effects are also known to be sensitive to anxiolytic effects of benzodiazepines, which were present in the mutants; consequently, the data cannot be conclusive (see Wisden and Stephens, 1999). Thus, the role of GABA-A subunits in mediating benzodiazepine-induced amnesia still needs to be investigated. Further studies using similar techniques have suggested that the $\alpha 2$ and not the $\alpha 3$ site is responsible for the anxiolytic effects of benzodiazepine (Low *et al.*, 2000; Crestani *et al.*, 2001), which makes the $\alpha 2$ subunit the most likely to mediate the anxiolytic effects of benzodiazepines and still leaves open the question about amnestic effects.

Zolpidem, a widely used imidazopyridine hypnotic (a non-benzodiazepine sedative-hypnotic) (Praplan-Pahud *et al.*, 1990) has been shown to induce sedation and muscle relaxant activity similar to that induced by other benzodiazepines, while at the same time producing less memory and cognitive impairment. However, the reports in the literature are contradicting, and amnestic effects of zolpidem may be present, depending on the dose and the task used (Evans *et al.*, 1990; Balkin *et al.*, 1992; Wesensten *et al.*, 1995; Berlin *et al.*, 1993). In a more recent study, the effects of triazolam (a benzodiazepine full agonist) were compared with those of zolpidem on specific memory tasks (Mintzer and Griffiths, 1999). Although both compounds had a similar spectrum of effects in several tasks on memory, there were certain memory functions in which their effects were distinctively different. In particular, triazolam impaired, while zolpidem did not, the ability of the subjects to retrieve spatial contextual information (i.e. information about the location where an event occurred). Similarly, in a retrieval of spatial route task (Balkin *et al.*, 1992), zolpidem did not impair performance. Spatial information is an important attribute in the

retrieval of memories and appears to be impaired in patients with cognitive disorders (Carlesimo *et al.*, 1994; Swainson *et al.*, 2001). On the other hand, zolpidem is a sedative drug, and at certain doses sedation can account for impairment in several memory tasks. Thus, similarities between zolpidem and triazolam or other full agonist benzodiazepines in some memory tasks could be understood as a result of the sedation present (Stephens *et al.*, 1991). If, as shown from studies *in vivo*, zolpidem binds with high affinity to $\alpha 1$ (by which its sedative effects are mediated) (Crestani *et al.*, 2000) and intermediate affinity to $\alpha 2$ and $\alpha 3$, but not at all to $\alpha 5$, then we can suggest that $\alpha 5$ subunits may be the benzodiazepine binding site that mediates the amnestic effects. Consistent with this idea, recent studies have shown that novel benzodiazepine receptor ligands, designed using pharmacophore/receptor models and selective for the $\alpha 5\beta 3\gamma 2$ benzodiazepine receptor subtype, enhanced memory when injected directly into the hippocampus in animals (Huang *et al.*, 2000). The ligand used in these studies was an inverse agonist at the $\alpha 5\beta 3\gamma 2$ benzodiazepine receptor subtype. Thus, from these data, and taking into account the discussion at the beginning of this section, it can be suggested further that new benzodiazepine inverse agonist ligands specific for the $\alpha 5$ benzodiazepine receptor subunit may be the future candidates for treating Alzheimer's dementia. These compounds should not present with the type of anxiogenic effects that have been reported previously when the nonspecific benzodiazepine partial inverse agonists were given to humans (Dorow *et al.*, 1983; Dorow *et al.*, 1987; Duka *et al.*, 1987).

Glutamate and Cognition

Glutamate is the principal excitatory neurotransmitter in the brain. It binds to bring about its actions to four types of glutamate receptors: *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) and kainate, which are ionotropic (all three are named after the pharmacological agents that bind to them), and a fourth metabotropic receptor. There are several glutamate receptor subunits identified: for the NMDA receptor, the subunits NR1, NR2A, NR2B, NR2C and NR2D; for the AMPA receptor, the subunits GluR1, GluR2, GluR3 and GluR4; for the kainate receptor, the subunits GluR5, GluR6, GluR7, KA1 and KA2; and for the metabotropic receptor, the subunits mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6 and mGluR7. The result of NMDA binding is to facilitate the calcium conductance. However, in order for this to happen, the neuronal membrane has to be excited already so that magnesium will not bind in the channel. Another important condition to be fulfilled before NMDA can open the calcium channel is that glycine should be present at its binding site. The role of NMDA and glycine receptor activity in cognition will be presented in the following section.

NMDA Receptors

It is now well established that NMDA receptors play a crucial physiological role in various forms of synaptic plasticity, including those involved in learning and memory. NMDA is important for the induction of activity-dependent synaptic modification, so called long-term potentiation (LTP) (Bliss and Collingridge, 1993), a mechanism related to memory formation and learning (Sakimura *et al.*, 1995; Davis *et al.*, 1992).

In animal studies, performance in spatial but also non-spatial memory tasks is found to be impaired in the presence of competitive and non-competitive NMDA blockers. In particular, impairment of both working and reference memories in a radial-arm memory task has been reported after the administration of NMDA antagonists (Maurice *et al.*, 1994; Pitkanen *et al.*, 1995; Zhang *et al.*, 2000). The effects of NMDA antagonists in long-term memory have also been established using a passive avoidance task (Mondadori and Weiskrantz, 1993). Many studies have also reported learning deficits induced by the NMDA antagonist in the Morris water-maze task, a spatial learning task (McNamara and Skelton, 1993; Morris *et al.*, 1986; Morris *et al.*, 1990). From studies with non-human primates, it is suggested that several of the effects of NMDA antagonists on memory reflect impairments on acquisition and encoding of information (Thompson *et al.*, 1987; Buffalo *et al.*, 1994; Morris *et al.*, 1986). Numerous studies with animals have linked the activity of NMDA receptors within the hippocampus to the animal's performance in memory-related tasks (Morris *et al.*, 1986; Morris *et al.*, 1990). Memory tasks that are sensitive to the deficits induced by NMDA antagonists (i.e. the Morris water-maze task), are also affected by hippocampal lesions.

The effects of NMDA antagonists on memory in humans have been explored less due to the psychotogenic effects of the available NMDA antagonists, which limits their use in the laboratory. Early studies with phencyclidine (PCP; a non-competitive NMDA antagonist) aimed at simulating psychotic symptomatology have shown impairment of memory function as well as psychomotor performance and selective attention shortly after its administration (Rosenbaum *et al.*, 1959; Cohen *et al.*, 1962). The structurally related anaesthetic ketamine is known to induce anterograde amnesia, i.e. information acquired after its administration cannot be retrieved later (Pandit *et al.*, 1971). Since the doses used were those used for anaesthesia, it was difficult from these studies to conclude anything about the specificity of ketamine effects on memory. Recent studies with ketamine in subanaesthetic doses have shown ketamine to be an amnestic in tests of delayed recall of words presented at different times after ketamine administration (Krystal *et al.*, 1994; Krystal *et al.*, 1998a). In a more recent study, Newcomer *et al.* (1999) demonstrated a dose-dependent effect of ketamine on tasks of immediate and delayed paragraph recall, as well as in the delayed match-to-sample task. In a word-recognition task, subjects were also found to be impaired in recognizing words presented after ketamine infusion, whereas recognition for words presented before ketamine was not impaired (Hetem *et al.*, 2000). It has also been shown that the effects of ketamine on memory using a visual word-recognition task (subjects had to recognize words as being new (first time shown) or old (seen previously)) are mediated by hippocampal structures (Grunwald *et al.*, 1999). The method used to demonstrate the functional activation of hippocampal structure was an analysis of limbic event-related potentials. Findings such as an increase of cyclic adenosine monophosphate (cAMP) in the hippocampus (CA1 area) by NMDA (Nguyen and Kandel, 1996), and more recently, the finding that the selective inhibitor of a type 4 cAMP-specific phosphodiesterase (PDE4), rolipram, antagonizes the effects of NMDA on memory and learning (Zhang *et al.*, 2000), have led to hypotheses of an important role of cAMP and PDE4 in signal-transduction mechanisms for those NMDA receptors that are involved in learning and memory. Such findings highlight the molecular mechanisms underlying the role of NMDA in memory function. However, as yet there are no useful compounds that interact with the NMDA receptors and that can be used in human studies as tools to understand the role of glutamate on memory.

Glycine Modulatory Site

The glycine B (strychnine-insensitive) site on the NMDA receptor is a modulatory site to which glycine binds, and, in the presence of glutamate, acts synergistically with glutamate to promote channel opening and excitatory neurotransmission. This glycine binding site on the NMDA receptor complex is distinct from the classic strychnine-sensitive receptor, which mediates glycine's inhibitory actions.

D-Cycloserine is a partial glycine agonist. According to the principles of partial agonist effects, it activates the synapse at low concentrations but has an antagonistic effect on endogenous glycine

at high concentrations. In a series of studies, it has been shown that D-cycloserine improves memory in normal (Pussinen *et al.*, 1997; Kishi *et al.*, 1998; Land and Riccio, 1999) and aged animals (Popik and Rygielska, 1999), and also attenuates memory impairments induced by NMDA antagonists (e.g. MK-801) (Kawabe *et al.*, 1998). Recently, it was also shown that D-cycloserine could attenuate the cognitive impairment induced in monkeys who have developed Parkinson's disease (Schneider *et al.*, 2000). Parkinson's disease is induced experimentally in monkeys after chronic administration of 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) (Gerlach *et al.*, 1991).

In humans, the effects of bioglycine, a biologically active form of the amino acid glycine, were tested on attention and memory in young and middle-aged men. It was shown that bioglycine, whilst improving retrieval from episodic memory, did not affect divided or focused attention in either group. It did, however, improve sustained attention in the middle-aged men (File *et al.*, 1999).

The numerous studies on the cognitive effects of NMDA antagonists on memory and related functions in animals contrast with the few studies on the effects of NMDA antagonists on memory function in humans. The most investigated drug has been ketamine. However, the psychotogenic effects of this compound, and of the other known NMDA antagonists that can be given in humans, limits the investigations into the role of NMDA receptor ligands on memory function.

THE ROLE OF AMINO ACID NEUROTRANSMITTERS IN DEMENTIA

The most common dementias, dementia of the Alzheimer's type and substance-induced persisting dementia, are due to a neuronal degeneration process that parallels the ageing process, except that the mechanisms underlying the initiation and progression of this degeneration process are still, for the most part, unknown. Other common dementias, such as that associated with HIV, are caused by an infection, although the mechanisms underlying the generation of these dementias are also unknown. The role of glutamate, or more generally of the excitatory amino acids, in neuronal degeneration processes is now well established. Glutamate facilitates learning and memory function, while at the same time it can induce neuronal degeneration if it is activated continuously, making the study of the role of glutamate in dementias challenging. GABA and glycine, two important neurotransmitters in the brain, interact with glutamate pathways to control glutamate release and glutamate neuronal activation, respectively, which makes the study of these two neurotransmitter systems and their relationship to dementias also of key importance.

In the previous sections, the roles of NMDA, GABA and glycine in memory and learning were highlighted under normal conditions or when applying experimental models of impaired cognitive function. In the following sections, their roles in the progression of the neuronal degeneration and, more directly, their relationship to a demented brain will be highlighted.

Alzheimer's Dementia

There is evidence that among the neurons most vulnerable to degeneration in Alzheimer's disease are those that utilize glutamate as a neurotransmitter. For instance, pronounced degeneration is observed in glutamatergic neurons of the entorhinal cortex and hippocampus (Braak and Braak, 1991; Hyman *et al.*, 1984; Pearson *et al.*, 1985). The involvement of a degenerative process with respect to glutamate is supported by a decrease in glutamic acid levels, as well as the loss of glutamate terminals in hippocampal and cortical regions in Alzheimer's disease (Cowburn *et al.*, 1990; Hyman *et al.*, 1987; Procter *et al.*, 1989). These findings suggest the importance of degeneration of glutamatergic neurons in the pathological manifestations of Alzheimer's disease (Greenamyre and Young, 1989; Myhrer, 1993; Palmer and Gershon, 1990).

The suggestion that glutamate plays a role in neuronal death due to excitotoxicity is not exclusive to Alzheimer's disease. Amino acid receptor-mediated excitotoxicity appears to contribute to neuronal death associated with several neurological disorders, including epilepsy, hypoxic ischaemic insult, and Alzheimer's disease (Choi, 1988). In a recent study (Aronica *et al.*, 1998), the distribution of NMDA receptors in the hippocampus was compared between Alzheimer's disease patients and controls; it was revealed that in the vulnerable regions, such as the CA1 region, immunolabelling of glutamate subtype receptors (GluR 1/2, GluR5/6/7 and NR1) was reduced in patients, presumably due to cell loss.

Studies have been carried out with post-mortem brains of Alzheimer's disease patients using proton (H^1) and phosphorus (P^{31}) magnetic resonance spectroscopy (MRS) as a technique to identify levels of several metabolites, among which were metabolites of excitatory and inhibitory amino acids. Most of these studies have reported elevations in L-glutamate, indicating that generally compounds related to excitatory neurotransmission increase in Alzheimer's disease (for a recent study, see Klunk *et al.*, 1996).

GABA and Alzheimer's Dementia

It is well established that GABA neurons are spared in Alzheimer's disease (Nagga *et al.*, 1999), whereas glutamate and cholinergic neurons are damaged at specific brain areas, including the hippocampus and the basal forebrain (see above). Studies that set out to examine the distribution of GABA-A receptor subunits in the hippocampus of patients with Alzheimer's dementia have revealed that the $\alpha 5$ subunit-containing GABA-A receptors is relatively spared (Howell *et al.*, 2000), whereas the $\alpha 1$ subunit-containing GABA-A receptors appear to be vulnerable as the disease progresses (Mizukami *et al.*, 1998). The roles of these variable responses of selected GABA subunits in the process of the disease is not clear. However, these data suggest that GABA released from the spared neurons in the hippocampus can still activate the intact $\alpha 5$-subunit-containing receptors. Since GABA interacts with glutamate neurons in the hippocampus (Walker *et al.*, 2001) and with cholinergic neurons in the basal forebrain (Casamenti *et al.*, 1986; Moore *et al.*, 1995) to inhibit the neuronal activation of glutamatergic and cholinergic neurons respectively, its effects upon the remaining glutamatergic or cholinergic neurons must further impair the availability of glutamate and acetylcholine in the hippocampus and cortex, respectively. An inverse agonist at the benzodiazepine site on the GABA-A receptor complex will reverse the GABA inhibition and allow more release of acetylcholine in the cortex and glutamate in the hippocampus. The pharmacology reflecting the relationships between GABA, glutamate and cholinergic neurotransmitter with memory has been demonstrated in several studies, and is summarized in a review by Izquierdo and Medina (1995). Thus, the idea of developing an inverse agonist as a treatment for Alzheimer's disease remains valid (Eid and Rose, 1999; see also discussion in the section on GABA and cognition above).

Glutamate and Alzheimer's Dementia

The role of glutamate in cognitive processes has been highlighted in several reviews, and was discussed earlier in this chapter. Although its role is complex, there are several explanations why dysfunction of glutamate neurotransmission could be an underlying mechanism of Alzheimer's disease. Histological results of neuronal

loss in Alzheimer's disease were associated with reduced glutamate activity in the neocortical pyramidal cells, which are part of the cortico-cortical connections, and also in other neurons in the cerebral cortex (Greenamyre and Maragos, 1993). Memory deficits, as discussed in a previous session, can be seen in animals and humans when glutamate antagonists are administered. Many of the impairments seen resemble the cognitive dysfunction seen in Alzheimer's disease, which further supports the idea that a reduction of NMDA may be the cause of the memory decline seen in Alzheimer's disease. This idea is supported further by the finding that the degree of loss of neocortical pyramidal cells shown to be associated with glutamatergic hypoactivity in Alzheimer's disease patients post-mortem (Greenamyre and Maragos, 1993) correlated well with the severity of cognitive deficits (Sumpter *et al.*, 1986).

In addition, glutamate exerts an excitotoxic action that leads to degeneration of the neurons. This action of glutamate has been especially highlighted in conjunction with ischaemic damage, when glutamate release dramatically increases. Recent evidence suggests that excessive release of glutamate, contributing to excitotoxic neuronal death, may be an aetiological mechanism in chronic neurodegenerative disorders, including Alzheimer's disease (Kornhuber and Weller, 1997). Two recent findings provide additional convincing evidence for a significant role of glutamate in Alzheimer's disease: first, the finding that amyloid precursor protein plays an important role in regulating glutamate levels in the synapse (Mattson *et al.*, 1999), and second that the β-amyloid protein, a key component of senile plaques, can enhance the neurotoxicity of glutamate (Blanchard *et al.*, 1997). Data from autoradiographic ligand-binding studies examining the glutamate receptor integrity in brains of Alzheimer's disease patients have shown conflicting results. Some studies report loss of glutamatergic receptors from cortical and hippocampal areas (Greenamyre *et al.*, 1985; Greenamyre *et al.*, 1987), whereas others report stable levels (Geddes *et al.*, 1986; Monaghan *et al.*, 1987). Several subunits of the NMDA receptor have been identified recently, and there are data to suggest that some subunits are more involved than others in learning and memory (Tang *et al.*, 1999). Thus, the inconsistency in the literature with regard to NMDA receptor density in Alzheimer's disease when compared with controls may reflect different vulnerabilities of the different subunit-protein-containing receptors. In a recent study, a reduction in several of the NMDA subunit protein levels was found in the hippocampus (NR1 and NR2B) and in the entorhinal cortex (NR2A and NR2B) of Alzheimer's disease patients' brains when compared with normal brains (Sze *et al.*, 2001). Importantly, the severity of the decrement correlated strongly with the magnitude of the cognitive impairments as measured by the mini mental state examination. When the NMDA subunit protein level was measured in early-stage Alzheimer's disease and compared with control brains, these differences were not found (Sze *et al.*, 2001). Based on this finding, the authors suggested that the changes in the glutamate subunit protein levels might be the result of a neuronal degeneration, part of the progress of Alzheimer's disease.

There is evidence to suggest that the neurodegeneration seen in Alzheimer's disease may be the result of a hyperactivation of the glutamate neurons. When glutamate is released under normal conditions in the synaptic cleft, where it remains for only 1–2 ms (Clements *et al.*, 1992), it induces a calcium influx into the cell and a short-term depolarization of the neuron, which serves acute physiological demands. If glutamatergic neurons are activated continuously, and glutamate remains for longer in the synaptic cleft, then an increase in calcium influx into the cell occurs. The calcium activates several catabolic enzymes, including nucleases, proteases and phospholipases, which may promote neuronal death. It is now widely accepted that increased activity of these enzymes, and accumulation of their toxic products, promotes neuronal death in hypoxia, ischaemia and trauma. Prolonged exposure to enhanced concentration of glutamate, which activates these enzymes, can therefore induce excitotoxicity *per se* (for a summary review, see Kornhuber and Weller, 1997).

The bimodal properties of NMDA antagonists with regard to cognitive functions, on the one hand to promote neuronal degeneration due to excitotoxicity and induce neuronal lesions, and on the other hand to promote neuronal plasticity, an essential mechanism underlying learning and memory, have challenged researchers when developing treatment approaches.

The role of NMDA antagonists to counteract excitotoxic insults due to either an excess of glutamate activity or other conditions has been well established (Parsons *et al.*, 1999). At the same time however, several reports in the literature have demonstrated the impairment in memory and learning induced by NMDA antagonists (see previous section).

If an impairment of cognitive functions in the first instance is the price to pay for delaying or even blocking a degenerative process that may not have led to any further impairment than the one the treatment itself induced, then an alternative approach should be followed. The non-competitive antagonists MK-801 and PCP, due to their low offset rate from the receptor and weak voltage dependency (Parsons *et al.*, 1999), tend to block the receptor for a long time and independently of its current activation. Because of this, these compounds can prevent possible excitotoxic events occurring, but at the same time reduce the responsiveness of the receptor to a physiological demand.

It has been suggested that memantine, a recently developed weak non-competitive antagonist at the NMDA site, may offer a better treatment approach to Alzheimer's dementia (Parsons *et al.*, 1999). Memantine, due to its receptor kinetics (a combination of fast offset and strong voltage dependency), dissociates rapidly from the receptor when the receptor is activated physiologically, but blocks the receptor when it is activated continually in a moderate fashion (Parsons *et al.*, 1998). Parsons *et al.*, (1999) suggest that memantine may act at the NMDA receptor like a potent Mg^{2+} ion. Thus, memantine, like Mg^{2+}, would leave the receptor channel in the presence of a depolarization event; however, memantine in contrast to Mg^{2+} will remain bound at the receptor if a moderate depolarization is prolonged, as in the case of chronic excitotoxic insults (Parsons *et al.*, 1995; Parsons *et al.*, 1999). An alternative hypothesis was put forward by Blanpied *et al.* (1997) when studying the tendency of non-competitive receptor ligands to be trapped in the NMDA channels. Using cultured rat cortical neurons, they showed that memantine blocked NMDA-activated channels by binding to a site at which it remained trapped after channel closure and agonist dissociation from the receptor. When memantine and the NMDA agonists were removed from the culture medium, a significant number (12%) of the blocked channels released the blocker, i.e. memantine exhibited partial trapping. On the basis of their findings, Blanpied *et al.*, using a simple kinetic model of blocker action, proposed that partial trapping guarantees that during synaptic communication in the presence of a blocker, some channels will release the blocker between synaptic responses. Thus, partial trapping (as in the case of memantine) will allow some receptors to be activated under physiological activation, whereas this will not be possible if the trapping is complete, as in the case of MK-801 or other types of ligands that undergo complete trapping.

There are no data from clinical studies to support the beneficial effects of memantine suggested here. Clinical evaluation of memantine with Alzheimer's disease patients is limited to two studies (Ditzler, 1991; Gortelmeyer and Erbler, 1992) that have demonstrated some functional improvement. However, several reports from preclinical studies have demonstrated the beneficial neuroprotective effects of memantine using animal models of Alzheimer's disease. In one such study, for example (Wenk *et al.*, 1995), NMDA was injected into the nucleus basalis of

Meynert, the main source of cholinergic efferents into the cortex, and the induced decrease in acetylcholinetransferase, indicating a reduction in cholinergic activity, was blocked if memantine was administered beforehand. When the behavioural sequelae of such NMDA-induced lesions were studied, the learning deficits seen in performance on a T-maze were no longer present if the animals were treated with memantine beforehand (Wenk *et al.*, 1994). Furthermore, learning deficits in a spatial learning task seen in animals with entorhinal cortex lesions (thought to be another model of Alzheimer's disease) were improved over time (performance reaching the levels of non-lesioned animals) when memantine, but not MK-801, was administered (Zajaczkowski *et al.*, 1996). In fact, MK-801 further enhanced the lesion-induced impairment of reference memory.

Thus, the ability of memantine to be neuroprotective and to slow down the degeneration of neurons, so restoring cognitive function, may not be in conflict with the known acute effects of NMDA blockers to impair neuronal plasticity (Collingridge and Singer, 1990). However, further studies in the clinical population, or alternatively better animal models of the disease, are required to confirm the hypotheses generated from the preclinical findings.

There are as yet no models of Alzheimer's disease developed in animals to allow the testing of memantine or other glutamatergic antagonists. The models referred to here are functional models dependent on discrete lesions, and do not relate closely to Alzheimer's disease. The recent development of transgenic mice, which appear to express mutations related to the human amyloid precursor protein and which show some features of the disease (Holcomb *et al.*, 1998), will allow the neuroprotective effects of glutamatergic antagonist to be tested in a more convincing manner.

Glycine and Alzheimer's Dementia

The role of the glycine B site at the NMDA receptor in Alzheimer's disease is not well investigated. A new drug, FK 960 (*N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate), a potential antidementia drug, has been developed. *In vitro* studies provide evidence for an interaction of FK 960 with either cholinergic or glutamatergic systems. FK 960 reversed the effect of scopolamine, which had abolished an increase in regional cerebral blood flow response to a vibrotactile stimulation in monkeys. However, FK 960 did not reverse the effect of (+)-3-amino-1-hydroxy-2-pyrrolidone (HA-966), an antagonist of the glycine modulatory site on the NMDA receptors, which had also abolished the increase in regional cerebral blood flow. These findings suggest that FK 960 may only bring about its effects by enhancing cholinergic neurotransmission (Tsukada *et al.*, 1999). There is a report on the beneficial effects of D-glycoserine, a partial agonist at the glycine B site, after short treatment. This improved the scores in the cognitive subscale of the Alzheimer's disease assessment scale for up to 3.0 points when given at a dose of 100 mg per day (Tsai *et al.*, 1998a; Tsai *et al.*, 1999). Some recent studies have also reported beneficial effects of D-glycoserine on cognition in healthy elderly people (File *et al.*, 1999).

Oestrogen and Alzheimer's Dementia

One interesting example of a substance with a complex profile with regard to its interaction with the amino acid neurotransmitters that also has a potent neuroprotective action is oestradiol. Oestrogen replacement therapy for postmenopausal women has proven beneficial in protecting against cognitive deterioration, and has also been shown to decrease the incidence of Alzheimer's disease (Costa *et al.*, 1999; Yaffe *et al.*, 2000a; Yaffe *et al.*, 2000b). Basic neuroscience findings provide an explanation for the mechanisms by which oestrogen may affect cognition. There is evidence that oestradiol increases dendritic spine density in hippocampal neuronal cell cultures (a function related to neuronal plasticity, by reducing GABA neurotransmission in hippocampus) (Murphy *et al.*, 1998). Oestrogen was also shown to enhance basal forebrain, hippocampus and cortex cholinergic activity by influencing the synthetic enzyme for acetylcholine (Gibbs and Aggarwal, 1998) and by increasing the number of cholinergic neurons (Miller and Franklin, 1999; Miller *et al.*, 1999). It has also been reported that oestradiol enhances compensatory synaptic sprouting in the outer molecular layer of the dentate gyrus in response to an entorhinal cortex lesion (Stone *et al.*, 1998). *In vitro* studies examining the protective effects of oestrogen against excitotoxic insults causing neuronal injury or death, such as hypoxia, ischaemia or excitatory amino acids, have also shown that oestradiol prevents neuronal injury and degeneration including cellular death (Weaver *et al.*, 1997; Harms *et al.*, 2001). There is some evidence that this neuroprotective effect may be due to a negative modulation of NMDA receptors (Weaver *et al.*, 1997).

On the basis of such findings, researchers have suggested that replacement of oestrogen in elderly females would reinstate, at least in part, cognitive abilities and may prevent the incidence of Alzheimer's disease. This proposal has received a measure of empirical support in the clinical setting. Kimura (1995) demonstrated that women receiving oestrogen replacement therapy outperform women not receiving such therapy on verbal fluency, perceptual speed, spatial awareness, motor control and articulation. Several studies since have confirmed the protection of hormone replacement therapy (HRT) against cognitive deterioration and incidence of Alzheimer's disease (Birge, 1996; Birge, 1997; Birge *et al.*, 2001; Costa *et al.*, 1999; Yaffe *et al.*, 2000b; but see Binder *et al.*, 2001). These reports on the effects of HRT on cognitive deterioration and/or incidence of Alzheimer's disease derive from studies with women who needed or wished to have the oestrogen replacement, and who had started the treatment mostly in the perimenopausal age band. We have published a study (Duka *et al.*, 2000) that was designed to investigate further the effects of oestrogen on memory in a group of healthy elderly females aged 55–65 years who had never previously taken HRT. We were able to demonstrate that even a 3-week treatment regime with oestrogens can improve aspects of learning and memory.

Although there are several empirical reports on the reduced incidence of Alzheimer's disease among women who were given HRT, there is only one prospective study testing the effects of oestradiol on the progression of Alzheimer's disease (Mulnard *et al.*, 2000). In this study, the progression of the disease over 1 year (at 2, 6, 12 and 15 months of follow-up) was compared between placebo and oestradiol treatment and evaluated on the basis of change on the clinical global impression scale, as well as on global measures of mood, memory, attention, language and motor function. There was no difference in the progression of the disease between the two groups. Thus, the findings from this prospective study did not support the role of oestrogen for the treatment of this disease; it did not, however, reject a protective role of HRT treatment for the development of Alzheimer's disease. In a previous small study with Alzheimer's disease patients in which cognitive tasks like the Stroop and Buschke verbal memory were used as primary measures, there were positive effects found on cognition following transdermal application of oestradiol (Asthana *et al.*, 1999). From the evidence presented here, it can be concluded that oestradiol effects on cognition and neuroprotection may be related, at least in part, to mechanisms involving the excitatory and inhibitory amino acids. For instance, the protective effect of oestradiol against neuronal death is thought to be due, among other factors, to an inhibition of NMDA receptors (Weaver *et al.*, 1997). The increase of dendritic spine density in the pyramidal cells of

the CA1 area of the hippocampus induced by oestradiol is thought to be related to an increase in NMDA binding and an increase in the sensitivity of the pyramidal cells to NMDA synaptic input, suggesting that the new spines and synapses induced by oestradiol are enriched in NMDA receptors. This effect of oestradiol to induce an increase in dendritic spine density is suggested to be due to the reduction of GABA inhibition by oestradiol in this hippocampal area (Murphy *et al.*, 1998).

Alcohol-Induced Dementia

It is well established that chronic alcohol abuse induces morphological, physiological and biochemical changes in the brain that are associated with cognitive impairment. The cognitive deficits can be attributed mostly to associated frontal pathology (Joyce and Robbins, 1991; Joyce and Robbins, 1993; Oscar-Berman, 1980; Tarter *et al.*, 1985; Stephens *et al.*, 1992; Fein *et al.*, 1990). The amnestic effects of alcohol, however, appear to be related more to hippocampal dysfunction (Melia *et al.*, 1996; Ryabinin, 1998). In chronic alcoholics, Korsakoff's syndrome or alcohol dementia may be present. Korsakoff's syndrome is characterized by severe anterograde memory impairment, whereas other cognitive abilities remain intact. Alcohol dementia, however, presents with additional cognitive deficits. Wernicke's encephalopathy is an acute neurological condition characterized by mental confusion, abnormal gaze, ataxia, and loss of recent memory (Langlais, 1995; Fadda and Rossetti, 1998). It is assumed that a basic mechanism, an alcohol-induced neuronal degeneration process, must be involved in the sequelae of chronic alcoholism.

Ethanol possesses a complex pharmacology (Littleton and Little, 1994), which includes facilitation of GABAergic transmission through an action at the GABA–benzodiazepine-chloride-channel complex (Suzdak *et al.*, 1986; Mehta and Ticku, 1988), antagonism of glutamatergic transmission through an action at NMDA glutamatergic receptors (Hoffman *et al.*, 1989; Lovinger *et al.*, 1989), and an action at 5-hydroxytryptamine (5-HT) 5-HT_3 (Lovinger and White, 1991) and $5HT_1$ receptors (Grant and Colombo, 1993). Alcohol also interacts with neuronal voltage-gated calcium channels (Twombly *et al.*, 1990; Littleton and Little, 1994). It is now well established that the neurophysiological and brain-damaging effects of alcohol are mediated mainly via the glutamatergic system. Alcohol acutely disrupts the glutamatergic neurotransmission by inhibiting the response of NMDA receptors. In a study with recently detoxified alcoholics, administration of ketamine hydrochloride, an NMDA receptor antagonist, induced ethanol-like subjective effects (Krystal *et al.*, 1998b). In addition, the chronic effects of ethanol appear to be the result of a promotion of neuronal toxicity through upregulation or changes in the functional state of NMDA receptors. For example, superfusion with ethanol of hippocampal slice preparations or primary cell cultures reduces excitatory synaptic transmission (Lovinger *et al.*, 1989; Lovinger *et al.*, 1990; Wirkner *et al.*, 1999) on to NMDA receptors, as measured by NMDA-activated membrane current. It is generally agreed that chronic ethanol treatment will increase the sensitivity of neurons to NMDA agonists (Blevins *et al.*, 1995). However, the findings with regard to an upregulation of NMDA receptors are less consistent.

Whereas several studies have reported an increase of NMDA receptor binding (Hoffman *et al.*, 1992; Hoffman *et al.*, 1995) or an upregulation of certain NMDA receptor subunits (e.g. Hu *et al.*, 1996; Follesa and Ticku, 1996), others report no change in NMDA receptor density or subunit levels (Chandler *et al.*, 1997; Blevins *et al.*, 1997). In another study in which the brains of alcoholic patients were compared with those of matching normal controls for their maximal binding and affinities of NMDA receptors, using as ligands competitive and non-competitive antagonists, no differences were found (Freund and Anderson, 1999). In a recent study, Nagy *et al.* (2001) investigated the chronic effects of ethanol in an *in vitro* preparation in which chronic but intermittent ethanol treatment was applied on primary cultures of cortical neurons. This kind of ethanol treatment is thought to provide a good model for the study of the mechanisms underlying the kindling-like phenomenon of withdrawal observed in humans (Ballenger and Post, 1978; Brown *et al.*, 1988). In the Nagy *et al.* study, the damage of neuronal cells when alcohol was withdrawn from the medium was measured in the absence and in the presence of an NMDA non-competitive antagonist MK-801, using as a marker lactate dehydrogenase (LDH) released into the culture media. The increase in LDH during withdrawal was reduced by either introduction of alcohol again or by addition of MK-801 into the culture media. Interestingly, the presence of a GABA-A receptor antagonist muscimol in the culture media did not show any effect, further supporting the view that NMDA mechanisms are involved in the excitotoxic effects of withdrawal from alcohol (Stephens, 1995; Nagy *et al.*, 2001). However, when cerebrospinal fluid (CSF) levels of excitatory amino acid neurotransmitters (aspartate, glycine and *N*-acetylaspartylglutamate) and of GABA, together with markers of oxidative stress, were measured in alcoholics 1 week and 1 month after alcohol withdrawal, the excitatory amino acid levels were found to be increased, while those of GABA were decreased. In addition, a relationship was found between the excitatory amino acid levels and the levels of oxidative stress markers. Thus, an increase in the activity of the glutamatergic system at withdrawal and subsequent brain damage may be caused partly by an inhibition of GABAergic inhibitory systems (Tsai *et al.*, 1998b).

It is well established that patients undergoing alcohol withdrawal are more likely to experience seizures if they have undergone previous episodes of detoxification (Booth and Blow, 1993; Brown *et al.*, 1988; Lechtenberg and Worner, 1991). Analogous findings have also been reported from animal experiments, which showed that the severity of the withdrawal intensity increased following several withdrawal episodes (Becker, 1994; Becker and Hale, 1993; Baker and Cannon, 1979; Kokka *et al.*, 1993). While the development of sensitization to repeated ethanol withdrawal is illustrated clearly by the increase in seizure sensitivity in both patients and animal models, it is less clear whether cognitive deterioration seen in alcoholism also undergoes a similar process of sensitization. Nagy *et al.* (2001), for example, have suggested that the processes seen during withdrawal may be relevant for increased excitability, but they may also initiate an excitotoxicity and loss of neurons.

Korsakoff's syndrome is always accompanied by nutritional deficiencies, in particular vitamin B1 (thiamine) deficiency. Administration of thiamine will reduce the acute symptomatology of Korsakoff's syndrome and will protect the patient from further impairment due to the condition (Cook, 2000). In addition, thiamine was found recently to improve amnestic symptomatology seen in alcoholics who otherwise did not show any of signs of Korsakoff's syndrome (Ambrose *et al.*, 2001). However, incidents of Korsakoff's syndrome following thiamine deficiency alone are rare (for an example, see Parkin *et al.*, 1991), and most cases with pure thiamine deficiency unaccompanied by chronic and excessive consumption of alcohol do not progress to Korsakoff's syndrome (Homewood and Bond, 1999).

Neuropathological studies have shown a significant decrease in volume and weight of the brain, whilst imaging techniques have revealed significant functional alterations of the frontal lobe (Hunter *et al.*, 1989; Hunter, 1990), followed by the thalamus, hypothalamus (Shear *et al.*, 1996; Sullivan *et al.*, 1999; Kril *et al.*, 1997; Harding *et al.*, 2000), basal nucleus of Meynert (Arendt *et al.*, 1983; Lotfi and Meyer, 1989; Butters, 1985), and the medial temporal lobe structures in Korsakoff's syndrome or alcohol dementia sufferers (Fadda and Rossetti, 1998). However, little is known

about the underlying mechanisms involved in alcohol-induced brain damage. As discussed above, there is accumulated evidence that withdrawal excitability may share a molecular mechanism of neurotoxicity linked to an increase in the NMDA neurotransmission. Recent research into the relationship between alcoholism and dementia has focused on the role of the nutritional deficiencies present in chronic alcoholism. Pyrithiamine, which induces thiamine deficiency, produces a range of neuropathological and behavioural abnormalities in rodents and has been used as a model of Wernicke–Korsakoff syndrome.

Studies on thiamine-deficiency-induced brain damage have again provided some evidence of an NMDA involvement in the development of Korsakoff's syndrome in chronic alcoholism. Pyrithiamine-treated animals showed a higher sensitivity to the amnestic effects of NMDA antagonists (Savage *et al.*, 1999). Neuronal degeneration seen in the thalamus of animals treated with pyrithiamine was found to be similar to the cytopathological changes induced by glutamate (Ikonomidou *et al.*, 1989). In addition, the neuronal degeneration was blocked with pretreatment with the NMDA antagonist dizolcipine. In another study, a neuroprotective effect of MK-801, a non-competitive NMDA antagonist, against pyrithiamine-induced neuronal damage in the thalamus has been reported. However, in this report the effect of the NMDA antagonist was attributed mostly to its anticonvulsant activity (Todd and Butterworth, 1998).

Recent reports in the literature have highlighted a relationship between homocysteine levels and alcoholism. Homocysteine is a sulphur-amino acid putative neurotransmitter at the NMDA receptors. Its role in vascular diseases has been known for some years. Its importance in relationship to alcoholism has been discussed only recently, however. In one study, homocysteine levels were found to be high in 29 chronic alcoholics compared with controls who underwent withdrawal from alcohol (Bleich *et al.*, 2000b). In another study, Bleich *et al.* (2000a) found higher levels of homocysteine in plasma samples of patients who suffered withdrawal seizures during withdrawal compared with alcoholics who did not. The authors also reported that the same patients had more alcohol in their blood at admission; however, homocysteine levels were the best predictor for the occurrence and intensity of seizures at withdrawal. High levels of homocysteine were also found in social drinkers who consume high amounts of alcohol (Koehler *et al.*, 2001). As discussed above, the development of seizures at withdrawal may be the result of a neuronal kindling mechanism associated with increased sensitivity of the NMDA receptor. It may therefore be reasonable to suggest that increased homocysteine (an agonist at the NMDA receptor) may be an additional factor involved in this phenomenon. A direct relationship between homocysteine and alcohol dementia has not yet been found; therefore, homocysteine may be involved in the damage of brain areas that are not part of learning and memory function.

HIV Dementia

Dementia due to HIV is the most common cause of dementia in children, young adults and middle-aged people, according to a recent review (Nath and Geiger, 1998). The dementia is characterized by cognitive decline, motor dysfunction and behavioural abnormalities associated with pronounced cortical atrophy (Atwood *et al.*, 1993; Power and Johnson, 1995). The toxic effects of the HIV-1 envelope glycoprotein gp120 was thought to be responsible at least in part for the dementia seen in HIV (Doble, 1999; Lipton, 1992c; Pugh *et al.*, 2000). *In vitro* studies showing that the toxic effects due to this protein coat were modulated by activation or blocking of glutamate receptors led to the hypothesis of an involvement of glutamate in these toxic effects (Lipton, 1992a; Lipton, 1992b). Further studies have demonstrated that the presence of macrophages generated during the infection process is essential for the toxic effects of gp120 to be manifested. Previous studies have shown that monocytes infected by HIV release small molecules that activate the NMDA receptors (Giulian *et al.*, 1990; Giulian *et al.*, 1993), confirming a relationship between excitatory amino acid activation and the dementia seen in HIV. The authors of these latter studies have proposed that this molecule may be quinolinic acid, a potent NMDA receptor agonist.

In the early 1980s, quinolinic acid was thought to be a non-physiological active metabolite of tryptophan. It is now suggested that quinolinic acid is a selective activator of the glutamate receptors sensitive to NMDA, which leads to an excitation of neurons seen as an increase in firing rate (Stone, 1993). Although the neurons themselves are not infected by the HIV-1 virus, at least part of the neuronal injury observed in the brain is thought to be mediated by NMDA receptor activation (Lipton, 1994).

Quinolinic acid is the only known endogenous compound that activates the glutamate receptors selectively for NMDA. Recent reports suggest strongly that quinolinic acid may be an important factor in the development of AIDS dementia. In patients with the AIDS–dementia complex, the levels of quinolinic acid in the CSF are increased up to 20-fold (Heyes *et al.*, 1989; Heyes *et al.*, 1991), and levels in the brain are reported to be 300-fold higher than in the CSF.

Children with HIV-1 infection also showed increased quinolinic acid in the CSF compared with controls. In post-mortem brains of AIDS patients, the levels of quinolinic acid were found to be approximately 20 times higher than in controls (23 pmol 10 mg *v*. 1 pmol 10 mg (Achim *et al.*, 1993). Changes in the quinolinic acid in the CSF might be the consequence of general brain deterioration. However, there are no reports of an increase of quinolinic acid in the CSF or the post-mortem brains of Alzheimer's disease patients. One study has reported high levels of kynurenic acid in all areas of the Alzheimer's diseased brain (especially in the striatum, which was found to contain double the normal levels) (Baran *et al.*, 1999). Kynurenic acid is another endogenous compound that blocks glutamate receptors. The authors suggested that the levels of kynurenic acid might have been enough to block the NMDA receptors to induce a decrease of memory.

These findings have led to the introduction of clinical studies to test the potential of NMDA antagonists in treating HIV dementia. At present, the psychogenic effects seen in HIV sufferers are treated by drugs such as neuroleptics or selective serotonin reuptake inhibitors (SSRIs), depending on whether psychosis or depression is the prominent clinical syndrome. Other mechanisms, e.g. an excess of concentration of chemokines (Rausch and Stover, 2001), as possible mechanisms underlying the excitotoxicity seen in HIV have also been discussed and supported by recent studies, but it is beyond the scope of this chapter to present mechanisms unrelated to amino acids.

Vascular Dementia

Vascular dementia results from brain damage at certain areas of the brain where blood supply has been limited due to the damage of arteries. The process is the result of neuronal necrosis following limited blood supply, i.e. hypoxia. Hypoxia results in excitotoxic events that must involve glutamate receptor activation. However, when the clinical picture of vascular or multi-infarct dementia is manifested, the processes that lead to the cell or brain area damage are no longer present. It may be that this could be the reason why there is very little information in the literature about the biological mechanisms underlying multi-infarct dementia.

Brain Injury

Traumatic brain injury is one of the leading causes of death and disability. Cognitive disorders and amnesia are some of the disabilities seen in traumatic brain injury. Like many of the cognitive disorders discussed in this chapter, in traumatic brain injury many excitotoxic processes take place that could be associated with NMDA release. One important difference in the case of traumatic brain injury is that a surprisingly good outcome can be seen in young patients, which is the result of compensation for lost function rather than recovery of the injured area.

The young brain that still retains plasticity is able to compensate, whereas an elderly brain has less plasticity and is more likely to suffer from degeneration. Thus, the management of cognitive disorders aims not only to stop the degeneration process but also to allow healthy tissue to compensate functionally. It should be commonly accepted that recovery is not possible.

SUMMARY

This chapter has looked at the role of amino acid transmitter systems in cognitive disorders. The main inhibitory amino acid neurotransmitter, GABA, and the main excitatory amino acid neurotransmitter, glutamate, in the brain are both implicated in the dementias, firstly by their direct participation in learning and memory processes, and secondly by their involvement in neuronal degeneration.

The first part of this chapter examined the function of GABA and glutamate in cognition. Most information on the role of GABAergic activity on cognition is derived from reports of the effects of benzodiazepine ligands on memory. Whereas benzodiazepines have an amnestic effect, partial inverse agonists at the benzodiazepine receptors enhance memory. However, the weakness of the effect and the psychotogenic side effects mean that these ligands are of little use in the treatment of Alzheimer's disease. The $\alpha 5$ GABA receptor subunit is now suggested to be the site that mediates the amnestic effects of benzodiazepines, therefore new benzodiazepine inverse agonist ligands specific for the $\alpha 5$ GABA receptor subunit may be the future candidates for treating Alzheimer's dementia. It is well established that NMDA plays a crucial role in learning and memory. NMDA antagonists have been shown to impair memory in animal studies, but as yet there are no useful compounds that interact with NMDA receptors that can be used in human studies as tools to understand the role of glutamate on memory.

The second part of this chapter explored the role of amino acids in dementia in Alzheimer's disease, alcoholic dementia and HIV. It is now well established that glutamate, or more generally the excitatory amino acids, play a part in neuronal degeneration processes. Glutamate facilitates learning and memory function, while at the same time it can induce neuronal degeneration if it is activated continuously. Dysfunction of glutamate neurotransmission could be an underlying mechanism of Alzheimer's dementia. A substance with a complex profile with regard to its interaction with the amino acid neurotransmitters is oestradiol. Oestrogen replacement therapy for postmenopausal women has proven beneficial in protecting against cognitive deterioration, and it has also been shown to decrease the incidence of Alzheimer's disease. There is some evidence that this neuroprotective effect may be due to a negative modulation of NMDA receptors.

It is now well established that the neurophysiological and brain-damaging effects of alcohol are mediated mainly via the glutamatergic system. Alcohol acutely disrupts glutamatergic neurotransmission by inhibiting the response of NMDA receptors. In addition, the chronic effects of ethanol appear to be the result of a promotion of neuronal toxicity through changes in the functional state of NMDA receptors. Studies on thiamine-deficiency-induced brain damage have again provided some evidence of an NMDA involvement in the development of Korsakoff's syndrome in chronic alcoholism, and recent reports have highlighted a relationship between levels of homocysteine, a neurotransmitter at the NMDA receptors, and alcoholism, although no direct relationship has yet been found between homocysteine and alcoholic dementia.

There is a confirmed relationship between excitatory amino acid activation and the dementia seen in HIV. Studies have shown that monocytes infected by HIV release small molecules that activate the NMDA receptors. The molecules may be quinolinic acid, which is suggested to be a potent and selective activator of the glutamate receptors sensitive to NMDA. These findings have led to the introduction of clinical studies to test the potential of NMDA antagonists in treating HIV dementia.

Thus, the proposal that amino acid transmitter systems, particularly GABA and glutamate, play a role in cognitive disorders has been backed by considerable evidence from both human and animal studies. Several promising compounds may in future provide relief from the neurodegenerative effects of dementia.

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XV-4

Neuropeptides and Cognitive Disorders

Garth Bissette

INTRODUCTION

Evidence for the role of neuropeptides in cognitive disorders includes several possible avenues: (1) the concentration of the neuropeptide may be found to be altered in brain regions or cerebrospinal fluid (CSF) of subjects with a cognitive disorder; (2) the neuropeptide may be associated with a metabolic or pathologic event that produces cognitive dysfunction; or (3) the administration of a neuropeptide receptor agonist or antagonist for therapeutic purposes may increase or decrease cognitive abilities. For the purposes of this review, we will not focus on neuropeptides located outside the central nervous system (CNS), and we will not attempt to incorporate the extensive literature on peripheral endocrine contributions to the cognitive state. We will review the existing data on cognitive alterations associated with CNS neuropeptides as found in both the human and laboratory animal literature.

Cognitive disorders are described in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association, 2000) as encompassing delirium, dementia and amnesia. Human cognition involves memory, visuospatial skills, reading, writing, mathematics, executive abilities and abstractive function. For most laboratory animals, only memory functions are directly assessable in determining cognitive abilities, although primates and some species of monkey may exhibit some executive and abstractive cognitive behaviours that can be quantitated reproducibly. Amnesia is characterized by a memory disorder in the absence of other cognitive impairments, while delirium is characterized by a disturbance in consciousness and cognition that develops over a short period of time. Dementia is characterized by a memory disturbance and one or more other cognitive deficits. Because delirium is a relatively rapid and usually transient phenomena, there is not as much research literature investigating the potential role of neuropeptides in this disorder. In contrast, the literature for both amnesia and dementia is more extensive — in the latter case, quite voluminous. This review will, necessarily, limit the coverage of these research domains to approximately the last decade, although seminal findings from earlier research publications will be mentioned.

NEUROPEPTIDE NEUROBIOLOGY

For a more comprehensive review than space allows here, see Owens *et al.* (2000) or Hokfelt *et al.* (1995). Neuropeptides are defined by convention as encompassing approximately 90 or fewer amino acids, with a combined molecular weight of less than 10 000 daltons. They are phylogenetically ancient molecules that have often been conserved across phyla to a remarkable degree. In fact, a yeast peptide mating factor shares a similar sequence of amino acids as the mammalian gonadotropin-releasing hormone (GnRH) neuropeptide. Neuropeptides are produced by transcription of DNA and translation of RNA into a protein product. This precursor protein or prohormone contains one or more copies of the active neuropeptide, which is cleaved from the precursor protein by specific processing enzymes during transport from the ribosome–Golgi complex to storage sites in vesicles. The active neuropeptide is then released into the synaptic gap, where it competes for access to the postsynaptic receptor. After binding to the receptor, the neuropeptide or its active fragment either is incorporated into the postsynaptic cell or dissociates from the receptor binding site.

Neuropeptide signalling is halted by degradation of the neuropeptide sequence into smaller fragments or individual amino acids, which are then incorporated into the general metabolic amino acid pool. Several specific peptidase enzymes that recognize particular sequences of amino acids cleave the active peptide into fragments and eventually single amino acids. No evidence for reuptake of intact neuropeptides by the presynaptic neuron has been described as occurs for the biogenic amine neurotransmitters.

Because only a few amino acids within the complete peptide sequence are required for binding to the neuropeptide receptor, it is possible for neuropeptide metabolic fragments to have activity at the receptor or for specific receptors to exist for the fragment. Furthermore, many neuropeptide families are composed of peptides with homologous sequences of amino acids but with separate messenger RNA and genes. If a peptide receptor recognizes the sequences held in common by the neuropeptide family, then it is possible to have several ligands competing for the binding site, usually with slightly different affinities. All neuropeptide receptors characterized to date use G-protein-coupled second messengers to transduce the signal at the postsynaptic membrane. Some neuropeptides, such as neurotensin or thyrotropin-releasing hormone (TRH), complex with a receptor that is then translocated from the neuronal membrane to the cell nucleus, where specific regulatory effects on gene expression ensue.

The physiological effects of neuropeptides continue for minutes to hours after binding to their receptors, compared with the seconds or minutes for many of the classical chemical and amino acid neurotransmitters. These characteristics are all the more remarkable because the concentrations of neuropeptides range from the picomolar to femtomolar, relative to the nanomolar to micromolar concentrations of the classical chemical neurotransmitters or the millimolar concentrations of certain amino acid neurotransmitters, such as glutamic acid.

Thus, neuropeptides possess unique ability to mediate chronic physiological effects, such as psychiatric symptoms. Neuropeptides themselves, however, have major limitations as direct therapeutic agents. Usually, they cannot be delivered orally, as gut peptidases are designed to destroy them. Unless designed for a physiological role via the circulatory system, most neuropeptides have quite short half-lives in blood and few are capable of penetrating the

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blood–brain barrier. Thus, much hope for direct manipulation of neuropeptide receptors rests in chemicals that have similar three-dimensional structures with the active amino acid sequence (epitope) of the neuropeptide of interest. Because the amino acids that compose the epitope recognized by the receptor are often not contiguous in the amino acid sequence, this task requires an understanding of tertiary protein structure in solution, which is a notoriously difficult problem. However, several pharmaceutical companies have developed chemical agonists or antagonists for proteins that bind to neuropeptides, and a few of these are currently in clinical trials.

Most neuropeptides can be measured directly only by immunoassay, which uses the unique ability of the immune system to recognize subtle differences in amino acid sequences of proteins. Radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) are used principally to measure quantitatively relative concentrations, while immunohistochemistry reveals precise cellular anatomic location. The information gained from such assays has both strengths and weaknesses. Strengths of quantitative assays include an assessment of relative concentration that approximates the contributions of extracellular synaptic availability and vesicular content of the target neuropeptide. Weaknesses of immunoassays are the possible recognition of similar epitopes in fragments of the active peptide or related peptides with homologous sequences, and the inability to determine functional meaning from concentration measures.

If the relative concentration of a neuropeptide differs across experimental groups, there are several mechanisms that could contribute to such changes. An increase in expression of the precursor protein product of the neuropeptide messenger RNA that is processed to the active neuropeptide in rates that exceed degradation by peptidases is one possibility. Other possibilities are decreased degradation relative to active peptide production, decreased release of the active peptide from vesicular stores with continued synthesis of new product, and a combination of these. The reverse can be said for decreased concentrations. Without evidence of releasability and evaluation of peptidase activity and mRNA expression, the turnover of a neuropeptide cannot be approximated. Having stated this, it must be said that one can come much closer to an understanding of synaptic availability with concentration evidence alone than one can with either mRNA content or peptidase activity alone. Knowledge of mRNA concentration alone is particularly limited as the expression level, mRNA degradation and synthesis rates, and precursor processing rate all affect active neuropeptide concentrations. Neuropeptide receptor populations are subject to similar constraints, although an increase or decrease in receptor numbers can give evidence for chronically decreased or increased release of their endogenous neuropeptide ligands, respectively.

Over 100 distinct, biologically active neuropeptides have been discovered that occur in the CNS of animals. Estimates are that there may be several hundred in total. A majority of these have specific receptors, but only a few have well-defined physiological roles. Those that have been implicated in cognitive disorders number between ten and 20, but only a few of these have been associated reproducibly with specific cognitive deficits.

ADRENOCORTICOTROPIC HORMONE

This 39-amino acid pituitary hormone (see Table XV-4.1) is derived from a larger 265-amino acid precursor, proopiomelanocortin (POMC), which contains sequences that give rise to beta and gamma lipotropin, beta-endorphin, and alpha, beta and gamma melanocyte-stimulating hormones. A sequence of seven amino acids in positions 4–10 of adrenocorticotropin hormone (ACTH) are also contained in the melanocyte-stimulating hormones. DeWied and colleagues were among the first to demonstrate that ACTH 4–10 could influence cognitive behaviours. Using electric foot shock to induce laboratory rats to climb a pole to escape, it was demonstrated that peripheral administration of ACTH 4–10 in physiologically large doses would increase the latency for extinction of this learned response. An analogue of ACTH 4–10 with the substitution of a dextrarotary phenylalanine residue in position 7 exhibited the reverse effect on extinction of an active avoidance response (van Nispen and Greven, 1982) and a [Met $(O_2)^4$, D-Lys^8, Phe^9-ACTH 4–9] analogue known as Organon 2766 with oral activity was developed for clinical use.

VASOPRESSIN

Release of ACTH is regulated physiologically by several hypothalamic neuropeptides, including corticotropin-releasing factor (CRF), urocortin and vasopressin (Table XV-4.1). Two types of peripheral vasopressin receptors (VP1 and VP2) have been identified (see Rinaman *et al.*, 1995 for review). It is not surprising that vasopressin has also been shown to be active in facilitating laboratory rat performance in a memory task (see Van Ree *et al.*, 1985 for review). Le Moal (1992) demonstrated that peripheral vasopressin may produce these effects by a generally aversive, systemic effect on peripheral vasopressin receptors rather than at CNS sites. Later work,

Table XV-4.1 Structures of neuropeptides implicated in cognitive disorders

ACTH	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe-OH
Vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Beta-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
LEK	Tyr-Gly-Gly-Phe-Leu-OH
MEK	Tyr-Gly-Gly-Phe-Met-OH
Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH
Dynorphin B	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr-OH
Galanin	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Val-Gly-Asn-His-Arg-Ser-Phe-Ser-Asp-Lys-Asn-Gly-Leu-Thr-Ser-OH
TRH	pGlu-His-Pro-NH_2
SRIF	Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
CRF	Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-Ala-His-Asn-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala-NH_2
Cholecystokinin	Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-OH

however, demonstrated that direct injection of vasopressin into rat brains could facilitate memory consolidation, and that this effect could be blocked by antagonists of the VP1 receptor. Using a vasopressin antagonist applied directly into the lateral septum, social recognition was reported to be impaired in male rats while spatial memory was unaffected (Everts and Koolhaas, 1999). Further evidence of vasopressin's role in social memory in males subjected to a vasopressin antagonist was demonstrated in rats receiving prenatal doses of an androgen antagonist, flutamide. Rats so treated were able to recognize familiar juveniles in spite of treatment with the vasopressin receptor antagonist (Axelson *et al.*, 1999), indicating that an intact androgen supply during development is crucial to the social recognition effects of vasopressin.

Initial double-blind, crossover clinical trials of an intranasally administered vasopressin analogue, desglycinamide-9-arginine-8-vasopressin, (Organon 5667), for memory improvement in patients with amnestic disorders such as Korsakoff's syndrome, Alzheimer's disease and head injury was not successful (Jennekins-Schinkel *et al.*, 1985). However, basal plasma vasopressin secretion in Korsakoff's syndrome subjects is elevated relative to non-alcoholic controls, and stimulation of vasopressin secretion with hypertonic saline produced fluctuations in vasopressin in Korsakoff's syndrome subjects that were seen to a lesser degree in non-amnestic alcoholics, indicating dysregulation of vasopressin in these disorders (Emsley *et al.*, 1995). Subjects with memory impairment due to electroconvulsive therapy (ECT) (Mattes *et al.*, 1990) and severe impairments due to Korsakoff's syndrome did not benefit from intranasal vasopressin or vasopressin analogues, but those with mild cognitive deficits due to diabetes or alcoholism did show improvement (Laczi *et al.*, 1987). While an early report of Organon 5667's potential effectiveness in improving memory of a list of low imagery words in Alzheimer's disease subjects has been reported (Peabody *et al.*, 1985), neither the Jennekins-Schinkel study (1985) nor a later, multicentre, double-blind, placebo-controlled trial of Organon 5667 in 115 Alzheimer's disease subjects over 84 days (Wolters *et al.*, 1990) showed promise as a treatment for dementia.

There have been two independent reports of altered secretion of vasopressin in alcoholics undergoing severe withdrawal symptoms. The first study found increased plasma vasopressin in withdrawn alcoholics relative to normal controls (Emsley *et al.*, 1987), while the second study reported increased vasopressin in the withdrawn alcoholic patients with delirium tremens, but decreased vasopressin secretion in the alcoholics after 10 days of withdrawal (Trabert *et al.*, 1992).

Vasopressin involvement in dementing disorders has not been researched extensively, but at least one report has claimed an increase in vasopressin levels in the temporal cortex of post-mortem Alzheimer's diseased brain (Labudova *et al.*, 1998). Most reports concerning vasopressin changes in Alzheimer's disease have not found any differences. No differences in control and Alzheimer's disease levels of vasopressin in the locus coeruleus have been observed, despite degeneration of noradrenergic cells in this region in late-stage Alzheimer's disease (van Zwieten *et al.*, 1994). The number of CRF neurons in the paraventricular nucleus of the hypothalamus that also colocalize vasopressin are increased similarly in Alzheimer's disease and controls with age (Raadsheer *et al.*, 1994), although vasopressin mRNA levels in the paraventricular nucleus of the hypothalamus and supraoptic nucleus remain unchanged in Alzheimer's disease and normal subjects (Lucassen *et al.*, 1997). There is some disagreement about the status of plasma vasopressin in Alzheimer's disease, with reports of no change in plasma or CSF relative to controls (Peskind *et al.*, 1995a) and reports of an increase in plasma but not CSF levels of vasopressin (Nadal *et al.*, 1994). Reports of increased numbers of vasopressin receptors in the choroid plexus have been published (Korting *et al.*, 1996), but vasopressin secretion after overnight water deprivation (Albert *et al.*, 1994) or due to hyperosmotic challenge (Peskind *et al.*, 1995b) does not reveal differences between Alzheimer's disease subjects and controls. These data indicate that vasopressin remains relatively unaltered in Alzheimer's disease, and argue against a vasopressin deficit underlying the memory disturbance seen in Alzheimer's disease. However, recent data indicate that vasopressin and bradykinin stimulate secretion of the amyloid precursor protein (APP) by activating alpha-secretase (Nitsch *et al.*, 1998). As alpha-secretase processing of APP yields less of the potentially toxic beta-amyloid 1–40, vasopressin receptor agonists may have a therapeutic role in Alzheimer's disease.

SUBSTANCE P

Substance P (Table XV-4.1) is an 11-amino acid member of the neurokinin peptide family that has been implicated in learning and memory functions in normal and lesioned laboratory animals (see Huston and Hasenohrl, 1995 for review). The memory-enhancing and -reinforcing effects of full-length substance P and C-terminal fragments of substance P are seen after nanomolar peripheral doses of substance P, and are accompanied by increased dopamine turnover in the nucleus accumbens but not the striatum. Others have reported that the long-lasting (21 days), memory-enhancing effects of post-training, peripherally administered substance P in rats reside in the N-terminus (substance P 1–7), with C-terminal fragments (substance P 7–11) being inactive (Tomaz and Nogueira, 1997). The substance P 1–7 N-terminal fragment has been shown to increase the *N*-methyl-D-aspartate (NMDA) receptor subunit NR2A in the hippocampus, periaqueductal grey and ventral tegmental areas and subunits NR2B and NR1 in the hippocampus and nucleus accumbens within a few hours of intracerebroventricular injection (Zhou *et al.*, 2000), indicating a glutaminergic mechanism for memory enhancement by substance P. This beneficial effect of exogenous substance P on memory is not limited to mammals, as goldfish also improve performance in an appetitive-motivated memory task after peripheral substance P injection (Mattioli *et al.*, 1997). Depending on where in the brain exogenous substance P is injected after training, either facilitation of memory (lateral hypothalamus; Staubli and Huston, 1979) or induction of amnesia (substantia nigra and amygdala; Huston and Staubli, 1978) have been reported. Inhibition of prolyl endopeptidase degradation of substance P is also associated with improvements in spatial memory in rats performing a water-maze task, although the possible contribution of other peptides, such as TRH and vasopressin, that are also metabolized by this peptidase cannot be ruled out (Toide *et al.*, 1997). In animal models of amyloid-induced deficits, where exogenous beta-amyloid was administered to rats (Nag *et al.*, 1999) or where endogenous beta-amyloid was overexpressed in transgenic mice (Tomidokoro *et al.*, 2000), substance P levels were decreased in brain regions containing senile plaques or in cortex, respectively. In another study using 18- and 26-month-old transgenic mice with the V717F mutation in APP, a decrease in substance P in nerve fibres around the granule cells of the hippocampus was observed (Diez *et al.*, 2000). The reversal of the amnestic effects of a variety of drugs or ECT by substance P has also been demonstrated in laboratory animals. Post-training peripheral administration of substance P reversed the amnestic effect of cyclohexamide or electroconvulsive shock (ECS) in mice and facilitated retention of foot-shock-enhanced passive avoidance (Schlesinger *et al.*, 1983). Similarly, post-training administration of substance P or its N-terminal fragment reversed diazepam-induced amnesia in rats performing an avoidance task (Costa and Tomaz, 1998).

Evidence for substance P involvement in human dementia symptoms can be found in studies using CSF and post-mortem brain. In a study comparing Alzheimer's disease subjects, patients with

multi-infarct dementia, and normal controls, significantly lower concentrations of substance P were found in Alzheimer's disease subjects relative to normal controls (Martinez, 1993). In a comparison of lumbar CSF from living patients with probable Alzheimer's disease and ventricular CSF from post-mortem, autopsy-confirmed Alzheimer's disease subjects, no differences were found in lumbar CSF levels of substance P between probable Alzheimer's disease patients and non-Alzheimer's disease controls, but higher substance P levels were found in late-onset versus early-onset Alzheimer's disease cases (Rosler *et al.*, 2001). The ventricular CSF levels of substance P were lower than post-mortem lumbar CSF levels, but no differences were seen between Alzheimer's disease patients and controls. In an elegant post-mortem immunohistochemical study using Alzheimer's disease subjects and non-demented controls with and without high levels of senile plaques, amygdala concentrations of substance P were found to be reduced in Alzheimer's disease subjects relative to the normal controls, although senile plaques contained dystrophic neurites staining for substance P in all cases (Benzing *et al.*, 1993).

In another immunohistochemical study, substance P neurons in the remaining developmental cortical subplate beneath the striate cortex of Alzheimer's disease subjects were found to be decreased relative to age-matched non-Alzheimer's disease controls (Ang and Shul, 1995). However, in a recent immunohistochemical investigation using post-mortem Alzheimer's disease tissue and age-matched non-Alzheimer's disease control tissue, no real differences in substance P staining intensity were seen in the hippocampus, frontal cortex or occipital cortex (Yew *et al.*, 1995). Substance P receptors are decreased in the striatum of Alzheimer's disease subjects relative to Parkinson's disease patients, but not controls (Rioux and Joyce, 1993), while the activity of the metalloendopeptidases that degrade substance P were found to be decreased in Alzheimer's diseased temporal cortex but not hippocampus or caudate nucleus post-mortem tissue, relative to non-Alzheimer's disease controls (Waters and Davis, 1995).

ENDOGENOUS OPIOIDS

The endogenous opioids comprise a family of neuropeptides that interact with one or more types of opioid receptors (for review, see Wagner and Chavkin, 1995). One of the possible cleavage products of the POMC precursor protein is beta-endorphin (Table XV-4.1), which is released from the anterior pituitary by the actions of CRF, vasopressin or urocortin on POMC cells. The enkephalins Met-enkephalin (MEK) (Table XV-4.1) and Leu-enkephalin (LEK) (Table XV-4.1) are derived by differential processing of the pro-enkephalin precursor protein. Four different bioactive products can be obtained from the pro-dynorphin precursor protein: dynorphins A and B (Table XV-4.1), and alpha- and beta-neoendorphin. The opioid receptors comprise three major subtypes: mu, delta and kappa opioid receptors. Unlike most neuropeptides, a chemical antagonist for opioid receptors, naloxone, has been known for some time. This panoply of possible agonists and multiple receptors provides a rich substrate for behavioural and physiological responses to painful stimuli.

Most of the evidence for a role for endogenous opioids in cognitive disorders is found for beta-endorphin, which can be measured and, more importantly, alterations interpreted, from blood samples. In delirium, beta-endorphin is decreased in spinal fluid relative to non-delirious age-matched controls, and this decrease correlates with performance on the mini-mental status exam (Koponen *et al.*, 1989b). A follow-up 1 year later saw continued deficits in beta-endorphin in CSF from delirious patients (Koponen and Riekkinen, 1990). However, beta-endorphin in blood was elevated in post-operative delirium (McIntosh *et al.*, 1985) and was not changed in alcoholics undergoing acute withdrawal (Brambilla *et al.*, 1988)

Much of the evidence for endogenous opioid actions in amnesia comes from animal studies. Peripheral administration of beta-endorphin produces retrograde amnesia in rats (Izquierdo *et al.*, 1980), and intracerebroventricular beta-endorphin or MEK produces a similar effect (Lucion *et al.*, 1982). In contrast to these findings, pentylenetetrazol seizure-induced amnesia is reversed by beta-endorphin administered peripherally (Baratti *et al.*, 1990). In a series of papers, researchers demonstrated the ability of dynorphin A to reverse passive-avoidance-task amnesia in mice produced by galanin (Kameyama *et al.*, 1994) or scopolamine (Ukai *et al.*, 1995), or carbon-monoxide-delayed amnesia for a Y-maze task in mice (Hiramtsu *et al.*, 1997). Others found that dynorphin would enhance avoidance performance in mice, while mu and delta receptor antagonists also had this effect (Ilyutchenok and Dubrovina, 1995). In a surprising report in light of the above data, these researchers reported that dynorphin agonists for delta 1 and delta 2 receptors could actually produce amnesia in mice in a variety of learning tasks (Ukai *et al.*, 1997). The latest contribution from this laboratory used beta-amyloid to induce amnesia in mice and found that dynorphin A (full-length, or with the first amino acid deleted) could improve performance in both passive-avoidance and Y-maze performance (Hiramatsu *et al.*, 2000).

Human evidence for endogenous opioid involvement in amnestic disorders includes a post-mortem brain study of Wernicke–Korsakoff's syndrome patients where, relative to controls, these subjects and alcoholics had increased beta-endorphin levels in the mammillary bodies, the site of neurodegeneration in this syndrome (Summers *et al.*, 1991). In another study implicating endogenous opioids in the production of amnestic symptoms, the opioid receptor antagonist naloxone was shown to reduce the adverse cognitive effects of ECT, including amnesia (Prudic *et al.*, 1999).

There is a large literature on the effects of endogenous opioids in dementing disorders, principally Alzheimer's disease. Beta-endorphin has been found to be increased in blood from clinically diagnosed 'probable' Alzheimer's disease subjects relative to cognitively normal, age-matched controls (Franceschi *et al.*, 1988), and others have seen a similar increase in beta-endorphin blood levels of demented Parkinson's disease patients (Rabey *et al.*, 1990). This elevation in plasma beta-endorphin levels was shown to be increased in Alzheimer's disease relative to normal controls, normal elderly controls, or multi-infarct dementia subjects (Rolandi *et al.*, 1992). The concentration of beta-endorphin in CSF from Alzheimer's disease subjects has been shown to be normal relative to controls and does not change with severity of dementia in Alzheimer's disease. However, Binswanger's disease subjects had increased levels of beta-endorphin in CSF (Lee *et al.*, 1990). Others have confirmed the finding of normal levels of beta-endorphin in CSF from Alzheimer's disease subjects (Martinez *et al.*, 1993). Dynorphin A (1–8) levels in CSF from Alzheimer's disease subjects have been reported to be reduced to around 60% of those for age-matched non-demented controls (Sunderland *et al.*, 1991).

Using post-mortem tissue, the amount of MEK immunohistochemical staining in striasomes in the striatum and globus pallidus of Alzheimer's disease subjects was similar to that seen in age-matched normal controls (Matsumoto *et al.*, 1990). This finding of normal MEK in striatal patches was later confirmed (Gearing *et al.*, 1997) and shown to have no association with beta-amyloid-containing senile plaques. Receptors for MEK and LEK (mainly delta opioid receptors) were reported to be decreased in the temporal cortex, amygdala and hippocampus of Alzheimer's disease subjects compared with age-matched controls, while the external globus pallidus was unchanged (Rinne *et al.*, 1993). In another report that assessed opioid receptor populations in Alzheimer's disease, the number (B_{max}) and binding affinity (K_d) of mu receptors were normal in the frontal cortex, amygdala and putamen, while the

number of kappa receptors was increased in the amygdala and the numbers of both kappa and delta opioid receptors were decreased in the putamen relative to age-matched controls (Barg *et al.*, 1993). In both Alzheimer's disease and Down's syndrome post-mortem tissue, the frontal cortex concentrations of LEK and dynorphin have been reported to be decreased relative to normal controls (Risser *et al.*, 1996).

GALANIN

Galanin (Table XV-4.1) was originally purified from the colon and pituitary. It is a 30-amino acid neuropeptide that generally inhibits the firing of neurons with which it communicates (see Bartfei, 1995 for review). Recently, a third galanin receptor, GAL3, was cloned in rats and humans (Smith *et al.*, 1998). The major evidence for galanin involvement in cognitive disorders is in Alzheimer's disease. Galanin is one of the only neuropeptides shown to increase in concentration in Alzheimer's disease, rather than decrease or show no change. Found in neurons of the nucleus basalis of Meynert, where acetylcholine-producing neurons degenerate in Alzheimer's disease, galanin has been shown to increase in concentration and inhibit cholinergic cell activity (see Crawley, 1996 for review). The concentration of galanin is increased significantly in post-mortem Alzheimer's disease tissue in several frontal, temporal and parietal cortical regions (Gabriel *et al.*, 1994), and temporal, but not occipital, cortical receptors for galanin are decreased (Ikeda *et al.*, 1995), which may indicate downregulation in response to increased galanin release. Others have reported galanin receptors to be increased in the hippocampus, decreased in the caudate nucleus, and unchanged in the frontal and entorhinal cortex of Alzheimer's disease compared with age-matched controls (Rodriguez-Puertas *et al.*, 1997). In contrast to these findings, confocal microscopic examination of nucleus basalis neurons in Alzheimer's disease demonstrated galanin hyperinnervation of basalis cholinergic neurons (Bowser *et al.*, 1997), and autoradiography indicated increased numbers of galanin receptors in the nucleus basalis in late-stage but not early-stage Alzheimer's disease tissue (Mufson *et al.*, 2000). Noradrenergic neurons of the locus coeruleus that express galanin mRNA are preserved in Alzheimer's disease tissue, indicating the specificity of the changes observed in the nucleus basalis (Miller *et al.*, 1999).

Animal evidence for galanin association with cognitive function includes administration of exogenous galanin to rats with or without 192IgG-saporin lesions of nucleus basalis cholinergic neurons. Exogenous galanin reduced accuracy in a delayed non-matching to position memory task in both lesioned and normal rats, and this effect on memory of the cholinergic lesion was not attenuated by administration of a galanin antagonist, M40 (McDonald *et al.*, 1997). A later paper from this group used the galanin antagonist in addition to a muscarinic M1 cholinergic receptor agonist and reported improvement of performance in this delayed non-matching to position memory task (McDonald *et al.*, 1998). Using transgenic mice with overexpression of a mutated human APP gene, galanin immunoreactivity was increased in several hippocampal neuronal fields and in the ventral cortex, while diffuse galanin staining in noradrenergic fibres of the hippocampus were no longer visible at 26 months of age in these transgenic mice (Diez *et al.*, 2000). Non-peptide galanin antagonists have also been developed and shown to reverse the inhibitory effects of galanin on acetylcholine release in rat hippocampal brain slices (Scott *et al.*, 2000).

THYROTROPIN-RELEASING HORMONE

There is little direct evidence for alteration of TRH (Table XV-4.1) in cognitive disorders, and TRH remains undisturbed in Alzheimer's diseased brain (Nemeroff *et al.*, 1989), yet a body of evidence does exist that supports a role for TRH or TRH analogues in the treatment of cognitive disorders (see Bennett *et al.*, 1997 for review). This tripeptide regulates the release of thyrotropin and prolactin from the anterior pituitary and is implicated in physiological regulation of body temperature (see Mason *et al.*, 1995 for review). Pharmacologically, TRH reverses the effects of drugs that produce depression of CNS activity, such as alcohol and barbiturates, and assists in neuronal recovery from damage through trophic effects. This arousal-like effect of TRH may be responsible for its beneficial effects in cognitive disorders, and TRH may be particularly useful in this regard as it produces CNS effects after peripheral administration. Using synthetic TRH to induce release of thyrotropin from the pituitary, a lack of dose response to TRH stimulation was observed in Alzheimer's disease subjects (Lampe *et al.*, 1988), indicating possibly reduced sensitivity of pituitary TRH receptors. However, other attempts to replicate this effect of TRH on thyroid-stimulating hormone (TSH) (Molchan *et al.*, 1991; Tsuboyama *et al.*, 1991; Albert *et al.*, 1993) or prolactin (Bille *et al.*, 1991) did not observe differences between Alzheimer's disease subjects and age-matched normal controls. Researchers at National Institutes of Health (NIH) used intravenous doses of TRH in Alzheimer's disease subjects and reported increased arousal and improvement in mood, as well as a modest increase in semantic memory (Mellow *et al.*, 1989). This ability to improve cognition was demonstrated later in normal subjects with transient scopolamine-induced memory impairment (Molchan *et al.*, 1990). A TRH analogue, posatirelin, has been used in the treatment of Alzheimer's disease, and after 3 months of daily intramuscular injections, in a variety of measures involving intellectual function, orientation and memory, the TRH-treated group were improved over groups receiving placebo or a reference drug, citicoline (Parnetti *et al.*, 1995). This same group of researchers assessed the effect of this analogue on vascular dementia subjects compared with placebo-treated controls; after 3 months of daily intramuscular injection, they found similar improvements as before in the posatirelin group (Parnetti *et al.*, 1996).

Animal studies have largely confirmed the clinical reports of beneficial effects of TRH analogues on memory task performance. Using a slowly released formulation of TRH administered subcutaneously, TRH blood levels were increased for up to 4 weeks, increased time was spent in the open arms of an elevated plus maze, and improved spatial memory performance was seen in a water-maze task of a strain of senescence-accelerated mice compared with a normally ageing strain (Miyamoto *et al.*, 1994). Effects were not seen immediately after injection, but were first present 10/days after initial injection of TRH. In a study using aged rats, inhibition of a peptidase that metabolizes TRH by the compound JTP-4819 improved spatial memory impairments in water-maze performance that were present in the aged rats relative to non-aged controls (Toide *et al.*, 1997). A TRH analogue with N-terminal substitutions that improves cognitive and motor recovery after a fluid percussion brain injury in rats has been developed that is devoid of analeptic or endocrine effects (Faden *et al.*, 1999). Others have demonstrated the ability of nicotinic receptor antagonists to abolish the neuroprotective effects of TRH in restoring consciousness to mice with concussive head injury (Lestage *et al.*, 1998).

CHOLECYSTOKININ

Cholecystokinin (CCK) is a 33-amino acid neuropeptide (Table XV-4.1) that shares sequence similarity with the gut hormone gastrin (see review by Beinfeld, 1995). There are two CCK receptors, CCKA and CCKB; the C-terminal 8-amino-acid sulphated fragment of CCK has activity at both receptor subtypes. Although normal levels of CCK are found in Alzheimer's diseased brain, there are several reports of cognitive effects of analogues interacting

with both CCK receptor subtypes. Intravenous administration of the CCK analogue ceruletide improved event-related potentials as measured by electroencephalography (EEG) in the brains of young, but not aged, subjects (Dodt *et al.*, 1996). Intravenous administration of the CCK-4 fragment has produced panic attacks in humans, and it has been suggested that this effect is due to CCK altering information processing (Aluoja *et al.*, 1997), while others have found that CCK-4 administration decreases short-term memory performance (Shlik *et al.*, 1998).

Animal evidence for CCK effects on cognitive processes indicates the possibility of more than one CCKB receptor subtype (see Dauge and Lena, 1998 and Fink *et al.*, 1998 for reviews). While the general consensus from animal studies supports the concept that CCKA agonists and CCKB antagonists improve memory function, exceptions have been noted. Using a two trial memory task with periods of 2 and 6 hours after initial training, the CCKB agonist BC 264 improved memory at the 6-hour timepoint after post-training intraperitoneal injection, while the CCKB agonist BC 197 disrupted memory at the 2-hour timepoint (Lena *et al.*, 1999). These results have been interpreted as indicating the possible existence of subtypes of CCKB receptors, as selective CCKB antagonists abolished these effects while selective CCKA antagonists were without effect. These effects of BC 264 were later extended to aged rats, and demonstrated that injection before training was not effective while post-training injection was effective (Taghzouti *et al.*, 1999). Using an olfactory recognition test of memory, the memory-enhancing effects of a CCKB antagonist were abolished by perforant path lesions, while this lesion did not alter CCKA agonist effects but a subdiaphragmatic vagotomy did (Lemaire *et al.*, 1994).

SOMATOSTATIN

Somatostatin (somatotropin-release inhibiting factor, SRIF; Table XV-4.1) is a neuropeptide that was originally isolated from the hypothalamus of sheep and pigs as a factor controlling the release of pituitary hormones (see Epelbaum *et al.*, 1994 and Rubinow *et al.*, 1995 for reviews). Somatostatin was shown to physiologically regulate the release of growth hormone and many other pituitary hormones by providing the inhibitory component of the dual regulation experienced by most of the anterior pituitary hormones. Upon further investigation, it was quickly realized that the distribution of SRIF outside of the hypothalamus was more indicative of a neurotransmitter role in addition to that as a hypothalamic releasing factor, and application of SRIF to neurons consistently inhibited their firing rate. It was subsequently found to often be colocalized within neurons containing the inhibitory transmitter, γ-aminobutyric acid (GABA) in the cortex. Two active forms have been identified that are cleaved from a larger precursor molecule and contain either 14 or 28 amino acids. Five molecular subtypes of SRIF receptors have now been cloned, and the specific peptidases contributing to the degradation of active SRIF have also been identified.

Somatostatin and Delirium

In a series of papers, researchers have demonstrated decreased SRIF concentrations in the CSF of patients with delirium. In the first paper (Koponen *et al.*, 1989a), elderly patients with delirium were shown to have significantly less SRIF in the CSF than elderly controls, and SRIF deficits were correlated with increasing cognitive dysfunction. In the next paper (Koponen *et al.*, 1990), SRIF concentrations in the CSF of delirious subjects were assessed acutely and 1 year after initial examination. Compared with age-matched non-delirious controls, SRIF was decreased in the delirious subjects' CSF and continued to decrease over time as cognitive deficits increased. These researchers then published a report of follow-up in elderly delirious subjects that encompassed 4 years from initial examination (Koponen *et al.*, 1994). As before, the SRIF concentrations in the CSF of delirious subjects continued to decline and was correlated with decreased scores on the mini-mental state exam. Schizophrenics with cognitive deficits have also been reported to have less SRIF in the CSF than normal controls, but the SRIF deficits in these subjects did not correlate with cognitive impairment (Reinikainen *et al.*, 1990).

Evidence for involvement of SRIF in amnesia is mostly found in animal studies. Using ECS to induce amnesia in rats trained in an active avoidance task motivated by foot shock, intracerebroventricular (ICV) administration of SRIF at doses of 4 μg/4 μl significantly increased the avoidance latency when administered immediately or up to 23 hours after ECS (Vecsei *et al.*, 1983); this effect was found to require the entire SRIF molecule, as both SRIF 3–6 and SRIF 7–10 were inactive (Vecsai *et al.*, 1984). Analogues of SRIF with dextrarotary amino acid substitutions (D-TRP8, D-Cys14) were later found to be effective in reversing ECS-induced amnesia (Vecsai *et al.*, 1986), although other analogues with deletions of N-terminal amino acid residues were not effective. Using cysteamine to temporarily deplete SRIF, Haroutunian *et al.* (1987) observed increased locomotor activity and deficits in retention of training performance in a passive-avoidance task. Up to 50% depletion of cortical SRIF was observed and preceded increased SRIF concentrations in the CSF. Thiamine deficiency (25 days) was used to produce impairment of an avoidance response, and this amnestic effect was correlated with decreased concentrations of SRIF in several brain regions, including the cerebral cortex, amygdala, thalamus, hippocampus and hypothalamus (Nakagawasai *et al.*, 2000). A single thiamine treatment after 14 days was sufficient to prevent both behavioural and SRIF neurochemical deficits.

Somatostatin in Alzheimer's Disease

Somatostatin concentration deficits in the cortex of Alzheimer's diseased brain post-mortem were the second neurotransmitter system to be implicated in Alzheimer's disease pathology after the discovery of the cholinergic deficits (see Bissette and Myers, 1992 and Vecsai and Klivenyi, 1995 for reviews). Among the peptides colocalized with GABA in cortical neurons, SRIF is the second most abundant after substance P and is more pervasive than either vasoactive intestinal peptide (VIP) or CCK, which do not appear to be principal targets of Alzheimer's disease pathology (Gabriel *et al.*, 1995; Mazurek and Beal, 1991). The major population of SRIF neurons affected in Alzheimer's disease are cortical interneurons that do not contain other peptides such as neuropeptide Y (Davies *et al.*, 1990; Gabriel *et al.*, 1993), with most subcortical regions, such as the nucleus basalis and hypothalamus, largely unaffected, although a recent report indicates that SRIF deficits in subcortical white matter do exist (Ang and Shul, 1995) in Alzheimer's disease. Another report (Gaspar *et al.*, 1989) has implicated SRIF 28 as the species of SRIF most involved in Alzheimer's disease pathology, and *in situ* hybridization surveys of messenger RNA for the pre-pro-SRIF precursor molecule have shown that residual synthetic capacity for SRIF exists in many regions where absolute SRIF concentrations are decreased (Dournaud *et al.*, 1994). This would indicate that the presynaptic neuronal machinery necessary to produce SRIF is capable of continued synthesis and could be a potential target for therapeutic intervention. A recent paper reported that blocking GABA-B presynaptic receptors that mediate SRIF release produced cognitive enhancing effects (Bonanno *et al.*, 1999). Further support for a dysregulation of SRIF metabolism is found in reports that claim that the amount of SRIF precursor is increased relative to the active form of the peptide in the CSF from Alzheimer's disease patients (Gomez *et al.*, 1986), and that SRIF 28

is the species reduced in the CSF from Alzheimer's disease patients (Yasuda *et al.*, 1995). However, the presumably postsynaptic SRIF receptors are not upregulated in an attempt to compensate for the reduced synaptic availability of SRIF; in fact, the opposite is the case. Several research groups (Beal *et al.*, 1985; Krantic *et al.*, 1992; Bergström *et al.*, 1991) have now reported SRIF receptor populations in post-mortem cortex to be decreased in number, but unchanged for ligand affinity, in Alzheimer's disease. In addition to the decreases in endogenous ligand concentration and receptor dysregulation, the peptidases that degrade the active forms of SRIF are also altered inappropriately (Weber *et al.*, 1992), degrading SRIF more efficiently in post-mortem Alzheimer's diseased cortex than peptidases from non-Alzheimer's diseased brain.

Attempts to address the SRIF deficit with long-acting SRIF analogues have been unsuccessful in reversing Alzheimer's disease dementia (Mouradian *et al.*, 1991), partly because of the decreased numbers of receptors and increased activity of peptidases, and uncertainty over SRIF access to the CNS after peripheral administration. The SRIF concentration deficit has been observed widely in CSF (see Bissette and Myers, 1992) as well as post-mortem brain tissue from Alzheimer's disease patients, and several papers have now described treatment-induced amelioration of these CSF deficits in SRIF concentrations that may correlate with cognitive improvement (Karlsson *et al.*, 1985; Widerlöv *et al.*, 1989; Alhainen *et al.*, 1991). Unfortunately, any disease that produces significant cognitive impairment seems to reduce SRIF concentrations in the CSF, as indicated by the temporary and reversible deficits in CSF SRIF during bouts of delirium (Koponen *et al.*, 1990). The amount of SRIF in the CSF correlates significantly with dementia severity in Alzheimer's disease (Tamminga *et al.*, 1987), and with cognitive decline in multiple sclerosis (Roca *et al.*, 1999), and it could be a useful measure of treatment response. While this lack of specificity prevents SRIF in the CSF from being useful as a diagnostic indicator for the presence of Alzheimer's disease, these data indicate that the concentration of SRIF in the CSF may be a useful biological marker for treatments that successfully improve cognitive state.

CORTICOTROPIN-RELEASING FACTOR

This 41-amino acid neuropeptide (Table XV-4.1) was isolated and characterized in the early 1980s by Vale. A binding protein sequesters CRF for quick release and provides a mechanism by which CRF signalling can be amplified quickly upon demand (for review, see De Souza and Grigoriadis, 1995). Outside of the hypothalamus, CRF is located in limbic system regions and within a population of cortical interneurons. A plethora of behavioural studies have implicated CRF systems as major mediators of behavioural responses to stimuli producing fear and anxiety, and CRF hypersecretion is suspected to be a mediator of some of the endocrine changes and behavioural symptoms of major depressive disorder in humans (Nemeroff *et al.*, 1984). Two major subtypes of CRF receptors, CRFR1 and CRFR2, are now known to exist (Chalmers *et al.*, 1995; Lovenberg *et al.*, 1995), and a portion of the bioavailable CRF is sequestered from receptors and peptidase degradation by the action of an endogenous CRF binding protein.

Corticotropin-Releasing Factor in Delirium

Comparing AD subjects with delirium with multi-infarct patients, researchers reported decreased plasma CRF in the evening and increased CRF and ACTH in the morning in the Alzheimer's disease subjects relative to the vascular dementia subjects (Suemaru *et al.*, 1991b). In a study of acute alcohol withdrawal patients, CRF in the CSF was correlated significantly and positively with CSF noradrenaline during early phases of withdrawal, and both these measures correlated with diastolic blood pressure (Hawley *et al.*, 1994).

Corticotropin-Releasing Factor in Alzheimer's Disease

CRF was first reported to be decreased in concentration in the frontal and temporal cortex of Alzheimer's disease post-mortem brain compared with non-Alzheimer's disease control tissues in 1985 (Bissette *et al.*, 1985). The concentration of CRF in the Alzheimer's disease group was not significantly different from the controls in subcortical regions such as the nucleus basalis or hypothalamus, although in this patient population the caudate nucleus had significantly diminished concentrations of CRF in the Alzheimer's disease group. The deficits in cortical CRF concentrations in Alzheimer's disease were quickly confirmed (De Souza *et al.*, 1986). These workers extended this finding to occipital and parietal cortical regions, and observed that the postsynaptic neurons containing CRF receptors were demonstrating an inversely proportional, regionally specific increase in CRF receptor number relative to the decrease in the concentration of the CRF endogenous ligand in an apparent attempt to compensate for the reduced synaptic availability of CRF. Based on the regional distribution of the upregulated CRF receptor population found in Alzheimer's disease, it is likely that subtype involved is the CRF_1 receptor (D. Chalmers, personal communication); previous work has shown that the increase in CRF binding in Alzheimer's diseased tissue is due to functional CRF receptors (Grigoriadis *et al.*, 1989). Immunohistochemical staining confirmed that the cortical CRF concentration decreases in Alzheimer's disease were due to degeneration of the interneurons of the cortex containing CRF (Powers *et al.*, 1987). Other areas of the brain, such as the hippocampus, hypothalamus and cerebellum, have been shown to retain substantial levels of CRF-immunoreactive fibres and cells in Alzheimer's disease (Kelley and Kowall, 1989). Most of the CRF-related changes reported in Alzheimer's disease have involved the cortical interneuron CRF circuits, and few groups have reported CRF concentration changes outside of the cortex or within the hypothalamus (for review, see Bissette, 1997). However, evidence of hypothalamic dysregulation in Alzheimer's disease has been reported for the response of the hypothalamic-pituitary-adrenal (HPA) axis to challenge with exogenous synthetic CRF (Hartzinger *et al.*, 1995). Others have reported elevations in the level of CRF mRNA in the post-mortem paraventricular nucleus of the hypothalamus in Alzheimer's diseased patients, and even greater elevations in patients with a clinical history of major depression during life (Raadsheer *et al.*, 1995). As elevations of CRF are reported in the CSF of depressed patients relative to controls (Nemeroff *et al.*, 1984), along with other indicators of increased secretion of hypothalamic CRF, it would seem possible, based upon the CRF mRNA elevations in the hypothalamus of patients with Alzheimer's disease, that CSF concentrations of CRF may also be elevated in Alzheimer's disease relative to non-Alzheimer's diseased patients if hypothalamic CRF contributes significantly to CSF concentrations. One research group has reported such an increase (Martignoni *et al.*, 1990), and demented patients with depression (Banki *et al.*, 1992) have also exhibited such increases in CSF concentrations of CRF. However, CSF concentrations of CRF in simple Alzheimer's disease have also been reported to be either unchanged (Nemeroff *et al.*, 1984; Molchan *et al.*, 1993; Pomara *et al.*, 1989; Jolkkonen *et al.*, 1990; Edvinsson *et al.*, 1993; Banki *et al.*, 1992) or decreased (Heilig *et al.*, 1995; May *et al.*, 1987; Mouradian *et al.*, 1986; Suemaru *et al.*, 1991a; Suemaru *et al.*, 1995), depending on the control and Alzheimer's disease patient populations being compared and the laboratory reporting the data. These disparities are almost certainly due to the uncertainty of the diagnosis of Alzheimer's disease in patients during life. Our group has observed some populations of Alzheimer's disease patients with a nonsignificant increase in CRF

in the post-mortem hypothalamus (Bissette *et al.*, 1985) and other Alzheimer's disease groups with relative decreases in hypothalamic CRF and SRIF that did achieve statistical significance (Bissette *et al.*, 1998). Thus, a consensus on the state of the CSF and hypothalamic CRF in Alzheimer's disease remains elusive.

Recently, a strategy (Behan *et al.*, 1995) was proposed whereby the apparently reduced synaptic availability of CRF might be restored to near-normal levels in Alzheimer's disease patients. Using shortened analogues of CRF to displace endogenous CRF from the CRF binding protein, the amount of free CRF in post-mortem Alzheimer's diseased brain was able to be increased to control levels. The amount of CRF binding protein was similar in the Alzheimer's diseased cortical tissue and in the normal control brain in the frontal, temporal, parietal and occipital cortices. The ligand that displaces native CRF from the binding protein does not produce anxiety in laboratory animals at doses that effectively improve memory and learning, as assessed with the Morris water-maze task. Once such compounds are developed that can cross the blood–brain barrier in sufficient quantities to similarly affect the CRF binding protein of living patients, it will be possible to determine which symptoms of Alzheimer's diseased are mediated by the CRF concentration deficits seen at the time of death.

In one of the most important papers on the neurochemistry of Alzheimer's disease that has been published, the Mt Sinai group (Davis *et al.*, 1999) demonstrated that CRF cortical deficits become significantly different from age-matched controls when a score of 2 (out of a possible 5) is reached on the clinical dementia rating scale. Somatostatin and acetylcholine were not decreased significantly until a score of 4 was achieved. These data can be interpreted as indicating an earlier involvement of CRF in the neurodegenerative process, and make approaches to normalizing the synaptic availability of CRF more urgent.

MISCELLANEOUS PEPTIDES

Other peptides that have been implicated in cognitive disorders but have not been assessed thoroughly include neuropeptide Y (NPY) and insulin-like growth factor 1 (IGF-1). NPY has been shown to be decreased in schizophrenia (Gabriel *et al.*, 1995) and unchanged in Alzheimer's diseased post-mortem brain (Gabriel *et al.*, 1993), but little evidence from animal studies supports a role for NPY in clinical disorders of cognition. The association of IGF-1 with growth hormone and cognitive function are reviewed by van Dam *et al.* (2000) and Dore *et al.* (2000). IGF-1 levels are unchanged in Alzheimer's diseased frontal cortex, hippocampus and cerebellum relative to controls, and IGF-1 receptor affinity and numbers are also unchanged. However, IGF-1 has been observed in neurites contained in senile plaques (Jafferali *et al.*, 2000). Others have observed plasma IGF-1 levels to be correlated positively with cognitive performance, as assessed by the mini-mental status exam in aged subjects (Rollero *et al.*, 1998); this was confirmed recently with elderly males (Aleman *et al.*, 2000).

In summary, it is obvious that much work remains to be performed in assessing the contribution of neuropeptide neurotransmitters to conditions resulting in cognitive impairment. However, the diverse physiological effects mediated by neuropeptides and the panoply of pharmacological targets provided by neuropeptide receptors ensures that this knowledge will continue to be pursued assiduously.

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XV-5

The Neuroendocrinology of Cognitive Disorders

Sonia J. Lupien

INTRODUCTION

For many years, endocrinologists and neuroscientists thought that hormones, which are biological products secreted by peripheral glands, did not access the brain and acted mainly at the level of the peripheral nervous system. However, in the early 1960s, the discovery of neuropeptides as substances that not only had classical endocrine effects but also affected brain and behaviour significantly extended our view of hormones and opened the door to new possibilities of hormonal actions on the brain (for a complete historical background, see De kloet, 2000). The idea of a central action of hormones was also supported by studies showing that long-term therapy with anti-inflammatory drugs (which are synthetic corticosteroids) led to significant mental and cognitive deficits known as 'steroid psychosis' (Clark *et al.*, 1952). As shown in Figure XV-5.1, the symptoms of steroid psychosis appear gradually with chronic administration of synthetic corticosteroids, and include emotional lability, anxiety, distractibility, pressured speech, sensory flooding, insomnia, depression and cognitive impairment.

Grade 1	Mild euphoria Lessened fatigue Improved concentration Elevated mood
Grade 2	Heightened euphoria Flight of ideas Impaired Judgement Insomnia Increased appetite Memory impairment
Grade 3	Anxiety Phobia Rumination Hypomania Depression
Grade 4	Psychosis

Figure XV-5.1 Chronology of psychiatric symptoms occurring after long-term exposure to high levels of synthetic corticosteroids, resulting ultimately in steroid psychosis

The presence of a mental disorder induced by exposure to high levels of a corticosteroid hormone strongly suggested that these substances could, in some way, access the brain and impact on behaviour. Such a hypothesis was appropriate, since in 1943, Harris had completed a series of landmark anatomical studies that established clearly that the central nervous system regulates the hypothalamic-pituitary-adrenal axis (HPA) (for an historical review, see Harris, 1972). Following the discovery of a significant effect of corticosteroid on brain function, a significant number of animal and human studies assessed the potential impact of this steroid hormone on learning and memory. It is clear that the neuroendocrinology of cognitive disorders encompasses the unique action of corticosteroids on memory function (for reviews, see Sherwin, 1999, McGaugh, 2000, McGaugh and Izquierdo, 2000 and Sara, 2000). However, in order to describe in detail the extent and nature of the impact of hormonal variations on animal and human cognitive function, we will use the corticosteroid model (one of the most well-described models to this day), with the idea in mind that many of the methodological factors that have to be controlled for in our study of the impact of corticosteroids on cognitive function are the same as those that are important in other studies measuring the effects of various hormones on animal and human cognitive processing.

THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The HPA (Figure XV-5.2) axis along with the sympathoadrenal system governs metabolic responses to the stresses of everyday life, as well as to the demands that prevail under conditions of chronic, severe stress (Sapolsky, 1992). In response to stress, hypothalamic neurons release corticotropin-releasing factor (CRF), which will trigger subsequent secretion and release of adrenocorticotropic hormone (ACTH) from the pituitary. The corticosteroids (corticosterone in animals, cortisol in humans) will be secreted and released from the adrenal glands in response to ACTH stimulation.

The responsivity of the HPA axis to stress is, in part, determined by the ability of the corticosteroids to regulate ACTH release (i.e. corticosteroid negative feedback). Circulating corticosteroids feed back on to the pituitary and specific brain regions to inhibit the secretion of releasing factors from hypothalamic neurons and pituitary ACTH (Jacobson and Sapolsky, 1991). In addition to pituitary and hypothalamic sites, there is now considerable evidence for the importance of the limbic system (particularly the hippocampus) and cortical areas (particularly the prefrontal cortex) in the regulation of HPA activity. This regulation is possible through an inhibitory role of these regions on HPA activity (Feldman and Conforti, 1985; Murros *et al.*, 1993; Moghaddam *et al.*, 1994; Bagley and Moghaddam, 1997; Shimizu *et al.*, 1997).

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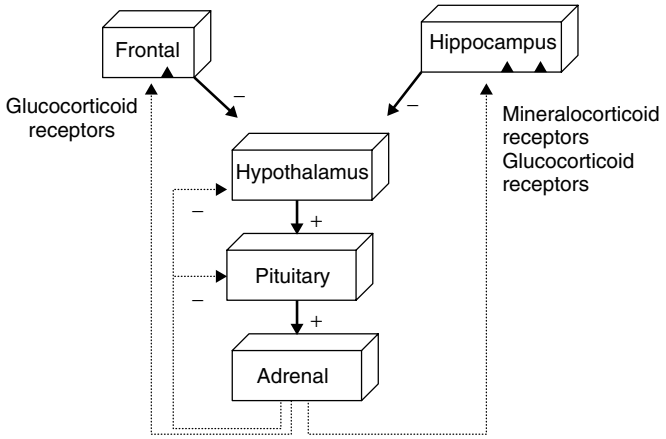

Figure XV-5.2 Schematic representation of the HPA axis. Following the perception of a stressor, the hypothalamus releases CRF, which activates the pituitary and leads to secretion of ACTH. The levels of ACTH are detected by the adrenal cortex, which then secretes corticosteroids. Corticosteroids will enter the blood circulation and act peripherally and centrally. At the level of the brain, both the frontal lobes and the hippocampus contain corticosteroid receptors, although the hippocampus contains both mineralocorticoid receptors and glucocorticoid receptors, while frontal lobes contains mostly glucocorticoid receptors. Both cortical structures inhibit the HPA axis. Along with inhibitory actions of the frontal and hippocampal region, circulating corticosteroids will act at the level of the hypothalamus to inhibit further secretion of CRF, and at the level of the pituitary to inhibit the secretion of ACTH. Circulating levels of ACTH also act at the level of the hypothalamus to inhibit secretion of CRF. The consequence of these negative feedback loops will be a significant decrease of circulating levels of corticosteroids after the end of the stressor

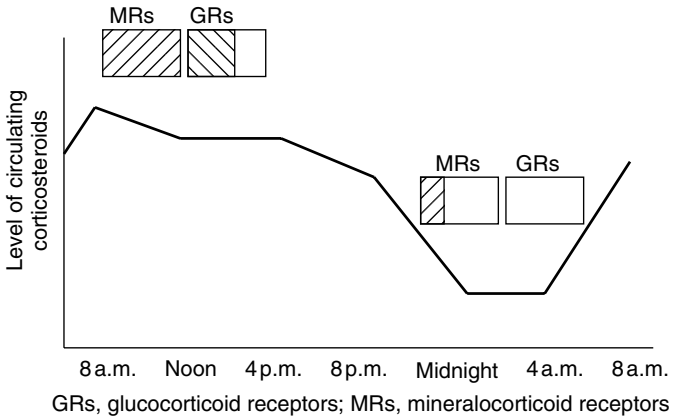

Figure XV-5.3 Circulating levels of corticosteroids at different times of the day in humans, and representation of the level of occupancy of mineralocorticoid receptors and glucocorticoid receptors at different times of day

IMPORTANT CHARACTERISTICS OF CORTICOSTEROIDS

Under basal conditions, cortisol secretion exhibits a 24-hour circadian profile in which cortisol concentrations present a morning maximum in humans (the circadian peak), with slowly declining levels during the late afternoon, evening and nocturnal period (the circadian trough), and an abrupt elevation after the first few hours of sleep. Circulating corticosteroids bind with high affinity to two corticosteroids receptor subtypes: the mineralocorticoid (type I) and glucocorticoid (type II) receptors. Although both receptor types have been implicated in mediating corticosteroid feedback effects (see Reul and De kloet, 1985), there are two major differences between mineralocorticoid and glucocorticoid receptors. First, mineralocorticoid receptors bind corticosterone with an affinity that is six to ten times higher than that of glucocorticoid receptors. As shown in Figure XV-5.3, this differential affinity results in a striking difference in occupation of the two receptor types under different conditions and time of day. Thus, during the circadian trough (the p.m. phase in humans and the a.m. phase in rats), the endogenous hormone occupies more than 90% of mineralocorticoid receptors but only 10% of glucocorticoid receptors. However, during stress and/or the circadian peak of corticosteroid secretion (the a.m. phase in humans and the p.m. phase in rats), mineralocorticoid receptors are saturated and there is occupation of approximately 67–74% of glucocorticoid receptors (Reul and De Kloet, 1985).

The second major difference between these two receptor types is related to their distribution throughout the brain. The mineralocorticoid receptor is present exclusively in the limbic system, with a preferential distribution in the hippocampus, parahippocampal gyrus, and entorhinal and insular cortices. On the contrary, the glucocorticoid receptor is present in both subcortical (paraventricular nucleus and other hypothalamic nuclei, hippocampus and parahippocampal gyrus) and cortical structures, with a preferential distribution in the prefrontal cortex (Meaney and Aitken, 1985; McEwen *et al.*, 1968; McEwen *et al.*, 1986; Diorio *et al.*, 1993). As we will see in the following section, the impact of corticosteroids on cognitive function can be best understood in terms of the differential effects of mineralocorticoid receptor and glucocorticoid receptor activation in both the hippocampus and frontal lobes, two brain structures critically involved in cognitive function (for a complete review, see De Kloet *et al.*, 1999).

ACUTE EFFECTS OF CORTICOSTEROIDS ON MEMORY

Effects on Memory Sustained by the Hippocampus

Since the seminal work performed by Scoville and Milner (1957) with amnesic patients who had undergone bilateral ablation of the hippocampus, it has been known that this structure plays a critical role in memory formation. Given the presence of corticosteroid receptors in the rodent and human hippocampus, it has been suggested that the corticosteroid modulation of hippocampal activity may underlie some aspects of the acute effects of corticosteroids observed in animal learning and memory processes. The effects of adrenal steroids on animal cognition, and the neural substrate, have been studied using, for the most part, three types of models that tap into hippocampal function. The first approach is to examine the neuroendocrine modulation of a physiological model of neuronal excitability that is relevant to memory. Hippocampal long-term potentiation (LTP) describes a long-lasting enhancement in synaptic efficacy that occurs in response to high-frequency electrical stimulation (Tyler and Discenna, 1987; Lynch *et al.*, 1988). LTP has many characteristics in common with memory, the most important being its rapid induction and long duration. The second approach is through the measure of associative learning, as defined by various aspects of conditioning behaviours. The third approach is through the study of spatial memory (Olton *et al.*, 1979; Jarrard *et al.*, 1984).

A number of rodent studies have reported that the induction of LTP in the hippocampus is blocked by the administration of corticosterone (Dubrovsky *et al.*, 1987; Filipini *et al.*, 1991). The role of corticosteroids in hippocampal LTP have been confirmed further

by studies showing that the acute administration of corticosteroids in the dentate gyrus of the hippocampus produces LTP (Filipini *et al.*, 1991; Pavlides *et al.*, 1993). In 1991, Bennett and coworkers reported the existence of a negative correlation between the magnitude of LTP in the CA1 population spike in the hippocampus and the level of circulating corticosteroids, thus suggesting a dose-dependent relationship between corticosteroids and their detrimental effects on LTP. One year later, Diamond *et al.* (1992) reported the presence of an inverted U-shaped relationship between the level of circulating corticosteroids and LTP. They described a positive correlation between corticosterone and primed burst potentiation (PBP; a low threshold form of LTP) (Bennett *et al.*, 1991) at low levels of corticosteroids, and a negative correlation between corticosterone and PBP at high levels of corticosteroids. These results provided strong support for the hypothesis that corticosteroids exert a concentration-dependent biphasic influence on LTP.

Direct effects of corticosteroids have also been observed in associative learning paradigms. For example, acute administration of either corticosterone or dexamethasone accelerates the rate of extinction of a shock-avoidance response (Bohus and Lissak, 1968; Bohus, 1970; Greidanus, 1970; Roozendaal and McGaugh, 1996). Similarly to studies performed on LTP, it has been shown that the effects of corticosteroids on animal cognition follow an inverted U-shaped relationship. In 1976, Kovacs and collaborators reported that low doses of corticosterone facilitate extinction of an avoidance response, while high doses of corticosterone delay the rate of extinction of the conditioned response. Finally, biphasic modulatory effects of corticosteroids were also reported using spatial memory paradigms (for a review, see Lupien and McEwen, 1997). Altogether, these results (obtained mainly in the rodent population) confirmed the important role of the hippocampus in explaining corticosteroid-induced cognitive impairments.

In humans, the effects of corticosteroids on learning and memory have been studied using, for the most part, measures of declarative and non-declarative memory. The logic for the inclusion of this amnesic dissociation is due to the fact that studies report that the hippocampus is essential for declarative memory but not for non-declarative memory (for a review, see Squire, 1992). Declarative memory is the conscious or voluntary recollection of previous information; non-declarative memory refers to the fact that experience changes the facility for recollection of previous information without affording conscious access to it (priming). Thus, this somewhat specialized role of the hippocampus serves as the basis for specific hypotheses regarding the relationship between increased cortisol secretion and impaired cognitive function in humans.

In general, the majority of human studies that have measured the impact of corticosteroids on cognitive function report impaired declarative memory function after acute administration of synthetic corticosteroids (for a complete review, see Lupien and McEwen, 1997). The first study performed on the acute effects of corticosteroids on human memory process was a dose–response study. In 1986, Beckwith and collaborators showed that the effects of hydrocortisone on human memory performance depend upon the dose administered. Only the highest doses of hydrocortisone (40 mg) enhanced recall when subjects were presented with lists of words. In 1993, Fehm-Wolfsdorf and collaborators reported that hydrocortisone administration in the morning (at the time of cortisol peak) impaired declarative memory function, while it had no effect on cognitive performance when administered at night. In 1996, Kirschbaum and collaborators showed that the oral administration of 10 mg hydrocortisone leads to a significant decrease in memory performance as tested 60 minutes after hydrocortisone intake. The results showed that subjects who received hydrocortisone treatment presented an impaired performance in the declarative memory task but not in the non-declarative memory task, thus suggesting that cortisol interacts with hippocampal neurons to induce cognitive deficits. More recently, De Quervain *et al.* (2000) tested the impact of an acute increase of corticosteroids as a function of the nature of memory processing. High doses of synthetic corticosteroids were administered before the acquisition of a list of words, or immediately after, or just before the retrieval of the list. The results revealed significant impairments in memory when the drug was administered just before retrieval, thus suggesting specific effects of corticosteroids on the retrieval of previously learned information.

Besides acute actions, delayed effects of corticosteroids were reported in memory studies of human subjects. In 1990, Wolkowitz and collaborators observed impaired memory performance in normal adults following 5 days' administration of high doses of prednisone (80 mg p.o. daily) but normal memory performance in another group of subjects following a more acute administration of 1 mg dexamethasone. In 1994, Newcomer and collaborators, using a 4-day administration procedure with 0.5, 1, 1 and 1 mg per day of dexamethasone in normal controls, reported impaired declarative memory performance (acquisition and recall) on the fourth day of treatment only. Similar results were obtained by the same group using hydrocortisone (Newcomer *et al.*, 1999). In both studies, no immediate or delayed effects of dexamethasone were observed on non-declarative memory or selective attention performance. Similar results were obtained by Schmidt *et al.* (1999) using a 4-day regimen of 160 mg prednisone. These results were in accordance with a hippocampal involvement in corticosteroid-related cognitive deficits and argued against a nonspecific effect of the steroid on attention and arousal.

In summary, the majority of studies performed in human populations tend to confirm the rodent literature reporting negative effects of corticosteroids on hippocampal-dependent forms of memory. Altogether, the rodent and human data have strengthened the view that stress hormones have a specific impact on the hippocampus.

Effects on Memory Sustained by the Frontal Lobes

Interestingly, there is evidence for a high density of corticosteroid receptors in the cerebral cortex of both rats (Meaney and Aitken, 1985; McEwen *et al.*, 1986) and humans (Sarrieau *et al.*, 1988). Binding studies in rats revealed a high retention of ^{3}H-CORT in the cortex, particularly in the medial prefrontal regions (McEwen *et al.*, 1968; Diorio *et al.*, 1993). Further studies in rats (Feldman and Conforti, 1985; Moghaddam *et al.*, 1994; Bagley and Moghaddam, 1997) and humans (Murros *et al.*, 1993; Shimizu *et al.*, 1997) have shown that the prefrontal cortex is a significant target for the negative feedback actions of circulating corticosteroids (Feldman and Conforti, 1985; Moghaddam *et al.*, 1994), which suggests that this area could play a significant role in the acute effects of corticosteroids on cognitive function.

Studies in nonhuman primates (Goldman-Rakic, 1987; Goldman-Rakic, 1995) and humans (Petrides and Milner, 1982; Owen *et al.*, 1990) showed that lesions of the dorsolateral prefrontal cortex (DLPFC) give rise to impairments in working memory. Working memory is the cognitive mechanism that allows us to keep a limited amount of information active for a limited period of time (see Baddeley, 1995). Thus, working memory impairments have been found in several experiments using a variety of delayed-task procedures. In these tasks, a temporal gap is introduced between a stimulus and a response, which creates the need to maintain the stimulus in a temporary memory storage. Data obtained in monkeys show that cells in the lateral prefrontal cortex become particularly active during delayed-response tasks, suggesting that these cells are actively involved in holding on to the information during the delay (Goldman-Rakic, 1990; Goldman-Rakic, 1995).

Neuropsychological evidence suggests that humans with prefrontal damage have impaired working memory (Luria, 1966;

Fuster, 1980; Damasio, 1985). These patients are also highly susceptible to proactive interference, and they perform poorly on neuropsychological tests that require response inhibition, such as the Wisconsin Card Sorting Test (WCST) (Stuss *et al.*, 1982; Shimamura, 1995). Moreover, recent neuroimaging data summarized and reviewed by Smith *et al.* (1998) (see also Dolan and Fletcher, 1997 and Ungerleider *et al.*, 1998) show a significant relationship between working memory processing and activation observed in the prefrontal cortex (Smith *et al.*, 1998; Ungerleider *et al.*, 1998).

Three studies performed in humans report that working memory is more sensitive than declarative memory to acute and short-term (10 days) increases in corticosteroids. In a study performed by Schmidt *et al.* (1999), young normal controls were administered with 160 mg prednisone for 4 days, and electroencephalogram (EEG) activity was measured on the fourth day of treatment. These authors found that participants treated with prednisone showed a significantly greater increase in self-reported negative emotion and significantly greater right frontal EEG activity compared with participants who were treated with placebo. More importantly, it was shown that the effects of prednisone on EEG activity were restricted to the frontal brain region. This finding is important in light of studies showing that the frontal cortex is implicated in the processing and regulation of emotion (Fox, 1991; Fox, 1994), and others showing that right frontal EEG activity is related to the processing of negative emotion in anxious and socially withdrawn adults (Davidson, 1993; Schmidt and Fox, 1994).

Young *et al.* (1999) administered 20 mg hydrocortisone for 10 days to young normal male volunteers and measured various cognitive functions in a randomized, placebo-controlled, crossover, within-subject design. They showed that this regimen of hydrocortisone led to deficits in cognitive function sensitive to frontal lobe dysfunction, but it did not impact on cognitive function sensitive to hippocampal damage. Similar results were obtained by our group (Lupien *et al.*, 1999a) using an acute dose–response neuroendocrine protocol. In this study, 40 young subjects were infused for 100 minutes with either hydrocortisone or placebo, and memory function was tested during the infusion period. The results revealed that performance on the working memory task decreased significantly, whereas performance on the declarative memory task remained the same following an acute elevation of corticosteroids. Curve-fit estimations revealed the existence of a significant quadratic function (U-shaped curve) between performance on the working memory task and changes in cortisol levels after hydrocortisone infusion. The results of these three studies suggest that in young people, working memory is more sensitive than declarative memory to an acute elevation of corticosteroids.

CHRONIC EFFECTS ON CORTICOSTEROIDS ON MEMORY

Due to obvious ethical reasons, most of the human studies that assess the long-term impact of hypercortisolaemia on memory function are correlational, although some have measured prospectively the impact of treatment on memory (Starkman *et al.*, 1999). In general, three types of condition associated with increases in circulating levels of corticosteroids have been studied, namely Cushing's disease (Starkman *et al.*, 1992; Starkman *et al.*, 1999), depression (Sheline *et al.*, 1996; Sheline *et al.*, 1999; Rajkowska *et al.*, 1999; Bremner *et al.*, 2000; Lucassen *et al.*, 2001) and unhealthy ageing (Davis *et al.*, 1986; Lupien *et al.*, 1998).

Clinical Disorders

Mental disturbances mimicking mild dementia, such as decrements in simple and complex attentional tasks, verbal and visual memory, encoding, storage and retrieval, have been described in depressed patients with hypercortisolism (Weingartner *et al.*, 1981; Cohen *et al.*, 1982; Rubinow *et al.*, 1984; Roy-Byrne *et al.*, 1986; Wolkowitz and Weingartner, 1988; Wolkowitz *et al.*, 1990), and in steroid psychosis following corticosteroids treatment (Hall *et al.*, 1979; Ling *et al.*, 1981; Varney *et al.*, 1984; Wolkowitz and Rapaport, 1989). Patients with Cushing's syndrome represent a powerful model in which to assess the association of hypercortisolaemia and memory impairment in humans, since these patients experience high levels of endogenous cortisol for periods of months or years. In 1992, Starkman and collaborators reported significant verbal memory impairment in patients with Cushing's disease. Using magnetic resonance imaging (MRI) of the brain, they showed that Cushing's disease patients have decreased hippocampal volume when compared with controls (Starkman *et al.*, 1992). In the same study, hippocampal volume was correlated negatively with plasma cortisol concentrations and correlated positively with scores on verbal learning and recall tasks. More recently, the same group (Starkman *et al.*, 1999) reported that therapeutic decreases in cortisol levels in Cushing's disease patients increased by 10% the hippocampal formation of treated patients, revealing some forms of reversibility of hippocampal atrophy in humans.

Similar atrophy of the hippocampus has been reported in depressed patients by Sheline *et al.* (1996) and Bremner *et al.* (2000). However, and in contrast with studies performed in Cushing's disease patients, the hippocampal atrophy reported to occur in depressed populations was observed in the absence of increased hypercortisolaemia (Sheline *et al.*, 1996; Sheline *et al.*, 1999) or the absence of cortisol measurement (Bremner *et al.*, 2000) in patient populations. In this context, it is particularly difficult to associate the observed hippocampal atrophy with the presence of hypercortisolaemia in this patient population.

Unhealthy Ageing

It is well known that the hippocampus is a brain structure that can atrophy as an organism ages (Jack *et al.*, 1998). Because prolonged exposure to corticosteroids has been reported to have detrimental effects on the hippocampus, a role of corticosteroids in the development of age-related cognitive impairments has been suggested (see Landfield *et al.*, 1978; Landfield *et al.*, 1981).

The results of studies performed on HPA activity during ageing in animals have indicated that in the rat, changes in HPA function are not an inevitable consequence of ageing, and individual differences in HPA activity are related closely to hippocampal dysfunction and cognitive impairments (Landfield *et al.*, 1978; Landfield *et al.*, 1981; Meaney *et al.*, 1988; Meaney *et al.*, 1991; Issa *et al.*, 1990; Yau *et al.*, 1995; Rowe *et al.*, 1997). In 1978, Landfield and collaborators showed that adrenal corticosteroid levels predict the magnitude of hippocampal neuron loss and cognitive impairments in the rat. These results suggested that in a normal population of laboratory rats, individual differences in HPA activity should serve as a predictor of age-related hippocampal pathology. This question was examined later by Issa *et al.* (1990), who screened a large sample of aged (22–28 months) rats using a test of spatial memory. The rationale was this: if HPA dysfunction is associated with hippocampal pathology and not merely with advanced age, then one would expect that a sample of aged, cognitively impaired (ACI) and aged, cognitively unimpaired (ACU) rats should differ considerably in HPA activity. Issa *et al.* used the Morris water maze to screen over 100 aged animals in order to select aged animals that were either cognitively impaired or unimpaired (>2 SD or <0.5 SD from the mean of 6-month-old animals; respectively). According to this criteria, about 30% of the animals were designated as ACI and a comparable percentage as ACU (underscoring the extreme variation in cognitive decline in aged rats) (see also Gage *et al.*,

1984 and Gallagher and Pellymounter, 1988). Interestingly, both groups of aged animals showed a loss of hippocampal neurons; however, the decrease in neuron density was substantially greater in the ACI rats.

The ACI animals showed increased plasma ACTH and corticosterone levels under both basal and post-stress conditions, whereas HPA activity in the ACU animals did not differ from 6-month-old controls. The increase in basal ACTH and corticosterone in the ACI rats was observed only in the p.m. phase of the cycle. Interestingly, while both aged groups showed a loss of hippocampal corticosteroid receptors (with no change in hypothalamic or pituitary receptor density), the loss was significantly greater in the ACI animals. The ACI rats also showed a significant decrease in hippocampal mineralocorticoid receptor density. These findings demonstrate the overall loss of hippocampal corticosteroid receptors in the ACI animals. Taken together with the findings of the Landfield studies, these data strongly suggest that long-term exposure to increased corticosteroid levels is selectively associated with the occurrence of hippocampal pathology and impaired cognition in later life.

Before 1991, the majority of human studies performed in order to measure whether basal cortisol levels increase with ageing in human populations were cross-sectional studies. In general, these studies revealed that basal cortisol levels generally do not change across age in healthy subjects (West *et al.*, 1961; Jensen and Blichert-Toft, 1971; Sherman *et al.*, 1985; Waltman *et al.*, 1991), although higher evening levels of plasma cortisol levels (Friedman *et al.*, 1969; Jensen and Blichert-Toft, 1971; Touitou *et al.*, 1982) and lower morning basal plasma cortisol levels (Maes *et al.*, 1994) have been reported in aged subjects, as well as a phase advance in their diurnal rhythm (Drafta *et al.*, 1982; Sherman *et al.*, 1985). This picture is consistent with animal studies indicating that in the rat, increased HPA activity is not a necessary consequence of ageing, but is significantly more prevalent in aged rats selected for spatial memory deficits than in cognitively unimpaired aged rats (Landfield *et al.*, 1978; Landfield *et al.*, 1981; Issa *et al.*, 1990).

However, interpretations of the results of earlier cross-sectional studies in healthy human subjects were somewhat compromised by the fact that HPA activity was often measured only once in young and elderly subjects in order to assess the existence of increased cortisol secretion in elderly subjects as a group. Since increased HPA activity does not seem to be a universal feature of ageing, this approach masked the individual differences that are common in aged populations and that are predictive of neuropathology in aged rats (McEwen *et al.*, 1986; Sapolsky *et al.*, 1986; Miller *et al.*, 1994). Moreover, the fact that each subject was measured only once might have obscured the age-related changes in cortisol levels in individual subjects. Indeed, animal data have shown that it is the cumulative exposure of the hippocampus to high levels of stress hormones that proves to be detrimental for an organism, rather than acutely high levels of stress hormones at one point in the individual's life (Landfield *et al.*, 1978; Landfield *et al.*, 1981).

Considering the importance of this issue, we examined a large sample of aged, healthy controls with hourly 24-hour sampling on a longitudinal basis (ranging from 3 to 6 years). Seventeen female and 34 male subjects aged 60–90 years participated in this longitudinal study. In order to have a measure of the change in cortisol levels over years for a particular subject, a simple regression analysis on plasma cortisol levels for each subject was conducted using year as the independent variable and the integrated 24-hour cortisol concentration (area under the curve) at each year as the dependent variable. The direction and amplitude of the slope of the regression line then served as the measure of the cortisol history per subject ('cortisol slope'). Indeed, the direction of the slope (positive = increasing cortisol levels with years, or negative = decreasing cortisol levels with years) gave us an indication of the changes in cortisol levels with time. The magnitude of the slope (e.g. 0.4 *v*. 0.7) gave us an indication of the rapidity of these changes over time (Lupien *et al.*, 1995).

Using this measure, we found considerable variation in plasma cortisol levels, as well as clear evidence for subgroups which show (1) a progressive year-to-year increase in cortisol levels with currently high levels (the increasing/high cortisol group), or (2) a progressive year-to-year increase in cortisol levels with currently moderate levels (the increasing/moderate cortisol group), or (3) a progressive decrease in cortisol levels with currently moderate cortisol levels (the decreasing/moderate cortisol group). We measured the endocrine and metabolic correlates of these subgroups and showed that there is no change in the circadian rhythm or corticosteroid binding globulin (CBG) levels in these three groups of subjects, nor are there any gender differences between men and women with regard to cortisol history or any other variables tested (Lupien *et al.*, 1995). We also measured the neuropsychological correlates of these subgroups and showed that the increasing/high cortisol group presents significant impairments in declarative memory when compared with the other two groups of subjects, although they do not present any impairment in non-declarative memory (Lupien *et al.*, 1994; Lupien *et al.*, 1998). This result has recently been duplicated in a larger population in which a decrease in declarative memory function was shown to be related to a significant increase of cortisol levels over a 1-year period (Seeman *et al.*, 1997). Then we measured the neuroendocrine correlates of these subgroups, particularly those concerned with levels of dehydroepiandrosterone-sulphate (DHEA-S) levels. We found that although DHEA-S levels were decreased in all subjects, this measure could not differentiate groups, nor was it related to cognitive function (Lupien *et al.*, 1999b). Next, we measured the environmental validity of our yearly laboratory cortisol measures by taking salivary samples for cortisol analysis at the subject's home four times a day over a 30-day period. Data revealed that cortisol levels clearly differentiate the subgroups over this longer period of time (Lupien *et al.*, 1998). Finally, we measured hippocampal volumes in subjects from the increasing/high cortisol and decreasing/moderate cortisol groups and reported a 14% significant decrease in the hippocampal volume of the increasing/high cortisol group when compared with the decreasing/moderate cortisol group. We also showed that hippocampal volume in this population is correlated significantly with both current salivary and dynamic (cortisol slope) plasma cortisol levels (Lupien *et al.*, 1998).

The results of our studies thus reveal that elderly human subjects showing a significant increase of cortisol levels over years with currently high cortisol levels are impaired on tests of memory known to be dependent upon hippocampal function, and present a significant 14% reduction in hippocampal volume, when compared with elderly subjects showing a decrease of cortisol levels over years with currently moderate cortisol levels. These results are in accordance with animal studies showing that cumulative exposure to high levels of corticosteroids has functional and structural consequences on the hippocampus (Landfield *et al.*, 1978; Landfield *et al.*, 1981).

MODULATORY INFLUENCES OF CORTICOSTEROIDS ON MEMORY

Although the majority of studies performed in human populations still report negative effects of corticosteroids on cognitive function (for a complete review, see Lupien and McEwen, 1997), various studies performed in rodents report a positive impact of corticosteroids on learning and memory (Micco *et al.*, 1979; Micco and McEwen, 1980; Bohus *et al.*, 1983; Veldhuis *et al.*, 1985; De Kloet *et al.*, 1988; Mitchell and Meaney, 1991).

Such a positive impact of corticosteroids on memory has been confirmed by hormone-replacement protocols. In a hormone-replacement protocol, the behaviour resulting from the absence of the hormone of interest is measured, then baseline hormonal levels are restored to normal values and the same behaviour measured again. It is postulated that if the hormone of interest has a real impact on the behaviour tested, then this behaviour should be restored to normal value after hormonal replacement (see Brown, 1998). Using this paradigm, many have reported that pretraining as well as post-training administration of corticosterone restores an impaired learned behaviour or extinction pattern induced by adrenalectomy (Micco *et al.*, 1979; Micco and McEwen, 1980; Bohus *et al.*, 1983; Veldhuis *et al.*, 1985; De Kloet *et al.*, 1988; Mitchell and Meaney, 1991). Veldhuis *et al.* (1982, 1985) have further shown that pretraining administration of corticosterone blocks the reduction in the pattern of exploratory behaviour observed after adrenalectomy. Because modulation of cortisol levels gives rise to a concomitant modulation of the learning and memory processes, direct implication of corticosteroids in memory function were postulated. The rodent studies summarized above, and hormone-replacement protocols, have shown that a physiological decrease of circulating corticosteroids is as detrimental to memory function as is a physiological increase.

The reason why hormone-replacement protocols might permit the assessment of the positive impact of corticosteroids on cognitive function relates directly to the differential involvement of mineralocorticoid and glucocorticoid receptors in cognitive function. Remember that circulating corticosteroids bind with high affinity to two corticosteroid receptor subtypes, the mineralocorticoid and glucocorticoid receptors. Although both receptor types have been implicated in mediating corticosteroid feedback effects (see Reul and De Kloet, 1985), there is a major difference between the receptors. Mineralocorticoid receptors bind corticosteroids with an affinity that is about six to ten times higher than that of glucocorticoid receptors. This differential affinity results in a striking difference in occupation of the two receptor types under different conditions and time of day.

Many studies performed in rodents have reported that the ratio of mineralocorticoid receptor/glucocorticoid receptor occupation is a major determinant of the direction of corticosteroid-induced cognitive changes (for a review, see De Kloet *et al.*, 1999). Figure XV-5.4 presents a scheme of the results obtained on LTP with variations in the levels of mineralocorticoid receptor and glucocorticoid receptor occupancy. LTP has been shown to be optimal when corticosteroid levels are elevated mildly, i.e. when the ratio of mineralocorticoid receptor/glucocorticoid receptor occupation is high (see Diamond *et al.*, 1992). In contrast, significant decreases in LTP are observed after adrenalectomy, when mineralocorticoid receptor occupancy is very low (Dubrovsky *et al.*, 1987; Filipini *et al.*, 1991), or after exogenous administration of synthetic corticosteroids (Bennett *et al.*, 1991; Pavlides *et al.*, 1993).

De Kloet *et al.* (1999) have reinterpreted the well-known inverted U-shaped function between circulating levels of corticosteroids and cognitive performance in line with the mineralocorticoid receptor/glucocorticoid receptor ratio hypothesis. In this view, cognitive function can be enhanced when most of the mineralocorticoid receptors and only some of the glucocorticoid receptors are activated (top of the inverted U-shaped function; increased mineralocorticoid receptor/glucocorticoid receptor ratio). However, when circulating levels of corticosteroid are decreased or increased significantly (extremes of the inverted U-shaped function; low mineralocorticoid receptor/glucocorticoid receptor ratio), cognitive impairments will result. The authors suggested that the negative view of corticosteroid actions on human cognitive function could be explained partly by limitations in previous human experimental designs, which did not allow differential manipulation of mineralocorticoid receptor and glucocorticoid receptor levels. In order to do this, such studies should measure cognitive function when corticosteroid occupancy is decreased (rather than increased), thus allowing functional measures of mineralocorticoid receptor/glucocorticoid receptor occupancy on learning and memory.

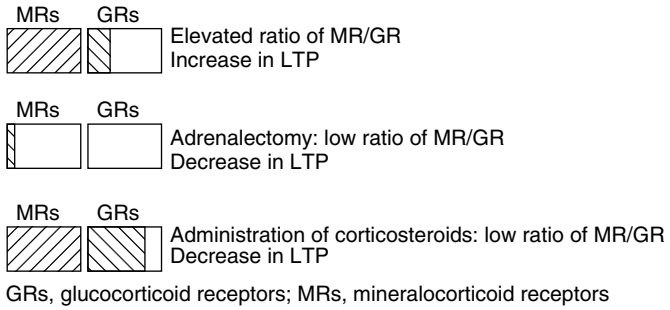

Figure XV-5.4 Level of occupancy of mineralocorticoid receptors and glucocorticoid receptors under different treatments, and its effects on LTP as a function of the ratio of mineralocorticoid receptors/glucocorticoid receptors

Acute Modulatory Effects of Corticosteroids

In order to test this suggestion, we have performed a hormone-replacement study in a population of young normal controls (Lupien *et al.*, 2002). In this protocol, we used a within-subject, double-blind experimental protocol in which we first induced a chemical lowering of corticosteroids levels by administration of metyrapone, a potent inhibitor of corticosteroids synthesis, and then restored baseline circulating corticosteroid levels with subsequent infusion of hydrocortisone. Memory performance of participants under each of these conditions was compared with that measured on a placebo day. The results showed that when compared with placebo, the pharmacological decrease of circulating levels of corticosteroids induced by metyrapone significantly impaired memory performance. Most importantly, we showed that this impairment was restored completely after hydrocortisone replacement. These results showed that corticosteroids can modulate memory function. We have suggested that this modulation can happen through a differential activation of mineralocorticoid receptors and glucocorticoid receptors. Indeed, during the metyrapone condition, mineralocorticoid receptor occupancy was low given the significant decrease of cortisol secretion induced by metyrapone. At this point, impairment in memory was observed. On the contrary, during the hydrocortisone-replacement condition, cortisol levels were restored to the levels typical of those measured in the a.m. phase, i.e. leading to a saturation of mineralocorticoid receptors, with partial occupancy of glucocorticoid receptors. This differential occupation thus led to an increased mineralocorticoid receptor/glucocorticoid receptor ratio and a restoration of baseline cognitive performance.

Chronic Modulatory Effects of Corticosteroids

As we have reported previously, results obtained from our longitudinal study of basal cortisol levels and cognitive function during human ageing show that elderly humans with an increase in cortisol levels with years and currently elevated cortisol levels at the time of testing present with both memory impairment and hippocampal atrophy (the increasing/high cortisol group) when compared with elderly people with a similar increase of cortisol levels with years but only moderate current cortisol levels at the time of testing (the increasing/moderate cortisol group). Given that the only difference between these two groups was their current cortisol levels, it was not clear whether the corticosteroid-related memory

deficits observed in the increasing/high cortisol group were related to their acutely high levels of corticosteroids at the time of testing (leading to a low mineralocorticoid receptor/glucocorticoid receptor ratio) or to a history of high corticosteroid levels (leading to hippocampal dysfunction and related memory impairments).

In order to examine this question, we measured the cognitive effects of our hormone-replacement protocol in elderly people from the increasing/high cortisol group and from the increasing/moderate cortisol group (Lupien *et al.*, submitted). We predicted that if the memory impairments of the increasing/high cortisol group were due to acute high levels of corticosteroid, then memory should benefit from an acute decrease in circulating levels of corticosteroid (which would restore the mineralocorticoid receptor/glucocorticoid receptor balance), while the memory impairment should be restored after hydrocortisone replacement. On the contrary, if the memory impairment of this group was due to their chronic exposure to high levels of corticosteroid (and thus possibly corticosteroid-induced brain dysfunction), then there should be no modulatory effects of the pharmacological manipulations on memory function. The results confirmed the chronic exposure hypothesis of memory impairments in the increasing/high cortisol group, as we showed that metyrapone treatment did not have any effect on memory performance, but hydrocortisone treatment significantly impaired memory. In contrast, we found that inhibition of cortisol production in the increasing/moderate cortisol group significantly impaired learning capacity and increased the rate of forgetting, while this pattern of cognitive impairments was reversed completely by subsequent administration of hydrocortisone.

Based on the results obtained in the increasing/high cortisol group, we suggested that the memory impairments in this group of elderly people are due to a relative loss of mineralocorticoid receptors, with normal or heightened sensitivity of glucocorticoid receptors. A loss of mineralocorticoid receptors would explain the absence of any metyrapone-induced memory effects in this population, as there are fewer mineralocorticoid receptors to bind circulating levels of corticosteroid, thus preventing any significant impact of the absence of corticosteroids on binding to mineralocorticoid receptors. Fewer mineralocorticoid receptors could also lead to a faster saturation of glucocorticoid receptors, leading to the increased sensitivity of glucocorticoid receptor activation and the induction of significant cognitive impairment after physiological increase in circulating levels of corticosteroid, as observed during the hydrocortisone-replacement condition. Our hypothesis is also supported by recent rodent (Herman and Spencer, 1998; Herman and Watson, 1995; Lopez *et al.*, 1998; Vazquez *et al.*, 1998) and human (Wetzel *et al.*,1995) studies showing that levels of mineralocorticoid receptors rather than glucocorticoid receptors are reduced markedly in response to chronic elevations in corticosteroid levels, such as those observed in elderly people with significant increases in corticosteroid levels with years.

Together, the results of these studies provide evidence that a time window for corticosteroid-induced cognitive impairments might exist and might be related to the balance in mineralocorticoid receptor/glucocorticoid receptor occupancy. Such a suggestion has important implications for studies showing that cumulative exposure to stress (Lupien *et al.*, 1998), depression (Sheline *et al.*, 1996) and/or corticosteroids (Lupien *et al.*, 1998; Starkman *et al.*, 1992) has detrimental effects on brain structures rich in corticosteroid receptors.

CONCLUSION

The data summarized in this chapter show that the best way to approach the effects of corticosteroids on animal and human cognitive function is to use a relative-impact model instead of a direct-impact model. A direct-impact model would imply that the hormone of interest has a direct and specific effect on a particular type of cognitive processing. In contrast, a relative-impact model implies that the effects of the hormone on cognitive function are relative and depend on a variety of factors that are known to be modulated by changes in the hormone of interest.

Hormones are known to be strong neuromodulators in that they tend to act on the brain by modulating an already existing connection between two cells (see Brown, 1998). In this sense, the effects of a hormone will always be relative. We have seen in this chapter that the effects of corticosteroids on animal and human cognitive function will be either positive or negative, depending on the dose and/or type of synthetic corticosteroid administered, the time of day, and the actual levels of corticosteroids achieved after administration of the synthetic compound. Given these data, one cannot view the impact of corticosteroids on cognitive function as being solely negative. Indeed, hormone-replacement protocols performed in both animal and human studies now show that corticosteroids are hormones that are necessary for adequate memory processing. Too little or too much of the hormone will have a negative impact on memory function, although these two effects are explained by very different receptor mechanisms. Given all these methodological factors to take into consideration in our study of the neuroendocrinology of cognitive disorders, we might come to the conclusion that no simple answer can be given to the impact of hormones on cognitive function. However, challenging questions still need to be studied.

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XV-6

Neuroinflammation in Neurodegeneration: Lessons from Alzheimer's Disease

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Alzheimer's disease is a neurodegenerative disorder characterized by accumulation of amyloid-β (Aβ) protein in the brain parenchyma and the walls of the leptomeningeal and parenchymal vessels. Furthermore, neurofibrillary changes and extensive neuronal loss are seen. Aβ aggregates and neurofibrillary changes are consistent features of Alzheimer's disease. Seen as neuritic plaques and tangles, they are considered to be hallmark lesions of the disease. Current post-mortem diagnosis of Alzheimer's disease is based on either semiquantitative assessment of neuritic plaques (Khachaturian, 1985; Mirra *et al.*, 1991) or regional assessment of tangles (Braak and Braak, 1991).

The aetiology of sporadic Alzheimer's disease is complex. The well-established risk factors include ageing, gender, and some genetic and environmental factors. No single cause have been identified that can be considered to be responsible for the disease, even though a combination of pathological events in genetically predisposed individuals may be considered to initiate and facilitate Alzheimer's disease pathology.

IS ALZHEIMER'S DISEASE AN IMMUNOLOGICAL DISORDER?

The idea that inflammation may contribute significantly to the pathogenesis of Alzheimer's disease has been supported as well as it has met resistance from both neuroscientists and immunologists. One of the greatest problems in evaluating the inflammatory parameters in Alzheimer's disease is related to the long progression of the disease. The possible contribution of inflammation in the pathogenesis of Alzheimer's disease is not stationary but varies with the progression of the disease, making the estimation of inflammation at any given moment difficult. The studies on brain tissue are made mostly in the final stages of the disease, and the analysis of blood and cerebrospinal fluid reflecting the events in the brain parenchyma are still quite scarce. The major objection has been related to the fact that Alzheimer's disease pathology lacks evidence for a classical immune-mediated response.

Some studies have reported a few T-cells in Alzheimer's diseased brains (McGeer *et al.*, 1989b; Rogers *et al.*, 1988), but numbers of inflammatory cells have generally been insignificant and results have not been convincing. Moreover, expression of molecules required for the recruitment of leucocytes from the blood, such as vascular adhesion molecule 1 and endothelial selectin, has not been shown in endothelial cells of brain capillaries in Alzheimer's diseased brains (Eikelenboom and Veerhuis, 1996; Eikelenboom *et al.*, 1998). Recently, however, many immunohistochemical, biochemical and molecular studies have indicated that inflammation is associated with Alzheimer's disease pathology, whereas the inflammation has been shown to be virtually nonexistent in brain tissue from normal aged individuals (McGeer and McGeer, 1999; Rogers *et al.*, 1996).

Various elements of the immune system, including features associated with both the innate and adaptive immune mechanisms, are associated with neuritic plaques and tangles. It is evident that a complex network of glial cells, cytokines, complement activation, growth factors, and oxidative stress is involved in Alzheimer's disease pathogenesis. Due to the highly interactive nature of these inflammatory subsystems, it is not plausible that any single pathway is more important than another in Alzheimer's disease pathophysiology. How, and to what extent, these inflammatory mechanisms contribute to cell death and evolution of other lesions in the Alzheimer's diseased brain remains to be resolved.

Table XV-6.1 lists the inflammatory mediators and proteases that have been found in association with amyloid plaques.

The complex inflammatory mechanisms may (1) modify the secretion and processing of the β-amyloid precursor protein (βPP); (2) modify the deposition or fibrillization of Aβ protein, a constant feature of Alzheimer's disease; (3) cause cell damage by the activation of the classic complement pathway, or by the release of potentially toxic products such as proteases, nitric oxide and free radicals; or (4) induce an abortive regenerative response in Alzheimer's diseased brains.

That the inflammatory response contributes to several crucial events in the pathogenetic cascade of Alzheimer's disease has been supported by various studies. Recent epidemiological data suggest that chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the risk of Alzheimer's disease (McGeer and McGeer, 1998; Rich *et al.*, 1995). The regional variability in the upregulation of inflammatory mechanisms is in concordance with the regional variability of Alzheimer's disease lesions. This is demonstrated clearly by the differences between cerebral and cerebellar pathology in Alzheimer's diseased brains (Brachova *et al.*, 1993; Kida *et al.*, 1995; Lue and Rogers, 1992). Whereas diffuse Aβ deposits are found in abundance in the cerebellum, neuritic plaques are virtually absent. On the other hand, the numbers of activated microglia and acute-phase proteins are lower in the cerebellum than in the cerebral cortical areas. Furthermore, most of the inflammatory mechanisms that seem to operate in the Alzheimer's diseased brain are cytotoxic in the periphery (Akiyama *et al.*, 2000). There are no reasons to think that the brain would be resistant to these toxic influences. Moreover, non-demented patients displaying some Alzheimer's disease pathology show only modest inflammatory reaction in their brains as compared with extensive neuroinflammation in demented patients with extensive Alzheimer's disease pathology (Hull *et al.*, 1995; Lue *et al.*, 1996). Further

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Table XV-6.1 Examples of inflammatory mediators and proteases that have been found in association with amyloid plaques

Complement proteins
C1–C9
Complement inhibitors
C1 inhibitor
Vitronectin
Clusterin (apoJ)
Protectin
C4-Binding protein
Acute-phase proteins
Amyloid P component
CRP
ACT
α2-Macroglobulin
α1-Antitrypsin
Cytokines and growth factors
IL-1
IL-6
TNF
TGFβ
BFGF
Chemokines and chemokine receptors
CXCR2
IP-10
MCP-1
CCR3
CCR5
MIP-1β
Others
ApoE
Proteoglycans (HSPG, CSPG, DSPG)
Heat-shock proteins (HSP-72, HSP-27)
Cathepsins (B, D, E, H, L, S)
Cystatins (A, C)
Thrombin
Tissue plasminogen activator
Integrins
ICAM-1

support for the notion that chronic inflammation contributes to the pathology of Alzheimer's disease comes from animal studies. In rats, chronic lipopolysaccharide (LPS) infusion into the fourth ventricle leads to spatial memory impairment and hippocampal pyramidal cell loss (Hauss-Wegrzyniak *et al.*, 1998), enlargement of the lateral ventricles and decrease of the hippocampal volume and the temporal lobe structures (Hauss-Wegrzyniak *et al.*, 2000), and a decrease of choline acetyltransferase activity within the basal forebrain region (Wenk *et al.*, 2000).

AMYLOID: A CENTRE OF INFLAMMATORY REACTION

The core protein of amyloid plaques is a 4–kDa peptide, Aβ, that is derived from a much larger precursor protein, βPP (Kang *et al.*, 1987). Several isoforms of βPP are generated by alternative splicing of mRNA transcribed from a single gene on chromosome 21 (Ashall and Goate, 1994). βPP is synthesized as an integral membrane molecule, whereas Aβ is secreted as a product of proteolytic cleavage of βPP (Selkoe, 1994).

Aβ accumulates in the neuropil either as diffuse protein aggregates (diffuse plaques) that contain non-fibrillar Aβ, or as fibrillary Aβ, a major constituent of neuritic plaques. The complex structure of neuritic plaques also includes abnormal neurites with paired helical filaments and activated glial cells (Wisniewski and Wegiel, 1995). It appears that the fibrillar but not the diffuse Aβ protein deposits are involved in neuronal degeneration. This is supported by the finding that microinjection of soluble Aβ into the brains of experimental animals does not induce neuronal degeneration (Winkler *et al.*, 1994). Furthermore, the presence of numerous diffuse plaques in brains from elderly non-demented individuals (Tagliavini *et al.*, 1988) and concomitant lack of neuritic pathology indicate that the mere presence of Aβ deposits seems insufficient to induce cytopathology. Many different laboratories have shown that aggregation or fibrillization of Aβ is necessary for neurotoxicity (Selkoe, 1994). Immunohistochemical studies have shown diffuse foci of Aβ without evidence of neuropil pathology (Tagliavini *et al.*, 1988), whereas in neuritic plaques the fibrillar Aβ peptide is associated with abnormal neurites, activated microglia and reactive astrocytes (Figure XV-6.1).

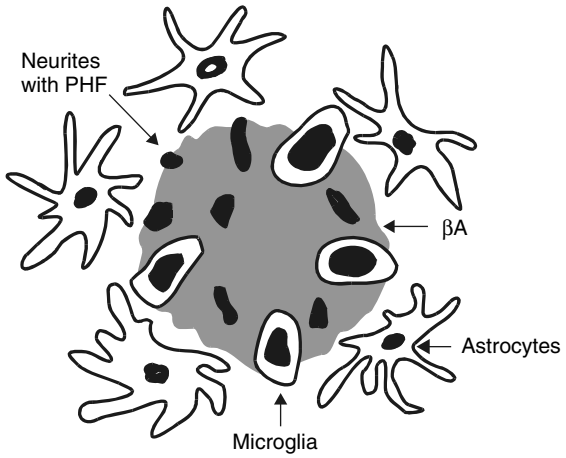

Figure XV-6.1 Schematic presentation of a neuritic plaque and location of glial cells in association with the plaque. Centrally located Aβ aggregates are surrounded by astrocytes. In the middle of the accumulated protein are seen microglial cells and neurites containing paired helical filaments (PHF) with hyperphosphorylated tau

Why does Aβ fibrillize or remain soluble? The stab-wound experiments and studies of transgenic mice show that neither overexpression of βPP alone, nor the presence of soluble Aβ protein, leads to amyloid formation (Fukuchi *et al.*, 1994; Sandhu *et al.*, 1991). Test-tube studies of synthetic Aβ peptides showed that fibril formation is influenced by Aβ peptide concentration, pH, length of Aβ peptide, and interaction with other proteins (Selkoe, 1994). Current data indicate that additional factors, such as some components of basement membrane or extracellular matrix, and Aβ carrier proteins, affect fibrillization of Aβ (Kisilevsky and Fraser, 1997). Amyloid component P or components of basement membrane and extracellular matrix may promote the initial steps in fibril formation (Leveugle and Fillit, 1994; Tennent *et al.*, 1995). The highly sulphated proteoglycans of extracellular matrix promote aggregation of Aβ peptide (Snow *et al.*, 1994), immobilize apolipoprotein E (apoE) in the amyloid aggregates (Ji *et al.*, 1993), and lead to aggregates of Aβ. The intermediating processes causing cell damage include neurotoxicity of fibrillar Aβ, oxidative injury and immune activation.

GLIAL CELLS

One of the major differences between diffuse and neuritic plaques is the presence of glial cells. Microglia and astrocytes are associated with neuritic plaques but are lacking from most diffuse plaques.

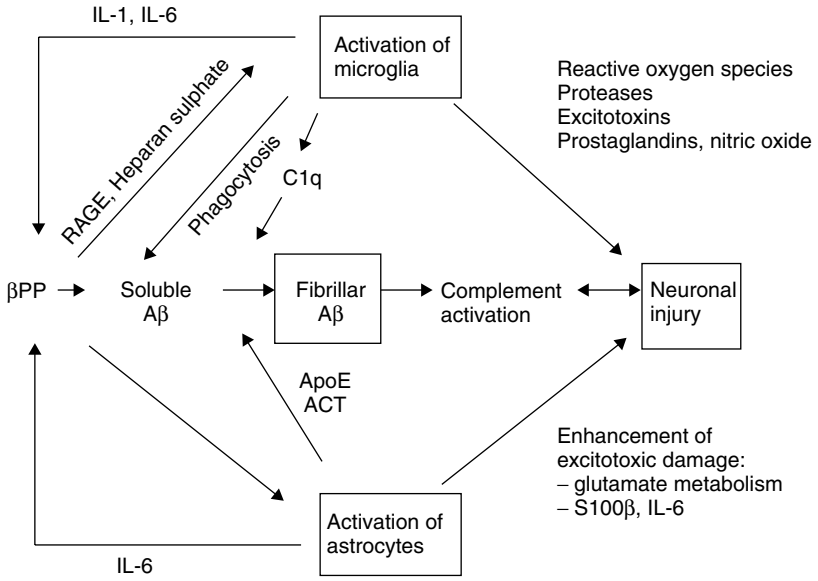

Figure XV-6.2 Activated glial cells produce substances that contribute to the production and fibrillization of Aβ and neuronal injury in the pathogenesis of Alzheimer's disease

The expression of microglial activation markers, such as class II major histocompatibility antigens, Fc-receptors, receptors belonging to the leucocyte adhesion family (β-2 integrins), and the vitronectin receptors, has been shown to be enhanced in Alzheimer's diseased brains (Akiyama *et al.*, 1991; Eikelenboom *et al.*, 1998; McGeer *et al.*, 1994; Overmyer *et al.*, 1999a). Moreover, microglia associated with neuritic plaques show intensive staining for many interleukins, such as interleukin 1 alpha (IL-1α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and tumour necrosis factor (TNF) (Eikelenboom *et al.*, 1994; McGeer and McGeer, 1999). Immunohistochemical studies have shown that a number of complement factors of the classical complement pathway and their inhibitors are localized in both diffuse and/or neuritic plaques in the neocortex and cerebellum in patients with Alzheimer's disease and Down's syndrome (Eikelenboom and Stam, 1982; Kalaria, 1993; McGeer *et al.*, 1994). While many acute-phase proteins (α1-antitrypsin, α1-antichymotrypsin (ACT), α2-macroglobulin C-reactive protein (CRP), amyloid P component) are found in neuritic plaques, only some of them (amyloid P component and ACT) are also present in diffuse plaques (Eikelenboom *et al.*, 1994; Kalaria, 1993). A variety of cell-adhesion molecules (integrins, intercellular adhesion molecule 1 (ICAM-1), vitronectin) are also found in association with neuritic plaques (Eikelenboom *et al.*, 1998).

Glial Activation in the Alzheimer's Diseased Brain

Present data suggest that a low-grade chronic inflammatory response is operating in Alzheimer's disease. Activated microglia seem to be the main immunocompetent cells contributing to the response. The signals leading to the microglial activation in the Alzheimer's diseased brain are not clear. Recent studies have shown that residues 13–16, the HHQK domain of the Aβ protein, bind to microglial cells (Giulian *et al.*, 1998), and the interaction between Aβ protein and microglia has been shown to be mediated by microglia-associated heparan sulphate. Other studies have shown that Aβ binds to microglia via the receptor for advanced glycated end-products (RAGE) (Yan *et al.*, 1996) or the scavenger receptor (El Khoury *et al.*, 1996). More recent data also imply CD40–CD40L interaction in microglial activation (Tan *et al.*, 1999). The interaction between Aβ protein and microglia may be essential for neurotoxicity.

Microglial activation may contribute to Alzheimer's disease pathogenesis and cell death in many ways (Figure XV-6.2). Activated microglia secrete proinflammatory cytokines, such as IL-1 and IL-6, and some growth factors, which have been shown to stimulate βPP mRNA and induce increased production and altered processing of βPP in neuronal or endothelial cell cultures (Del Bo *et al.*, 1995; Forloni *et al.*, 1992; Ohyagi and Tabira, 1993; Vasilakos *et al.*, 1994). In turn, Aβ stimulates glial cells in culture to produce cytokines such as IL-1, TNF-α and basic fibroblast growth factor (BFGF) (Araujo and Cotman, 1992; Meda *et al.*, 1995), and thus enhances the vicious circle. Many studies have shown that Aβ stimulates microglia via the same signalling pathways that are employed by classical immune stimuli (McDonald *et al.*, 1997; McDonald *et al.*, 1998). *In vitro* studies suggest that Aβ by itself is a weak activator of microglia, whereas Aβ in association with augmenting proinflammatory signals stimulates a robust microglial response (Araujo and Cotman, 1992; Yates *et al.*, 2000). While LPS or interferon gamma (IFN-γ) have not been detected in Alzheimer's diseased brain, macrophage colony-stimulating factor (M-CSF) is elevated in cerebrospinal fluid of Alzheimer's disease patients (Du *et al.*, 1997). The important role of M-CSF is supported further by the finding that stimulation of RAGE receptor induces M-CSF (Yan *et al.*, 1997), whereas RAGE receptor is suggested to be a ligand for Aβ. The expression of the receptor of M-CSF (M-CSFR) is increased on plaque-associated microglia (Akiyama *et al.*, 1994), and M-CSF has been shown to augment microglial activation by Aβ (Murphy *et al.*, 1998). Increased expression of M-CSFR has also been shown after mechanical brain injury or ischaemia (Raivich *et al.*, 1998; Wang *et al.*, 1999). Investigators have also reported expression of M-CSFR in AβPPV717F mice that show extensive microglial activation (Murphy *et al.*, 2000). The latter data suggest that M-CSF and M-CSFR have pathophysiological relevance in Alzheimer's disease.

A possible role of microglia in the plaque evolution could be direct synthesis of βPP and Aβ by the cells. Electron-microscopical studies have indicated that newly formed amyloid fibrils appear in the deep cytoplasmic membrane infoldings and channels of

microglia (Wegiel and Wisniewski, 1990; Wisniewski *et al.*, 1989; Wisniewski *et al.*, 1990). Furthermore, there are data indicating that microglia may be involved in conversion of non-fibrillar Aβ into insoluble fibrils (Shirahama *et al.*, 1990; Wisniewski *et al.*, 1989). Many studies have also shown that microglia phagocytose exogenous fibrillar Aβ (Shaffer *et al.*, 1995; DeWitt *et al.*, 1998).

In addition, there are many indirect mechanisms through which microglial activation in the Alzheimer's diseased brain might be harmful. Activated microglia secrete a variety of other neurotoxic factors, such as eicosanoids, free radicals and nitric oxide, as well as proteases, protease inhibitors and excitotoxins (Cotter *et al.*, 1999; Eikelenboom *et al.*, 1994; Espey *et al.*, 1997; Lipton and Gendelman, 1995; Giulian *et al.*, 1995; McGeer and McGeer, 1989b). *In vitro* experiments demonstrate that activation of microglia by Aβ leads to the production of reactive nitrogen intermediates and neuronal cell injury (Meda *et al.*, 1995; Goodwin *et al.*, 1995; Li *et al.*, 1996). Investigators have shown that a neurotoxic amine secreted by plaque-associated activated microglia destroys hippocampal pyramidal cells (Giulian *et al.*, 1995). Moreover, activated microglia secrete glutamate (Piani *et al.*, 1992), which may influence synapses and dendrites without killing the entire neuron.

Whereas the microglia are located in the plaques in the midst of the Aβ protein, surrounding the protein aggregates are numerous reactive astrocytes displaying fibrous processes and increased immunoreactivity for glial fibrillary acidic protein (GFAP) (Mandybar and Chuirazzi, 1990; Pike *et al.*, 1995; Overmyer *et al.*, 1999a). The extent of GFAP correlates with both the density of neocortical neurofibrillary tangles (Harpin *et al.*, 1990; Overmyer *et al.*, 1999b) and the number of neuritic plaques (Overmyer *et al.*, 1999b).

Aβ has been shown to induce stellate cell morphology and proliferation of astrocytes *in vitro* (Araujo and Cotman, 1992; Hu *et al.*, 1998; Kerokoski *et al.*, 2001; Singh *et al.*, 1994), although the mechanisms of this proliferative effect of A$\beta(1-42)$ are unknown. Aβ also stimulates the production of cytokines or nitric oxide in astrocyte cell cultures (Araujo and Cotman, 1992; Hu *et al.*, 1998; Pike *et al.*, 1994). The positioning of astrocytes in the surrounding of the protein aggregates is such that they may create a barrier that prevents Aβ phagocytosis. Moreover, astrocytes secrete proteoglycans that greatly inhibit the microglial attack (DeWitt *et al.*, 1998). On the other hand, reactive astrocytes secrete acute-phase proteins, which may contribute to Aβ fibrillogenesis (Koo *et al.*, 1991). For example, α1-ACT has been shown to enhance fibril formation of Aβ (Fraser *et al.*, 1993; Ma *et al.*, 1999).

Astrocytes are involved in supportive functions of the brain. They maintain ionic homeostasis, remove neurotransmitters such as glutamate thus preventing excitotoxicity, release growth factors, and participate in inflammatory responses. Another mechanism by which astrocytes may contribute to Alzheimer's disease pathogenesis is disruption of homeostatic and neurotrophic functions. Cytokines may stimulate production of prostaglandins (Parpura-Gill *et al.*, 1997; Katsuura *et al.*, 1989), which can elevate the extracellular concentration of glutamate by inducing its release from astrocytes (Bezzi *et al.*, 1998) or inhibiting its reuptake by astrocytes (Rothstein *et al.*, 1993). These changes will augment the excitotoxic damage.

Many protease inhibitors have potential neurite growth-promoting activity, and they may be released from astrocytes in response to neuronal degeneration (Kalaria, 1993). Investigators have demonstrated signs of a regenerative process in Alzheimer's diseased brains that may involve a complex network of proteases, cytokines, growth-promoting factors, protease inhibitors, integrins and adhesion molecules (Eikelenboom *et al.*, 1994). The relationship between apoE and Aβ has been studied widely since the apoE ε4 allele was shown to be the most important risk factor for the development of Alzheimer's disease (Corder *et al.*, 1993; Saunders *et al.*, 1993). ApoE is involved in transport and cellular uptake of lipid complexes via the low-density lipoprotein receptor (LDL-R) and the low-density lipoprotein receptor-related related protein (LRP) receptor (Mahley, 1988). Both of these receptors are expressed in human brain (Rebeck *et al.*, 1993; Rebeck *et al.*, 1995). ApoE binds to Aβ (Strittmatter *et al.*, 1993), and is colocalized with Aβ in plaques (Namba *et al.*, 1991). It has been suggested that LRP-mediated uptake of apoE/Aβ complexes may be a mechanism of Aβ clearance from the neuropil (Rebeck *et al.*, 1993; Rebeck *et al.*, 1995), and the risk associated with the ApoE genotype would then be related to the clearance of the Aβ from the neuropil. This is also supported by the high load of Aβ in the brain tissue of individuals carrying one or two copies of the apoE ε4 allele (Alafuzoff *et al.*, 1999).

Another important acute-phase protein implicated in the clearance of Aβ is α2-macroglobulin (α2-MG). This binds to a variety of other proteases, including ACT, forming a complex that is removed by endocytosis via α2-MG receptor (α2-MACR)/LRP (Borth, 1992). Moreover, the same endocytic pathway may operate in the clearance of many other inflammatory proteins, such as apoE, APP, IL-1β, and even Aβ itself (Borth, 1992; Narita *et al.*, 1997), and thus attenuate Aβ fibril formation and inflammation in the Alzheimer's diseased brain.

In cerebrospinal fluid, soluble Aβ forms complexes with apoE and apolipoprotein J (apoJ) (Ghiso *et al.*, 1993). Binding of Aβ with carriers or chaperon proteins, including transthyretin (Schwarzman *et al.*, 1994) and apolipoproteins E, J and A1 (Ghiso *et al.*, 1993; Koudinow *et al.*, 1994; Strittmatter *et al.*, 1993; Zlokovic *et al.*, 1994), is now considered a physiological mechanism in mediating solubility, transport and clearance of Aβ.

INTERLEUKIN 1 AND 6 AND BRAIN ACUTE-PHASE RESPONSE

In 1991, Vandenabeele and Fiers suggested that amyloidogenesis in the Alzheimic diseased brain could be due to an IL-1/IL-6-mediated acute-phase response. Since then, the role of proinflammatory cytokines IL-1α, IL-1β and IL-6 in the Alzheimer's disease pathogenesis have been studied extensively. Overexpression of these molecules has been shown consistently in the Alzheimer's diseased brain (Cacabelos *et al.*, 1994; Griffin *et al.*, 1998; Strauss *et al.*, 1992; Wood *et al.*, 1993).

Griffin *et al.* (1998) and Sheng *et al.* (1996) have promoted a hypothesis whereby microglia-associated IL-1 overexpression has a role in the pathogenic cascade in Alzheimer's disease. IL-1 overexpression seems to occur early in the plaque formation (Griffin *et al.*, 1989). IL-1 promotes synthesis and processing of βPP (Goldgaber *et al.*, 1989; Buxbaum *et al.*, 1992), astrocyte activation (Griffin *et al.*, 1989), and formation of dystrophic neurites and neuritic plaques (Mrak *et al.*, 1995). IL-1 can activate astrocytes to produce acute-phase proteins and other cytokines, such as S100β, IL-6 and interleukin 8 (IL-8) (Das and Potter, 1995; Sheng *et al.*, 1996). Elevation of protein and mRNA levels of S100β (Marshak *et al.*, 1992) in the Alzheimer's diseased brain is of particular interest, since the neurite-promoting actions of this cytokine may be a driving force in the development of dystrophic neurites (Mrak *et al.*, 1996). Animal studies have, on the other hand, implicated IL-1 in memory deficits and reduced long-term potentiation in ageing rat hippocampus (Lynch, 1998), and in hippocampal cell damage (Araujo, 1992). IL-1 also increases acetylcholinesterase activity and may thus exacerbate cholinergic dysfunction in Alzheimer's disease (Li *et al.*, 2000).

IL-6 is expressed in the nervous system during development and affects cell growth and differentiation (Satoh *et al.*, 1988; Nakashima *et al.*, 1999). Normal adult central nervous system (CNS) contains barely detectable levels of IL-6, but the expression of IL-6 is elevated strongly under pathological conditions (Frei *et al.*, 1989; Vallieres and Rivest, 1997). Receptors for IL-6 have been detected on neurons (Marz *et al.*, 1996), and many studies

have indicated that IL-6 has an important role in neuronal survival and function (Campbell, 1998; Gadient and Otten, 1997). On the other hand, chronically elevated levels of IL-6 may be destructive, as shown in the experiments with transgenic mice overexpressing IL-6 (Heyser *et al.*, 1997). These mice exhibited age-related neuropathology and deficits in avoidance learning. Upregulation of IL-6 in the Alzheimer's diseased brain seems to be an early phenomenon, as immunohistochemical studies have shown colocalization of IL-6 with diffuse plaques before development of neuritic pathology (Hull *et al.*, 1996). IL-6 may modulate βPP metabolism (Ringheim *et al.*, 1998), influence neuronal functions via induction of ERK1/ERK2 and STAT3 activation (Schuman *et al.*, 1999), and increase synthesis of plaque-associated acute-phase proteins, such as α2-MG (Ganter *et al.*, 1991; Strauss *et al.*, 1992).

Glial activation and overexpression of both IL-1 and IL-6 may be a driving force for the amyloid-associated acute-phase response. A variety of acute-phase reactants, including the pentraxins serum amyloid protein (SAP) and CRP and the protease inhibitors α1-ACT, α1-antitrypsin and α2-macroglobulin, have been shown in association with amyloid deposits. The role of these molecules in fibrillization and clearance of Aβ has already been discussed. The other interesting aspect of brain acute-phase response is that βPP has a number of properties in common with acute-phase proteins. IL-1 increases synthesis of βPP (Goldgaber *et al.*, 1989), and its expression is upregulated after tissue damage (Banati *et al.*, 1994). βPP may enhance neuroinflammation by inducing IL-1 and nitric oxide synthase (Berger and Harmon, 1997). On the other hand, βPP has neurotrophic and neuroprotective effects after transient ischaemia or excitotoxic injury (Masliah *et al.*, 1997; Smith-Swintosky *et al.*, 1994).

COMPLEMENT ATTACK IN THE ALZHEIMER'S DISEASED BRAIN

The complement system seems to be involved intrinsically in cerebral amyloidosis, and increasing evidence links complement activation in the progression of pathology in Alzheimer's diseased brains (see Akiyama *et al.*, 2000). Some studies suggest that complement production in the Alzheimer's diseased brain may be as great as that in the liver, the primary source of complement in the periphery (Yasojima *et al.*, 1999). Complement pathways may contribute to Alzheimer's disease pathology indirectly, via interactions with Aβ, or directly, causing neuronal damage.

The pattern of complement fragments in Alzheimer's diseased brains indicates activation of the classical complement pathway (Kalaria, 1993; McGeer *et al.*, 1994). Lack of immunoglobulins in neuritic plaques indicates that instead of immune complex-mediated complement activation, antibody-independent mechanisms are operative in Aβ plaques. The classical complement pathway consists of tens of components that are activated sequentially in an amplifying cascade (Akiyama *et al.*, 2000). The cascade is activated when C1q binds to activating molecules, such as antibodies, CRP, SAP and DNA. Unless inhibited, the reaction leads to formation of C5b-9, the membrane attack complex (MAC), which assembles on a cell membrane creating a transmembrane channel, which permits the diffusion of ions and small molecules across the membrane. This may lead to disruption of calcium homeostasis, and ultimately result in cell lysis. Many studies have consistently shown that fibrillar Aβ (Jiang *et al.*, 1994; Rogers *et al.*, 1992; Webster *et al.*, 1997b), and also tau-containing neurofibrillary tangles (McGeer *et al.*, 1989a; Rogers *et al.*, 1996), can directly activate the classical complement pathway and lead to formation of MAC *in vitro*. A direct linkage between Aβ and complement activation was first established by Rogers *et al.* (1992), who showed that Aβ could activate the classical complement pathway in an antibody-independent manner. Other studies have showed that C1 activation is dependent on aggregation of Aβ (Jiang *et al.*, 1994; Webster *et al.*, 1997a). An amino acid sequence on the human C1q A-chain within the collagen-like domain of C1q (residues 14–26) contains five cationic side chains that bind in a charge-based fashion to anionic side chains in the N-terminus of human Aβ (Jiang *et al.*, 1994). C1 activation by Aβ corresponds to that by other non-immune activators, such as CRP, RNA and DNA, that contain a high density of negative charges, thus providing the possibility of multivalent binding (Cooper, 1985). One requirement for C1 activation by Aβ is that the peptide is in a beta-pleated sheet, multimeric form (Webster *et al.*, 1997a). Inhibition studies have implicated Aβ residues 1–11 in C1q binding (Webster *et al.*, 1997b), and at least two negatively charged residues, Asp 7 and Glu 11, as critical for the C1 activation (Velazquez *et al.*, 1997). The latter leads to generation of C3 and C4 fragments. Recent studies have also shown that Aβ forms covalent ester-linked complexes with C3 activation fragments. After incubation of fibrillar Aβ with a complement source, the C5a activation fragment is generated, which mediates formation of the proinflammatory C5b-9 membrane attack complex (Bradt *et al.*, 1998; Webster *et al.*, 1997c). In addition to the classical pathway, fibrillar Aβ can directly activate the alternative complement pathway in serum, as well as in mixtures of purified proteins of the alternative pathway (Bradt *et al.*, 1998). The activation of complement pathways by fibrillar Aβ appears to be a specific process, as other peptides of similar size and charge, such as fibrillar amylin, lack the ability to activate complement pathways (Bradt *et al.*, 1998).

Immunohistochemical data indicate that complement activation occurs *in vivo* in Alzheimer's diseased brains. Levels of mRNA of complement proteins are upregulated in Alzheimer's diseased brains (Shen *et al.*, 1997). Many complement proteins, including C1q, C4, C3, C5, C6, C7, C8, C9, activation fragments of C3 and C4, and MAC colocalize with Aβ deposits and neurofibrillary tangles (Alafuzoff *et al.*, 1987; Eikelenboom and Stam, 1982; McGeer *et al.*, 1989a; Rogers *et al.*, 1992). The covalently bound C3b molecules in neuritic plaques may remain bound and provide a nidus for chronic complement activation (Bradt *et al.*, 1998). Many studies have shown that MAC is fixed in membranes of dystrophic neurites (Itagaki *et al.*, 1994; Webster *et al.*, 1997c), which show signs of cells under complement attack: blebbing and endocytosis of their membrane at the site of fixed complement (Webster *et al.*, 1994). C5b-9 complexes may not be directly cytotoxic for neurons, since they bear CD59 (Morgan and Gasque, 1996), but they may alter functional properties of the neurons over time. The upregulation of complement defence proteins, including C4 binding protein, vitronectin and clusterin (apoJ), gives further support for the hypothesis that complement activation is a pathophysiologically relevant process in the Alzheimer's diseased brain and contributes to neurodegeneration. ApoJ is expressed by several cell types, and in neurons apoJ mRNA is induced in response to excitotoxins (Morgan *et al.*, 1999). In the Alzheimer's diseased brain, apoJ is expressed particularly in dystrophic neurons.

In addition to complement-mediated cell damage, plaque-associated complement activation products, such as C5a, provide chemotactic and activating signals to microglia and astrocytes. Glial cells express complement receptors, including C1qR, CR1, CR3, CR4, C3aR and C5aR (Gasque *et al.*, 1997; Lacy *et al.*, 1995; Webster *et al.*, 2000a). Activation of complement and resulting chemotactic signals may be one explanation for the presence of reactive astrocytes and activated microglia in the close proximity of amyloid plaques. Interestingly, C1q inhibits Aβ uptake by cultured microglia (Webster *et al.*, 2000b), and may thus influence the clearance of extracellular Aβ.

The proteins of the complement pathways may also contribute to amyloid formation in Alzheimer's diseased brains. Aβ/C1q binding may also enhance fibrillization of Aβ (Webster *et al.*, 1995). C1q is a hexamer that contains six identical subunits and C1q A-chains.

Thus, C1q may nucleate and stabilize the formation of Aβ fibrils by binding multiple Aβ molecules in its beta-pleated configuration. Furthermore, many studies have shown that apoJ binds Aβ and may be an important carrier of Aβ in blood (Matsubara *et al.*, 1996; Shayo *et al.*, 1997). There is evidence that apoJ facilitates Aβ transport across the blood–brain barrier (Zlokovic *et al.*, 1994; Shayo *et al.*, 1997). Some studies have also indicated that binding of apoJ to Aβ may decrease the formation of oligomeric Aβ (Choi-Miura and Oda, 1996; Matsubara *et al.*, 1996).

Complement involvement in the brain is not dependent on disruption of the blood–brain barrier, since neurons, astrocytes, microglia and oligodendrocytes synthesize most of the proteins of the complement system (Morgan and Gasque, 1996). *In situ* hybridization studies indicate that neurons can express virtually all proteins of the classical complement pathway (Shen *et al.*, 1997), and expression of complement mRNA increases after brain injury (Pasinetti *et al.*, 1992). Many studies have shown upregulation of C1qB and C4 in neurons in Alzheimer's diseased brains (Akiyama *et al.*, 2000).

Neurons also express receptors for complement molecules such as C3a and C5a (Davoust *et al.*, 1999; Nataf *et al.*, 1999), but the functional significance remains to be determined. Moreover, activated microglia and astrocytes secrete cytokines such as IL-1, IL-6 and TNF-α, which can stimulate secretion of C1s and C1r, and cultures of Alzheimer's disease microglia constitutively secrete C1q (Akiyama *et al.*, 2000).

IMMUNOGENETICS IN ALZHEIMER'S DISEASE

Previous studies have suggested that the presence of HLA-A2 antigen increases the risk of Alzheimer's disease. Payami *et al.* (1991) reported that the combined odds ratio from 12 different studies was 1.35, which was statistically significant but did not imply a strong association. However, two different studies suggested that HLA-A2 might be an important risk factor, particularly in men with early-onset Alzheimer's disease (Payami *et al.*, 1991; Small and Matsuyama, 1986). On the other hand, more recent studies have suggested that the presence of HLA-DR4 antigen seems to protect against the development of Alzheimer's disease (Curran *et al.*, 1997). However, this effect was not found in patients carrying the apoE4 allele.

Because α1-ACT has been indicated in the pathogenesis of Alzheimer's disease, the ACT gene is considered to be a candidate gene involved in Alzheimer's disease. One study reported that homozygotes for the ACT*A allele had a 1.5-fold increased risk of Alzheimer's disease as compared with other subjects (Kamboh *et al.*, 1995), but this could not be confirmed in another study (Haines *et al.*, 1996). However, recent studies showed that the concomitant presence of the ACT T,T and IL-1beta T,T genotypes increased the risk of Alzheimer's disease (odds ratio 5.6) and decreased the age at onset of the disease (Licastro *et al.*, 2000).

Another plaque-associated acute-phase protein that has been implicated in the development of amyloidosis is α2-macroglobulin. Blacker *et al.* (1998) first reported a genetic association between polymorphism in the α2-macroglobulin gene and Alzheimer's disease. Since then, many studies have confirmed this association (Liao *et al.*, 1998; Myllykangas *et al.*, 1999; Dodel *et al.*, 2000). However, the genotype of the α2-macroglobulin gene showing association with Alzheimer's disease has been different in these studies, and no association was found in some studies (Crawford *et al.*, 1999; Wavrant-DeVrieze *et al.*, 1999).

Recently, polymorphism of interleukin genes has been associated with Alzheimer's disease. Investigators have reported an association between the C allele of a variable number of tandem repeat polymorphism in the 3′ flanking region of the IL-6 gene (IL-6vntr) and delayed initial onset and reduced Alzheimer's disease risk (Papassotiropoulos *et al.*, 1999). This allele has previously been shown to influence the regulation of IL-6 expression and to reduce IL-6 activity in humans (Murray *et al.*, 1997). No association between IL-6 gene polymorphisms and Alzheimer's disease risk was found in other studies (Bagli *et al.*, 2000; Bhojak *et al.*, 2000). However, a strong linkage disequilibrium between IL-6vntr and IL-6prom was found, suggesting an interaction between IL-6vntr and IL-6prom that modifies Alzheimer's disease risk (Bagli *et al.*, 2000).

Four independent studies (three clinical, one using post-mortem material) have now shown increased risk for Alzheimer's disease associated with certain polymorphisms in the genes encoding the alpha and beta isoforms of IL-1 (Grimaldi *et al.*, 2000; Du *et al.*, 2000; Nicoll *et al.*, 2000; Rebeck, 2000). The IL-1A 2/2 genotype was associated with an increased risk of early-onset Alzheimer's disease (Grimaldi *et al.*, 2000; Rebeck, 2000) and late-onset Alzheimer's disease (Du *et al.*, 2000; Nicoll *et al.*, 2000), and with an earlier age of onset, carriers of this genotype showing an onset of disease 9 years earlier than IL-1A C/C carriers (Grimaldi *et al.*, 2000). However, IL-1A genotype does not change the rate of progression of Alzheimer's disease (Rebeck, 2000). Homozygosity for both allele 2 of IL-1A and allele 2 of IL-1B conferred even greater risk for Alzheimer's disease (odds ratio 10.8) (Nicoll *et al.*, 2000). There was no evidence for an interaction between the IL-1A and the apoE ε4 polymorphisms (Du *et al.*, 2000). IL-1 genotypes may confer risk for Alzheimer's disease through IL-1 overexpression. Recent studies showed that a common polymorphism within ACT and IL-1β genes affected plasma levels of ACT or IL-1β, and Alzheimer's disease patients with the ACT T,T or IL-1β T,T genotype showed the highest levels of plasma ACT or IL-1β (Licastro *et al.*, 2000).

TRANSGENIC ALZHEIMER'S DISEASE MOUSE MODELS AND NEUROINFLAMMATION

The development of transgenic animals that partly replicate Alzheimer's disease pathology and display some of the hallmark lesions of Alzheimer's disease pathology provides a means to monitor various aspects of the disease. Extensive Aβ deposition and development of thioflavine positive plaques with associated activated astrocytes and microglia have been observed consistently in different models (Frautschy *et al.*, 1998; Masliah *et al.*, 1999; Mehlhorn *et al.*, 2000; Stalder *et al.*, 1999). However, neuronal loss has been modest, and there are no reports of paired helical filaments, i.e. tangles, in these models. Differences in mouse strains have been reported that may affect the extent of the neuropathology observed in transgenic Alzheimer's disease models.

One potentially significant difference involves the activation of complement. Some strains are deficient in C5, which is a critical component of the MAC (Pasinetti, 1996). Other studies have reported complement proteins associated with amyloid aggregates in transgenic models that overexpress mutant human βPP genes. However, the mouse C1q lacks two of the positively charged arginine residues in the A-chain critical for the binding of fibrillar Aβ (Jiang *et al.*, 1994; Velazquez *et al.*, 1997). Mouse C1 is activated less effectively by fibrillar Aβ than human C1 (Webster *et al.*, 1999). Transgenic mice overexpressing mutant forms of human presenilin 1 or 2 produce rodent Aβ, which may potentially activate mouse C1 more efficiently than human Aβ. However, despite overproduction of Aβ, these models lack the accumulation of fibrillar Aβ (Borchelt *et al.*, 1997; Holcomb *et al.*, 1998) that activates complement. Thus, these mice lack the appropriate substrate for Aβ-mediated complement activation.

The acute-phase response is also quite different in mouse compared with that in human (Szalai *et al.*, 1995; Zahedi and Whitehead, 1993). However, recent data have shown that some

models display enhanced microglial staining for the proinflammatory cytokines TNF-α and IL-1β (Benzing *et al.*, 1999), increased expression of M-CSFR on microglia (Murphy *et al.*, 2000), and IL-6 immunoreactive astrocytes surrounding fibrillar Aβ (Benzing *et al.*, 1999). These mice show evidence of increased ubiquitin (Smith *et al.*, 1998) and AT-8 immunoreactivity (Hsiao *et al.*, 1996), suggesting neuritic damage.

RELATIONSHIP BETWEEN NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND ALZHEIMER'S DISEASE

Data from 20 retrospective epidemiological studies and two prospective, longitudinal studies (Rich, 1995; Stewart *et al.*, 1997; McGeer and McGeer, 1998; Prince *et al.*, 1998) have suggested that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) may delay the onset and slow the progression of Alzheimer's disease. NSAID consumption may reduce the risk of Alzheimer's disease by up to 50% (Breitner *et al.*, 1995; Stewart *et al.*, 1997). In studies, there was a greater risk reduction when NSAID treatment began more than 2 years before diagnosis (Stewart *et al.*, 1997) and in subjects younger than 75 years old (Prince *et al.*, 1998). Studies on clinically normal patients have reported that NSAID use did not affect the plaque counts in the mesial temporal cortex, whereas others showed that the use of NSAIDs decreased the number of activated microglia (Mackenzie and Munoz, 1998). Also, some investigators reported that long-term NSAID had no effect on the progression of the pathological changes in Alzheimer's disease patients, despite a beneficial effect on cognitive performance (Halliday *et al.*, 2000), whereas others have shown that regular use of NSAIDs resulted in a reduction in glial activation in the Alzheimer's diseased brain (Alafuzoff *et al.*, 2000).

The mechanisms by which NSAIDs may reduce the risk of Alzheimer's disease is unknown. One possibility is that they decrease amyloid-associated neurotoxic inflammatory reaction (Netland *et al.*, 1998). NSAIDs may also attenuate the production of inflammatory cytokines and other products of reactive glia, such as apoE, which have been implicated in amyloid deposition.

NSAIDs may also directly reduce production of Aβ, as suggested in transgenic models and *in vitro* experiments (Dzenko *et al.*, 1997; Lim *et al.*, 2000; Netland *et al.*, 1998). *In vitro* experiments have shown that cyclo-oxygenase (COX) inhibitors decrease production of prostaglandins that may induce neuronal degeneration (Pasinetti, 1998). Another target for NSAIDs is the stimulation of peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ agonists inhibit Aβ-stimulated expression of IL-1 and TNF-α by microglia (Combs *et al.*, 1999). *In vitro* experiments have shown that inflammatory mediators modulate bPP metabolism and production of Aβ (Del Bo *et al.*, 1995; Forloni *et al.*, 1992; Vasilakos *et al.*, 1994), and contribute to the conformational change of Aβ into neurotoxic fibrillary form.

Recently, investigators reported that chronic use of ibuprofen at a dose high enough to inhibit COX and stimulate PPAR-γ reduced levels of IL-1β, glial fibrillary acidic protein (GFAP), activated microglia, dystrophic neurites, amyloid plaques and Aβ in ageing transgenic mice, which overexpress the 695-amino acid form of human βPP containing a double mutation found in a Swedish kindred with familial Alzheimer's disease (Lim *et al.*, 2000). These results indicate that ibuprofen can modulate the development of some aspects of Alzheimer's disease pathology.

CONCLUSIONS

Even though Bolsi noted in 1927 argentophilic reactive cells in the surrounding of senile plaques in Alzheimer's diseased brains, the significance of glial cells in Alzheimer's disease has only recently received attention. Identification of microglia in the Aβ protein aggregates, i.e the plaque configuration, indicates that inflammatory mechanisms operate in the pathogenesis of Alzheimer's disease. It seems unlikely that inflammatory mechanisms are the initiators of Alzheimer's disease, but they are likely to be of importance in the progression of the disease. Much evidence has linked the Aβ protein to microglia, the principal immune effector element of the brain. Microglia have been implied to be involved in the synthesis, fibrillization and clearance of this deleterious protein found constantly in Alzheimer's diseased brains. Furthermore, microglia have been linked to neuritic pathology and neuronal death via cytokines, complement activation and acute-phase reactions. Astrocytes, producers of numerous cytokines after stimulation, have also been linked to the hallmark lesions of Alzheimer's disease. There is no doubt that inflammation is involved in the pathogenesis of Alzheimer's disease, but the question still remains: how?

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XV-7

Psychophysiology: Event-Related Potentials and Psychophysics in Dementia

Claire Murphy and Spencer Wetter

Dementia is defined in the *Diagnostic and Statistical Manual of Mental Disorders* 4th edition (DSM-IV) as a deficit in memory and in at least one additional cognitive domain (American Psychiatric Association, 1994). Two major subdivisions of dementia have been posited: cortical and subcortical (Brandt, 1991; Brandt *et al.*, 1988; Butters *et al.*, 1978; Butters *et al.*, 1985; Folstein *et al.*, 1990; Huber *et al.*, 1986; Salmon *et al.*, 1989). Cortical dementias (most prevalently Alzheimer's disease) are marked by early neuropathology in areas of the cerebral cortex (often the temporal and frontal lobes). Subcortical dementias (e.g. Huntington's disease and Parkinson's disease) result in early damage to subcortical areas such as the basal ganglia and substantia nigra. These two forms of dementia have been dissociated clinically by differing patterns of neuropsychological deficits. Specifically, cortical dementias are associated with deficits in recall and recognition memory, aphasia, agnosia and apraxia (Brandt *et al.*, 1988; Huber *et al.*, 1986; Salmon *et al.*, 1989). Subcortical dementias result in slowed thinking, difficulties in recall but not recognition memory, and deficiencies in sustained attention and cognitive flexibility (Folstein *et al.*, 1990; Brandt, 1991).

The electroencephalogram (EEG) is a measure of ongoing neuroelectrical brain activity recorded from the scalp. The event-related potential (ERP) is a brain potential derived from averaging the EEG signal during a recording epoch that corresponds to evaluation of a sensory stimulus. The components of these electrophysiological responses reflect brain function and integrity. The use of electrophysiological techniques to better understand and quantify the deficits associated with dementia have been studied extensively in the past two decades, beginning with Goodin *et al.* (1978). Such techniques offer an objective and quantitative measure of sensory and cognitive functioning, without the cognitively laden demands of neuropsychological testing. They can be useful in understanding severely demented patients, and they may prove useful in detecting individuals at preclinical or very early stages of dementia. Certain electrophysiological components (e.g. the latency of the P3 component of the auditory ERP) may be useful in differentiating demented from other psychiatric patients (Figure XV-7.1). The purpose of this chapter is to review the empirical evidence for electrophysiological performance in individuals with dementia, with an emphasis on later components of brain potential waveforms because these are highly associated with the cognitive changes resulting from dementia.

ALZHEIMER'S DISEASE

Alzheimer's disease is a progressive neurodegenerative disorder marked by severe memory loss and dementia resulting from the formation of neuritic plaques and neurofibrillary tangles in the brain. Risk factors for Alzheimer's disease include age (Katzman and Kawas, 1994; Farrer *et al.*, 1995), low education (Katzman, 1993; Mortimer and Graves, 1993; Stern *et al.*, 1999), positive family history (van Duijn *et al.*, 1991; Farrer *et al.*, 1995), past head trauma (Heyman, 1998; Rasmusson *et al.*, 1995), Down's syndrome (Wisniewski *et al.*, 1985; Motte and Williams, 1989), and the ε4 allele of the apolipoprotein E gene (ApoE) (Corder *et al.*, 1993a; Poirier, 1993). Definitive diagnosis of Alzheimer's disease is made only by post-mortem autopsy or biopsy (McKhann *et al.*, 1984). When the patient is living, a provisional diagnosis of probable Alzheimer's disease can be obtained by clinical examination, neuropsychological testing, history, and the exclusion of other disorders. Diagnosis is imperfect, hence the development of additional assessment tools to improve the sensitivity and specificity of early diagnosis is of great interest. The potential for psychophysiological measures to aid in the diagnosis of Alzheimer's disease is of particular interest.

Auditory P300 in Alzheimer's Disease

The potential clinical utility of the auditory ERP has received considerable attention. Early components of the ERP (N1, P2 and N2) are considered to be more exogenous measures that reflect the processing of the stimulus by auditory cortex (Polich, 1993). The P300 (P3) component is more endogenous in nature. It reflects an individual's ability to attend to and evaluate a stimulus (Kramer and Strayer, 1988; Wickens *et al.*, 1983), and its latency is considered to be a measure of stimulus classification speed (Donchin and Coles, 1988). The P3 is typically elicited during the 'oddball' paradigm, in which a standard stimulus with a high probability of occurrence is presented along with a target stimulus with a low probability of occurrence. The processing of the target stimulus (the oddball) elicits the P3. Early studies suggested that individuals with Alzheimer's disease may produce abnormal P3s, specifically lower amplitudes and longer latencies, and that these might be associated with the degree of dementia (Goodin *et al.*, 1978; Squires *et al.*, 1980a; Pfefferbaum *et al.*, 1984). Polich (1991) reviewed eight studies investigating auditory ERPs in Alzheimer's disease, which exhibited a great deal of variability in the percentage of patients who had P3 latencies two or more standard deviations above the mean for normal controls. The range of these percentages was 13–83% (Table XV-7.1).

Considerable controversy has ensued over the clinical utility of the auditory P3. Squires *et al.* (1980a) and Gordon *et al.* (1986) reported that 74% and 80%, respectively, of demented individuals had prolonged auditory P3 latency, compared with 3% and 12% of psychiatric patients diagnosed with depression and schizophrenia. Goodin (1990) suggested that these findings show promise in the

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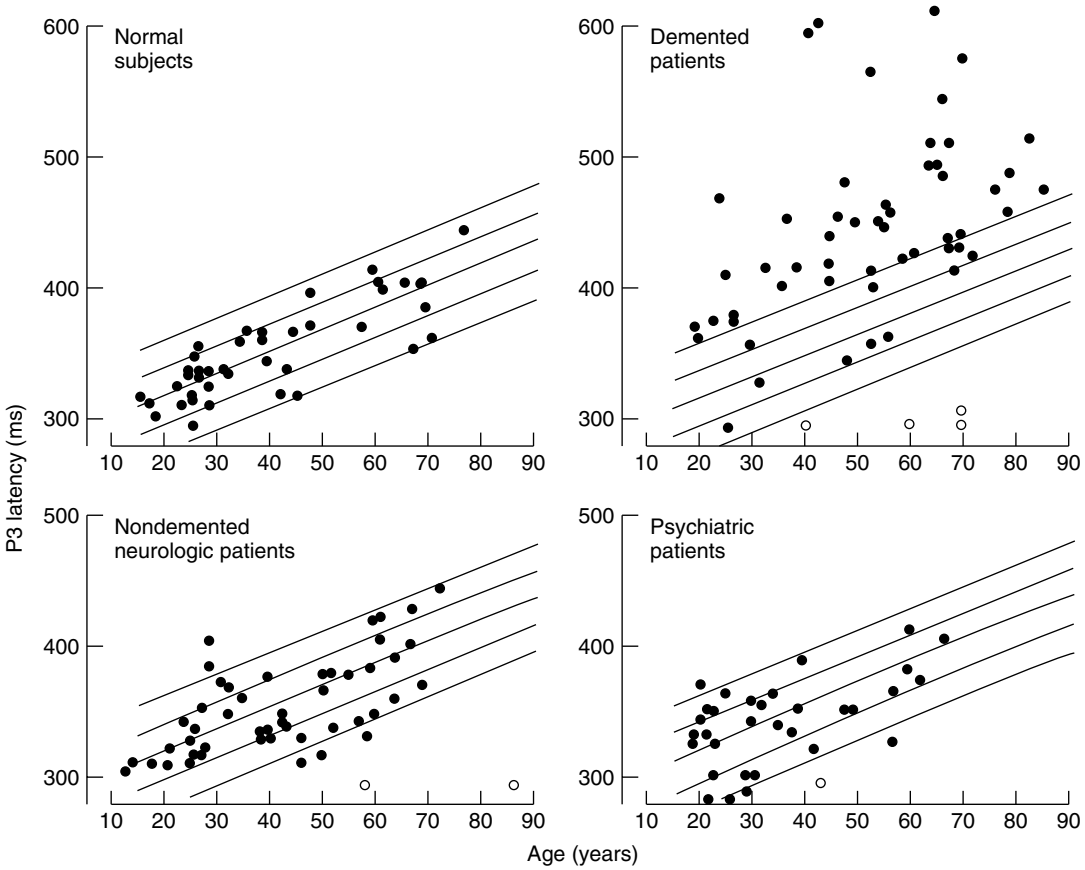

Figure XV-7.1 Auditory P3 latency by age from normal subjects, demented subjects, non-demented neurologic patients, and psychiatric patients. Reproduced by permission from Polich, J., 1991. P300 in the evaluation of aging and dementia. *American Journal of EEG Technology* 31, 201–231

Table XV-7.1 Characteristics of auditory P3 dementia studies (from Polich, 1991)

Study	Dementing illness	*n*	Age	Mini-mental state examination	Patients P3 > 2 SD (%)	Task and performance
Goodin *et al.* (1978)	Pre-senile, organic, CVA, etc.	27	55.3*	20.7	70	Count: 2–3 errors
Squires *et al.* (1980a)	Alzheimer's, CVA, etc.	58	50.7*	19.6	80	Count: 'instructions had desired effects'
Syndulko *et al.* (1982)	Alzheimer's	12	66.9	20.9	83	Count: NR
Brown *et al.* (1983)	Alzheimer's, multi-infarct, Parkinson's	18	74.0	14.7	72	Count: NR, tapping task for some patients
Pfefferbaum *et al.* (1984)	Alzheimer's, alcohol-related, CVA, etc.	37	56.8	22.4	22–30	Button press; ERPs: correct response only
Polich *et al.* (1986)	Alzheimer's, CVA, alcohol-related	39	70.6	3.9**	31	Finger-tap: 'most patients had little difficulty'
Gordon *et al.* (1986)	Primary, degenerative multi-infarct	20	77.0	≤25.0	63	Count: patients reminded of task; often wrong
Patterson *et al.* (1988)	Alzheimer's, multi-infarct	15	71.1	18.3	13	Button press: ERPs, correct response only

*Estimated; **mean Reisberg scale; CVA, cerebrovascular accident; NR, not reported.

utility of the P3 to differentiate dementia from depression (defined as 'pseudodementia' at the time). He also suggested that the P3 might be potentially useful in tracking the course of a degenerating disorder, testing response to treatment, or detecting dementia in those at risk. Pfefferbaum *et al.* (1990) also examined the existing literature at the time, but they concluded that the auditory P3 is neither consistently large nor specific enough to any particular disorder to be depended upon for diagnosis. More recent research has supported longer latencies and smaller amplitudes in Alzheimer's disease patients, but with varying degrees of sensitivity and specificity (Filipovic *et al.*, 1990; Verleger *et al.*, 1992; O'Mahony *et al.*, 1994; Cohen *et al.*, 1995; Yamaguchi *et al.*, 2000). Interestingly,

two studies found delayed P3 latency only at the Fz electrode site, with normal latencies at Cz and Pz sites (Verleger *et al.*, 1992; O'Mahony *et al.*, 1994). Onofrj *et al.* (1991) reported that a subset (37%) of patients with dementia (AD, multiple sclerosis, and progressive supranuclear palsy) did not produce a P3 component from the auditory oddball paradigm, suggesting that some patients with dementia (regardless of type) may have severe damage to areas of the brain that generate the P3. Cohen *et al.* (1995) again suggested the potential clinical utility of the auditory P3 by reporting that (1) P3 latency and amplitude (using an active oddball paradigm) predicted functional outcome (activities of daily living) in dementia, and (2) P3 latency (using a passive oddball paradigm) was the best predictor of mortality among dementia patients (even more than neuropsychological test performance). Such results support the potential utility of electrophysiological techniques to predict functional outcome of demented patients. However, if the auditory P3 is to be clinically useful, then much work needs to be done to reduce variability, identify more homogeneous patient groups for research purposes, and further understand the cognitive processes associated with the auditory oddball P3 (Polich and Kok, 1995; Barrett, 2000).

The N400 Effect

Another ERP component that has provided useful information regarding impaired and spared abilities in AD is the N400. This negative component reflects a semantic expectancy, such that the presentation of a sentence with an incongruent or unexpected final word results in a large N400 effect (Kutas and Hillyard, 1980). When the final word is as expected, the N400 is significantly smaller. The N400 effect has been shown to be smaller in AD to incongruent words (Castaneda *et al.*, 1997; Ostrosky-Solis *et al.*, 1998; Schwartz *et al.*, 1996), which likely reflects the difficulties AD patients have with processing semantic information. Ford *et al.* (1996) found that unlike controls (who had large N400s to semantically unprimed words and small N400s to semantically primed words), AD patients produced large N400s to semantically primed words. This appears to reflect a breakdown in semantic knowledge (the stem of the sentence does not prime the final word properly). However, some semantic knowledge in AD appears to be spared. For instance, recent researchers (Hamberger *et al.*, 1995; Ford *et al.*, 1996; Ford *et al.*, 2001) have reported that AD patients demonstrate an N400 priming effect. For example, for these patients, words that did not match pictures had greater N400 amplitude than those that matched pictures (Ford *et al.*, 2001). Interestingly, AD patients were unable to name many of the pictures, yet they still demonstrated the N400 effect. Such evidence suggests that even though AD patients have anomia, knowledge of the pictures was intact enough to activate cortical responses. These findings suggest that the N400 may provide useful information about the degeneration of semantic knowledge in AD.

Visual Evoked Potentials in Alzheimer's Disease

Visual evoked potentials (VEPs) were first suggested to be delayed in populations with dementia in 1976 (Visser *et al.*, 1976). The most frequently used stimuli have been visual flashes, which elicit flash VEPs (FVEPs). These have been reported to be delayed in Alzheimer's disease in a number of studies (Harding *et al.*, 1981; Cosi *et al.*, 1982; Wright *et al.*, 1984; Philpot *et al.*, 1990). However, not all investigators have demonstrated such a delay in mild-to-moderate Alzheimer's disease (Coben *et al.*, 1983; Ray *et al.*, 1991). Coben *et al.* (1983) did report latency delays for VEPs elicited by the presentation of a chessboard that was shifted either to the left or right, and they suggested that this paradigm might be more useful than FVEPs in identifying visual processing deficits in Alzheimer's disease. Swanwick *et al.* (1996) combined the FVEP with the auditory P300 in a single study. The authors reported that the FVEP might be more sensitive to cognitive dysfunction than the P300. Using a discriminant function analysis, they combined two FVEP measures (P2 and N2) and two auditory ERP measures (N2 and P3). This function proved to correctly classify subjects with a sensitivity of 80% and a specificity of 80%. Thus, a combination of ERP paradigms (perhaps in conjunction with neuropsychological data) might well be required to accurately predict outcome in the individual Alzheimer's disease patient.

Olfaction in Alzheimer's Disease

Alzheimer's disease is associated with impairment in odour identification, odour recognition memory and odour threshold, and these olfactory deficits may be useful as behavioural markers that contribute to accuracy in early assessment of Alzheimer's disease (Murphy, 1999; Murphy *et al.*, 1987: Murphy *et al.*, 1990; Nordin *et al.*, 1995; Nordin and Murphy, 1996; Nordin and Murphy, in press; Serby *et al.*, 1985). Both Alzheimer's disease patients and those at risk for the disease show deficits in odour identification, suggesting that this measure may be particularly useful in early diagnosis (Murphy, 1999). Alzheimer's disease patients score in the normal range on the picture identification test (PIT), demonstrating that they have the cognitive ability to perform an odour identification test (Morgan *et al.*, 1995), and they have normal taste threshold, indicating that they are capable of performing a threshold task (Murphy *et al.*, 1987: Murphy *et al.*, 1990). People at risk for Alzheimer's disease because of mild cognitive impairment show deficits in odour identification (Nordin and Murphy, 1996). The sensitivity and specificity of odour identification testing for Alzheimer's disease is very high and rivals many tests used in existing batteries for neuropsychological assessment of the disease (Morgan *et al.*, 1995; Morgan and Murphy, in press; Murphy, 1999). The olfactory deficits seen in Alzheimer's disease may be related to the existence of neuritic plaques and neurofibrillary tangles throughout olfactory-related brain structures. Indeed, the magnitude of olfactory threshold impairment is related to the degree of dementia (Murphy *et al.*, 1990).

The earliest changes in Alzheimer's disease patients take place in the mesial temporal lobe, particularly in the entorhinal and transentorhinal areas important for olfactory processing (Braak and Braak, 1992; Braak and Braak, 1994; Braak and Braak, 1997; Esiri and Wilcox, 1984; Pearson *et al.*, 1985; Price *et al.*, 1991). Indeed, disconnection of the entorhinal and transentorhinal areas from the hippocampus has been hypothesized to underly dementia in Alzheimer's disease (Braak and Braak, 1992; Braak and Braak, 1994; Braak and Braak, 1997). The very high sensitivity and specificity of odour identification tasks in discriminating between Alzheimer's disease patients and normal people suggests that they reflect the presence of underlying neuropathology. Significant neuropathological changes are seen in areas critical to processing olfactory information, even in the early stages of Alzheimer's disease. Murphy *et al.* (submitted) investigated whether performance on olfactory tasks (odour threshold and odour identification) was related to volumetric magnetic resonance imaging (MRI) measures of mesial temporal areas central to olfactory information processing and important in the neuropathology of early Alzheimer's disease. Participants were patients with probable Alzheimer's disease and normal age-matched controls, all diagnosed at the UCSD Alzheimer's Disease Research Centre. Robust relationships were observed between mesial temporal lobe volumes and olfactory functional measures in both Alzheimer's disease patients and controls. Relationships between left hippocampal volume and the functional

measures were greater for Alzheimer's disease patients than for controls, and for the odour identification test ($r = 0.85$) and the Boston naming test than for odour or taste threshold. The findings suggest a neural substrate for the breakdown in functional performance on verbally mediated olfactory tasks in Alzheimer's disease.

Techniques for recording the olfactory ERP (OERP) have been developed (Kobal *et al.*, 1992; Murphy *et al.*, 1994; Pause *et al.*, 1996; Morgan *et al.*, 1999; Murphy *et al.*, 2000). Olfactory stimuli elicit the same waveform components (N1, P2, N2 and P3) as auditory and visual stimuli in ERP paradigms. Morgan and Murphy (in press) investigated olfactory functioning in Alzheimer's disease using the OERP. Figure XV-7.2 illustrates the finding that OERP latencies were significantly longer in Alzheimer's disease patients than in age- and gender-matched normal controls. These latencies were associated with dementia status and classified correctly up to 92% of participants. The OERP proved to be more sensitive at correctly classifying Alzheimer's disease patients and controls than the auditory ERP. The combination of the San Diego odour identification test and the OERP P3 latency resulted in a correct classification rate of 100%. These findings suggest that the OERP, in addition to the measures mentioned above, is a useful technique in the assessment of Alzheimer's disease.

Apolipoprotein E

A key risk factor for Alzheimer's disease is the presence of the $\varepsilon 4$ allele of the ApoE gene. ApoE binds to β A4 peptide, which is the primary component of senile plaques (Strittmatter *et al.*, 1993). It

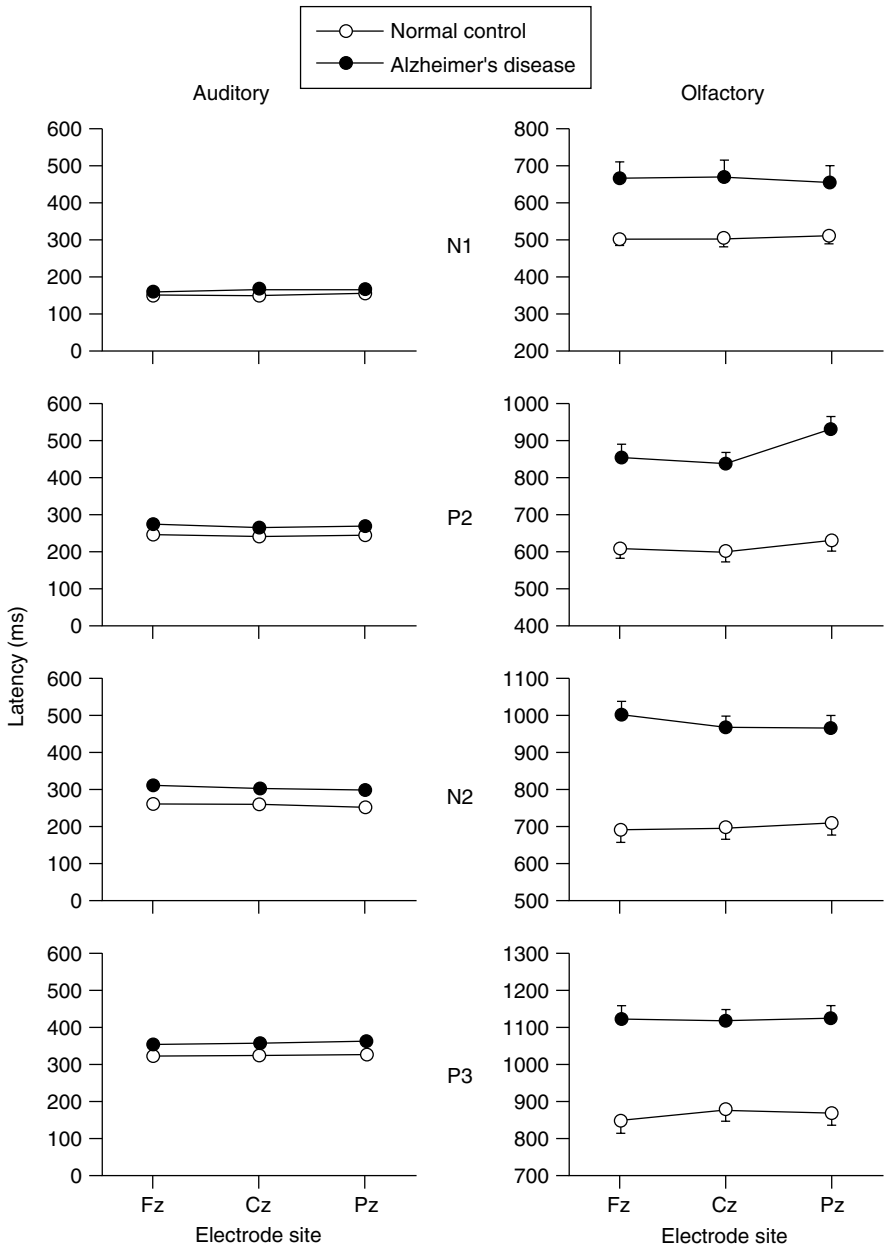

Figure XV-7.2 Auditory and olfactory ERP latencies as a function of electrode site for normal controls and Alzheimer's disease patients (from Morgan and Murphy, in press)

has also been implicated in the formation of neurofibrillary tangles (Namba *et al.*, 1991). The presence of at least one $\varepsilon 4$ allele has been strongly associated with Alzheimer's disease. It has been linked to both familial and sporadic Alzheimer's disease (Corder, *et al.*, 1993a; Corder *et al.*, 1993b; Poirier *et al.*, 1993; Saunders *et al.*, 1993).

The ApoE $\varepsilon 4$ allele has also been linked to deficits in olfactory functioning. Bacon *et al.* (1998) demonstrated that elderly individuals with at least one $\varepsilon 4$ allele demonstrated poorer odour threshold sensitivity than those without the $\varepsilon 4$ allele in the year that they were diagnosed with Alzheimer's disease. Murphy *et al.* (1998) demonstrated that non-demented elderly individuals with at least one $\varepsilon 4$ allele showed poorer performance at odour identification than those without the $\varepsilon 4$ allele. Of particular interest for the present discussion, Wetter and Murphy (2001) used the OERP to investigate older people with and without the $\varepsilon 4$ allele. Older individuals who are $\varepsilon 4$ allele positive demonstrated delayed latencies of the OERP compared with $\varepsilon 4$ allele-negative individuals. Such delays were not present in the auditory ERP (see Figure XV-7.3) for these individuals who are $\varepsilon 4$ allele positive. The magnitude of the OERP latency delays in the $\varepsilon 4$ allele-positive individuals was approximately 100 ms, in comparison with Alzheimer's disease patients,

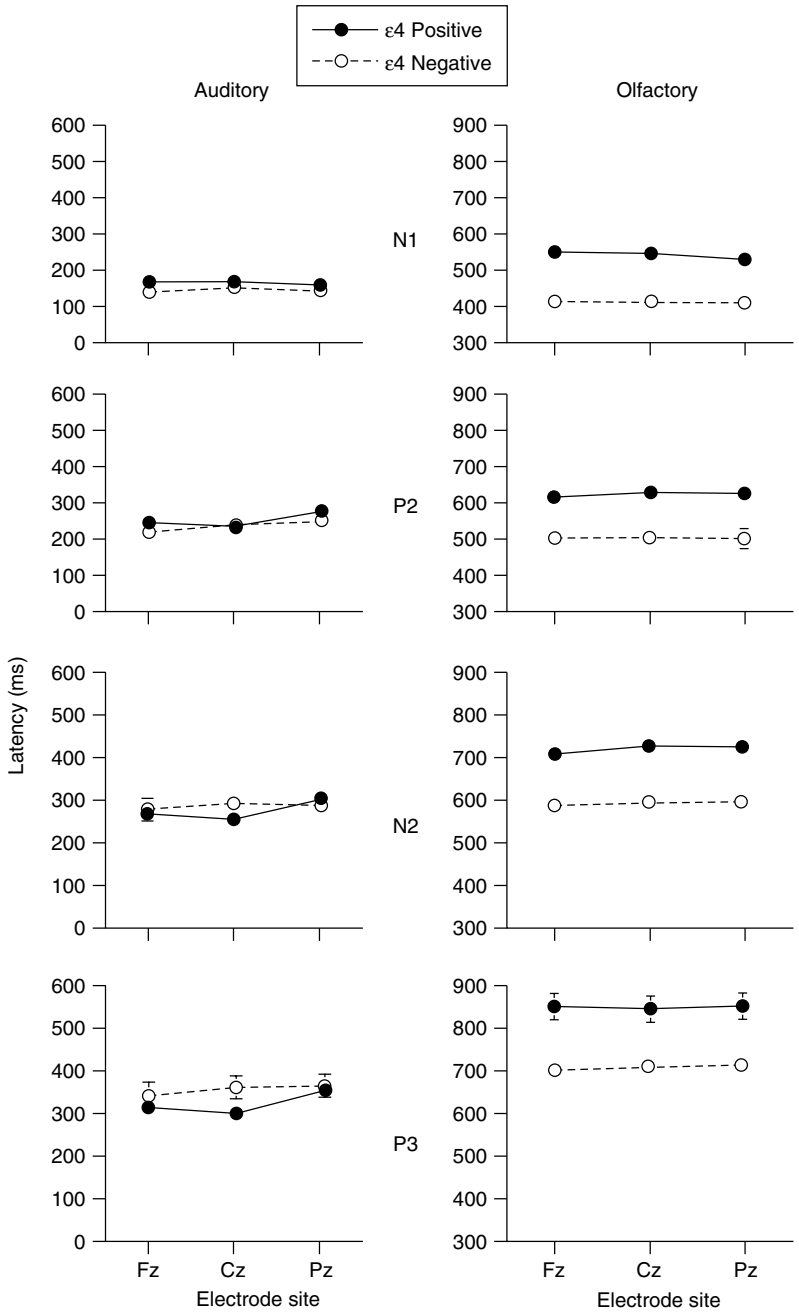

Figure XV-7.3 Auditory and olfactory ERP latencies as a function of electrode site for ApoE $\varepsilon 4$-positive and -negative individuals. Reprinted from *Neurobiology of Aging*, 22, Wetter, S. and Murphy, C., Apolipoprotein E $\varepsilon 4$ positive individuals demonstrate delayed olfactory event-related potentials, 439–447, Copyright (2001), with permission from Elsevier Science

who showed latency delays of approximately 200 ms relative to controls (Morgan and Murphy, in press). Thus, delays in the processing of olfactory information among elderly ApoE ε4-positive individuals appear to be on a continuum between normal controls and patients with Alzheimer's disease, indicating that these individuals, or a subset of them, might be demonstrating early signs of Alzheimer's disease.

DOWN'S SYNDROME

Patients with Down's syndrome who live to the fourth decade inevitably develop the clinical and neuropathological features of Alzheimer's disease: senile plaques and neurofibrillary tangles (Oliver and Holland, 1986; Schapiro and Rapoport, 1988; Brugge *et al.*, 1994; Nelson *et al.*, 1995). Wisniewski *et al.* (1985) demonstrated that the neuropathology increases with age, and that the number of plaques is associated with the degree of dementia. Adults with Down's syndrome and patients with Alzheimer's disease demonstrate a similar cholinergic deficiency (Yates *et al.*, 1980) and a degeneration of neurons in the nucleus of Meynert in the basal ganglia (Coyle *et al.*, 1983). Furthermore, there has been an increased prevalence of Down's syndrome births in families that have had a prevalence of pre-senile Alzheimer's disease (Heston *et al.*, 1981; Heyman *et al.*, 1983).

Since the initial event in Alzheimer's disease is damage in the entorhinal and transentorhinal areas of the brain, areas that process olfactory information (Price *et al.*, 1991; Braak and Braak, 1997), deficits in olfactory functioning resulting from this damage may prove to be an early indicator of Alzheimer's disease in Down's syndrome and in the general population (Morgan *et al.*, 1995; Nordin and Murphy, 1996). Early work demonstrated deficits in odour identification ability in Down's syndrome (Warner *et al.*, 1988; Hemdal *et al.*, 1993; Murphy and Jinich, 1996), and documentation of impairment in odour detection thresholds confirmed that the deficits were, indeed, olfactory (Murphy and Jinich, 1996). Both odour identification and odour threshold show greater impairment in older people with Down's syndrome than in children with Down's syndrome, suggesting that the impairment in olfactory function reflects increased neuropathology (Nijjar and Murphy, in press). Significant losses were also seen in odour recognition memory (Murphy and Jinich, 1996). Wetter and Murphy (1999) used

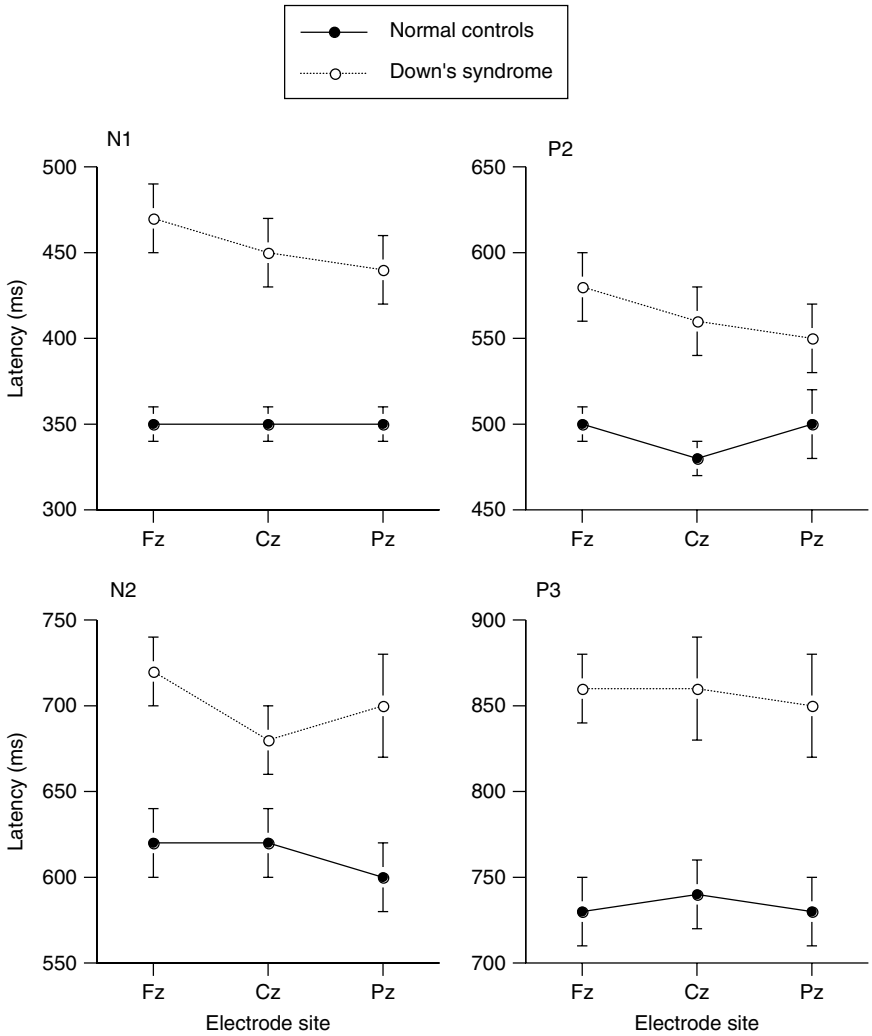

Figure XV-7.4 OERP latency as a function of electrode site for normal controls and Down's syndrome patients. Reprinted from Clinical Neurophysiology, 110, Wetter, S. and Murphy, C., Individuals with Down's syndrome demonstrate abnormal olfactory event-related potentials, 1563–1569, Copyright (1999), with permission from Elsevier Science

the OERP to investigate odour processing in the brains of older people with Down's syndrome. The people with Down's syndrome showed significantly longer latencies in both the sensory (N1, P2 and N2) and cognitive (P3) components of the OERP than normal controls (see Figure XV-7.4). Odour threshold was associated significantly with sensory OERP components. In addition, Down's syndrome subjects with a higher level of dementia showed significantly longer P3 latencies than those with lower dementia levels. Taken together, these studies suggest that measures of olfactory dysfunction in Down's syndrome may be useful in identifying incipient dementia in this population.

ERPs from other sensory modalities have been recorded in individuals with Down's syndrome. Research in auditory ERPs has demonstrated abnormalities in both early and late components. Abnormalities in brain stem responses in Down's syndrome include short latencies in some components, increased latencies in others, and/or lower amplitudes in general (Squires *et al.*, 1980b; Galbraith *et al.*, 1983; Sersen *et al.*, 1991; Kakigi and Kuroda, 1992; Miezejeski *et al.*, 1994). The latency of the auditory P3, a cognitive component of the ERP, has been demonstrated to increase compared with normal controls (Blackwood *et al.*, 1988), and progressively as an individual with Down's syndrome ages (Muir *et al.*, 1988). Other studies of auditory ERPs demonstrated that latencies of late components were delayed in Down's syndrome (Vieregge *et al.*, 1992; Diaz and Zurron, 1995).

Visual ERPs have also been recorded in individuals with Down's syndrome. As with auditory ERPs, VEP components are latency delayed in Down's syndrome (Kakigi *et al.*, 1993; Prasher *et al.*, 1994). In addition, amplitudes are significantly lower in Down's syndrome compared with normal controls (Kakigi *et al.*, 1993). As in adults, a P3 delay in rare auditory-visual events has also been demonstrated in children with Down's syndrome (Lincoln *et al.*, 1985). This delay suggests that the abnormalities in ERP components in the auditory and visual modalities in young Down's syndrome individuals may reflect their inherent cognitive deficits.

PARKINSON'S DISEASE

Auditory Event-Related Potential Deficits in Parkinson's Disease

Parkinson's disease is marked by destruction of the nigrostriatal dopaminergic system (Hornykiewicz, 1966), and is associated with motor (tremor), psychiatric (apathy, depression, irritability), and cognitive (memory retrieval deficits, bradyphrenia, initiation problems, and lack of cognitive flexibility) symptoms. The cognitive symptoms are consistent with those of a subcortical dementia, which were first identified in progressive supranuclear palsy (Albert *et al.*, 1974). These symptoms have been associated with deficits in the latency of auditory ERPs. Specifically, later components (N2 and P3) have been shown consistently to be delayed in demented Parkinson's disease patients (Goodin and Aminoff, 1986; Goodin and Aminoff, 1987b; O'Donnell *et al.*, 1987; Gil *et al.*, 1989; Hautecoeur *et al.*, 1991; Kutukeu *et al.*, 1998). Some evidence has demonstrated a differential effect of Parkinson's disease on early ERP components, with N1 delayed and P2 intact (Goodin and Aminoff, 1986; Goodin and Aminoff, 1987b). However, not all research is consistent with these findings (Hansch *et al.*, 1982; O'Donnell *et al.*, 1987; Hautecoeur *et al.*, 1991; O'Mahony *et al.*, 1993). Non-demented patients with Parkinson's disease demonstrate no ERP delays (Goodin and Aminoff, 1987a; Hautecoeur *et al.*, 1991; Tanaka *et al.*, 2000), suggesting that it is the dementia rather than other symptoms of Parkinson's disease that affects sensory information processing. P3 latency is also associated with deficits on neuropsychological measures in Parkinson's disease (for a review, see Ruzicka and EI Massioui, 1993).

Olfactory Deficits in Parkinson's Disease

Meta-analysis of psychophysical testing of odour identification, recognition and detection threshold suggests olfactory impairment in Parkinson's disease (Mesholam *et al.*, 1998), but the literature also suggests that olfactory impairment is not seen in all patients (Ansari and Johnson, 1975; Serby *et al.*, 1985; Doty *et al.*, 1988; Quinn *et al.*, 1987; Ward *et al.*, 1983). Ansari and Johnson (1975) found increased odour thresholds in less than half the Parkinson's disease patients they tested. Ward *et al.* (1983) examined odour detection and qualitative discrimination in 72 patients and reported odour identification deficits in more than half of them. Doty *et al.* (1988) confirmed odour threshold and odour identification deficits in Parkinson's disease. Deficits in odour identification in Parkinson's disease patients are typically reported to be independent of age, disease stage and duration (Quinn *et al.*, 1987; Ward *et al.*, 1983; Wszolek and Markopoulou, 1998). Dopaminergic or cholinergic treatment does not appear to affect odour identification impairment (Doty *et al.*, 1992a) or olfactory thresholds (Quinn *et al.*, 1987) in Parkinson's disease patients. Conditions similar to idiopathic Parkinson's disease have also been examined for olfactory functional deficits. Odour identification and odour detection thresholds are normal in both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Doty *et al.*, 1992b) and progressive supranuclear palsy, which can be misdiagnosed as Parkinson's disease because of similar motor symptoms (Doty *et al.*, 1993). Interestingly, although an important characteristic of most patients with Parkinson's disease is tremor, patients with essential tremor do not show olfactory impairment (Busenbark *et al.*, 1992).

Olfactory Event-Related Potentials in Parkinson's Disease

Hawkes and colleagues examined OERPs in a small number of Parkinson's disease patients (Hawkes *et al.*, 1997; Hawkes and Shephard, 1998). They reported delayed brain response at P2 in a small number of patients, but with amplitudes comparable to controls. Interestingly, Hawkes *et al.* (1997) also reported pathological results in a group of eight cadavers that indicated the presence of Lewy bodies in every olfactory bulb, particularly in the anterior olfactory nucleus. A study published by Barz *et al.* (1997; see also Kobal and Hummel, 1991) found prolonged OERP latencies (N1, P2 and P3) in Parkinson's disease patients who were and were not taking antiparkinsonian medications. Interestingly, those who were taking the medications demonstrated more pronounced latency deficits than those who were not on medication. In addition, ERP latencies of all Parkinson's disease patients were similar to normal controls when stimulated with trigeminal stimuli. This finding suggests that the delays in OERP latency are specific to the olfactory system and likely reflect neuropathology in central brain areas that process olfactory, but not trigeminal, information.

HUNTINGTON'S CHOREA

Huntington's disease is an autosomal dominant and progressive neurological disease, characterized by movement disorder (chorea) and progressive cognitive deterioration. The disease is without cure, unremitting, and progresses to a typical 'subcortical' dementia. The latter is exemplified in bradyphrenia, impaired attentional mechanisms, problem-solving deficits, and a memory deficit in retrieval of information in fluency and recall tasks, but not in

recognition tasks. Patients encode information but have difficulty with retrieval of information without a cue (Butters *et al.*, 1978; Brandt, 1985; Butters *et al.*, 1985). Patients with Huntington's disease have neuropathy in the basal ganglia, principally the caudate and less in the putamen (Dom *et al.*, 1976; Vonsattel *et al.*, 1985), with significant loss in the entorhinal cortex (Braak and Braak, 1992) and the frontostriatal system.

Event-Related Potentials in Huntington's Disease

ERPs in the auditory and visual modalities have proven to be abnormal in Huntington's disease. Individuals with Huntington's disease demonstrate lower amplitudes than normal controls in both auditory (Josiassen *et al.*, 1984) and visual (Josiassen *et al.*, 1984; Lawson *et al.*, 1984; Hennerici *et al.*, 1985) modalities. In Huntington's disease patients, the cognitive P3 component of the ERP has consistently shown delayed latencies in both auditory (Rosenberg *et al.*, 1985; Filipovic *et al.*, 1990) and visual (Lawson *et al.*, 1984; Rosenberg *et al.*, 1985) modalities. Latency delays in early auditory ERP components have also been observed (Goodin and Aminoff, 1986; Hoemberg *et al.*, 1986). In addition to these findings in Huntington's disease patients with clinical symptoms, two studies have demonstrated abnormal ERPs in individuals at risk for Huntington's disease. Hoemberg *et al.* (1986) found that people at risk for Huntington's disease had delayed N2 and P3 latencies in an auditory ERP paradigm compared with controls. Josiassen *et al.* (1988) demonstrated lower ERP amplitudes in auditory and visual ERPs for people at risk for Huntington's disease compared with clinical and normal controls. Such findings suggest that early neuropathology of Huntington's disease can be detected using ERP paradigms.

MULTIPLE SCLEROSIS

Multiple sclerosis is marked by motor, psychiatric and cognitive abnormalities. Motor deficits include ataxia, ophthalmoplegia, nystagmus, facial palsy and dysarthria (Reder and Antel, 1983). Depression, emotional lability and disinhibition are common psychiatric symptoms (Rabins *et al.*, 1986; Honig *et al.*, 1992). Cognitive abnormalities are seen in the areas of attention, slowed information processing, and memory retrieval, with language function spared (Peyser *et al.*, 1980; Heaton *et al.*, 1985; Jennekens-Schinkel and Sanders, 1986). Research on auditory ERPs has reflected this cognitive decline. Much emphasis has been placed on the P3 component, which is delayed in multiple sclerosis (Polich *et al.*, 1986; Onofrj *et al.*, 1991; Geisser *et al.*, 1992; Honig *et al.*, 1992; Triantafyllou *et al.*, 1992; Gil *et al.*, 1993). Other components with delayed onset are N1 (Geisser *et al.*, 1992), P2 (Geisser *et al.*, 1992; Gil *et al.*, 1993), and N2 (Triantafyllou *et al.*, 1992; Gil *et al.*, 1993). P3 latency has been associated with demyelinative brain lesions, cognitive impairment, and duration of the disease (Geisser *et al.*, 1992; Honig *et al.*, 1992; Gil *et al.*, 1993). Geisser *et al.* (1992) demonstrated that N1, P2 and P3 latencies were delayed in demented multiple sclerosis patients but not in non-demented patients, suggesting that these electrophysiological abnormalities are related to the cognitive and not the other clinical symptoms of multiple sclerosis. Cooling of patients in order to alleviate sensory and motor disturbances does not appear to affect the latency of the auditory P3 (Geisler *et al.*, 1996).

Impairments in taste and odour identification have been reported in patients with multiple sclerosis (Catalanotto *et al.*, 1986; Cohen, 1965; Doty *et al.*, 1984; Pinching, 1977), although not without controversy. Neuropathological changes in these patients include the appearance of demyelinating plaques in a number of areas of the brain, including the frontal lobe and the mesial temporal lobe, areas important in olfactory function. Yousem *et al.* (1998) demonstrated not only impairment in odour identification (University of Pennsylvania Smell Identification Test, UPSIT) in approximately one-third of the sample of multiple sclerosis patients studied, but also a significant relationship between poorer performance on the UPSIT and increased numbers of plaques detected by MRI in the inferior frontal lobe and the temporal lobe. More recently, Doty *et al.* (1999) examined both UPSIT performance and plaque load in the inferior frontal and temporal lobes in five patients studied on three or four separate occasions over an 18–20-month period. They reported a close association between remission and exacerbation of the disease and plaque numbers and UPSIT scores, supporting the hypothesis that olfactory loss in multiple sclerosis reflects fluctuating plaque numbers. Thus, inconsistencies among earlier studies may well be related to patient selection and the disease status: patients in remission would be expected to demonstrate better performance, and samples of patients with greater disease progression would be expected to demonstrate greater olfactory impairment than samples with patients in early stages of the disease. Geisler *et al.* (2000) investigated OERP performance in multiple sclerosis and found that individuals had delayed latencies and lower amplitudes than normal controls.

AGEING

The ageing process affects brain anatomy and function. Of particular interest, ageing shares with neurodegenerative diseases some common pathology in the frontal and mesial temporal lobes. In the absence of dementia, senile plaques are often observed in elderly people in mesial temporal areas (Price *et al.*, 1991), and there are significant age-related losses in the hippocampus and frontal lobes (Jernigan *et al.*, 2001). The degree of neuropathy in normal ageing distinguishes it from neurodegenerative disease.

The auditory brain stem response is a sensitive indicator of hearing impairment and shows significant effects of age. The latency of the auditory P3 is delayed in old age and is considered to reflect the slowing of the brain with age (Goodin *et al.*, 1978). Similarly, the latency of the P3 components of the visual ERP and the OERP are delayed significantly in elderly people and have been demonstrated to be sensitive to the cognitive decline in old age (Kugler *et al.*, 1996; Murphy *et al.*, 2000).

Investigations of lifespan samples have demonstrated significant effects of age on the auditory and visual ERPs (See Polich, 1991 and Polich, 1997 for reviews). The ERPs showed significant effects of age, the most salient of which were a decrease in amplitude, an increase in latency, and a change in scalp distribution from posterior maximal to more equalized activity at midline sites. P3 amplitude was smaller and the latency shorter for the auditory than for the visual modality, and age effects were larger for P3 amplitude in response to auditory than to visual stimulation (Polich, 1997). Amenedo and Diaz (1998) reported that P2 amplitude increased linearly with advancing age at frontal sites but remained unchanged in middle-aged and elderly subjects. The results suggest age-related changes in inhibitory processes that prevent allocation of attentional resources to the task. Latencies of N2 and P3 increased linearly at Fz, Cz and Pz. Of interest, Bashore (1989) reported that older individuals who are more fit (defined by VO_2 max., i.e. maximum oxygen volume) show larger P3 amplitudes and shorter P3 latencies than older individuals who are less fit. These results are generally consistent with other data that suggest that cognitive function is facilitated by physical fitness in older people.

The P3 component becomes more frontally oriented in elderly subjects (Amenedo and Diaz, 1998; Fabiani *et al.*, 1998; Friedman *et al.*, 1997). Through adolescence and young adulthood the P3 is most robust when recorded from parietal locations. The shift

in elderly people is towards more equal signal intensity from midline sites, thus shifting the distribution in frontal areas relative to central and parietal sites, although the extent of this shift is modulated by task and processing characteristics (Friedman *et al.*, 1997). This may reflect the need to allocate more resources to the initial orienting process or to other frontal lobe functions, such as executive function in older individuals. Compromise of frontal lobe function as a result of neurodegenerative processes in the frontal lobes has been hypothesized (Fabiani *et al.*, 1998; Friedman *et al.*, 1997). Fabiani *et al.* (1998) showed that elderly subjects with frontal-maximal P3 scalp distributions had lower performance on standard neuropsychological tests of frontal lobe function than elderly subjects who showed posterior-maximal P3 scalp distributions. Amenedo and Diaz (1998) also reported a more uniform P3 scalp distribution in elderly subjects that only decreased significantly with advancing age in men. Consistent with a cognitive rationale for the age-related changes in P3 scalp distribution, the sensory component N1 shows no differences in scalp distribution between young and old subjects (Friedman *et al.*, 1993; Ford and Pfefferbaum, 1991).

Impairment of olfactory function as a result of normal ageing is well-documented (Murphy, 1983; Murphy, 1986; Murphy, 1993; Murphy, 1999; Murphy, *et al.*, 2000; Murphy and Cain, 1986; Schiffmann, 1993; Schiffmann, 1997). Olfactory threshold sensitivity decreases in the elderly (Murphy, 1983; Venstrom and Amoore, 1968), and suprathreshold perception of the intensity of odours also decreases (Murphy, 1983). The ability to identify odours falls significantly over the lifespan (Murphy, 1981; Murphy, 1986; Schiffman, 1977), although women are slightly better at identifying odours than are men at all ages (Doty *et al.*, 1984). Memory problems are particularly salient in the elderly. Age impacts all aspects of odour memory: recognition, recall and identification (Murphy *et al.*, 1991; Murphy *et al.*, 1997). Recognition memory for odours is quite good in the young, remaining above chance for 6 months or longer. Elderly people's recognition memory for odours falls to chance between 1 hour and 2 weeks (Murphy *et al.*, 1991). Odour recall also shows significant impairment with age (Murphy *et al.*, 1997) when tested in a manner similar to the neuropsychological standard, the California Verbal Learning Test (Delis *et al.*, 1987). Thus, regardless of the psychophysical test, elderly people show poorer performance at detecting, perceiving, identifying, recognizing and recalling odours.

Assessment of olfactory function with the event-related brain potential has revealed both sensory and cognitive impairment in the elderly. The early sensory components of the OERP show longer latencies and lower amplitudes to odour stimulation in older people (Murphy *et al.*, 1994; Murphy *et al.*, 2000; Morgan *et al.*, 1997). Specifically, N1–P2 amplitude decreases by 1.5 μV per decade, and P2 latency increases by 2 ms per decade, as one ages from 20 to 70 years (Murphy *et al.*, 2000). These components are associated strongly with psychophysical measures of olfactory functioning, especially detection threshold (Murphy *et al.*, 1994). Older males tend to have the greatest deficits as indicated by the OERP, perhaps due to greater brain atrophy in the temporal lobes than females (Morgan *et al.*, 1997). The P3 shows significant effects of age on the speed of olfactory cognitive processing in the elderly, with an amplitude decrease of 2 μV per decade and a latency increase of 2 ms per decade from 20 to 70 years (see Figures XV-7.5 and XV-7.6) (Morgan *et al.*, 1999: Murphy *et al.*, 2000), with the most striking effects beginning to be apparent when participants reach the age of 50 years. In all of these functional measures, there is greater variability in performance in the elderly, suggesting that some older individuals are more resistant to the effects of age (Murphy, 1983; Murphy *et al.*, 2000).

It is important for healthcare providers to be educated about the loss of olfactory function in the elderly, and to understand that as elderly people are largely unaware of this loss (Nordin *et al.*, 1995),

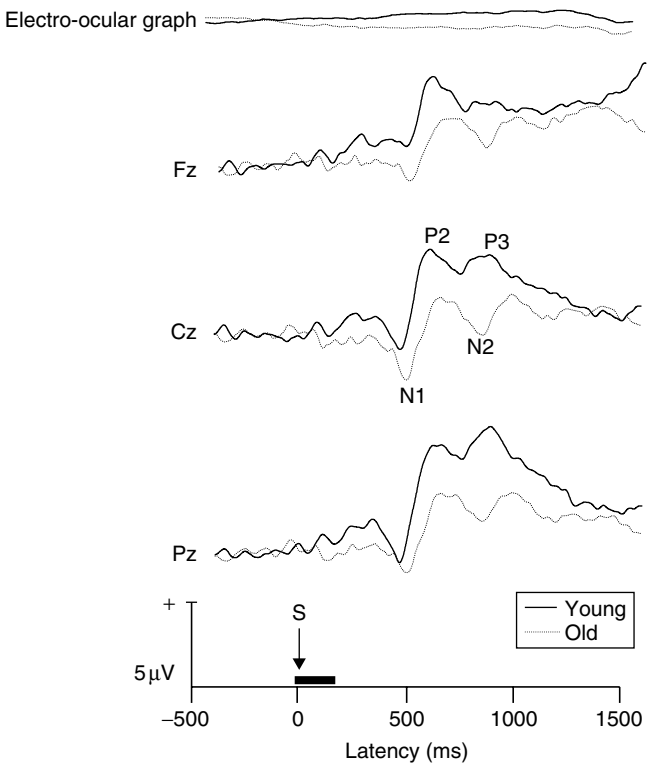

Figure XV-7.5 Grand averaged OERPs for each electrode site for young and older adults. The arrow on the voltage/time scale indicates stimulus onset (from Morgan *et al.*, 1999)

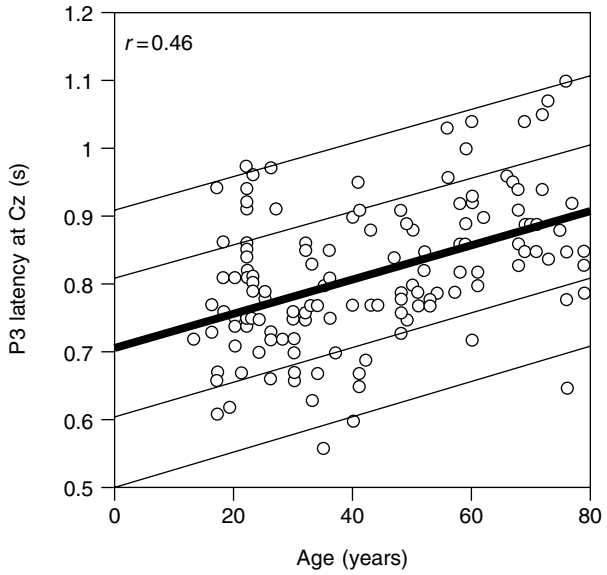

Figure XV-7.6 Rate of increase in latency of the P3 component (±1 and 2 SD) of the OERP, Cz electrode site, across the lifespan. Reprinted from *International Journal of Psychophysiology*, 36, Murphy, C., Morgan, C.D., Geister, M.W., Wetter, S., Covington, J.W., Madowitz, M.D., Nordin, S. and Polich, J.M., Olfactory event-related potentials and aging: normative data, 133–145, Copyright (2000), with permission from Elsevier Science

they are less likely to report impairment or to realize that they are at nutritional or safety risk.

CONCLUSION

The prevalence and debilitating nature of dementia has emphasized the need to deepen our understanding of the phenomenon in order to facilitate discovery of, and apply appropriately, treatments that will stop or slow the decline in cognitive functioning as they become available. Psychophysical and electrophysiological tests can be sensitive and specific in correctly classifying patients with dementia from controls. More investigations are needed to differentiate subtypes of dementia (e.g. vascular, Lewy body, Alzheimer's type) using psychophysical, neuropsychological, neuroimaging and neuropathological techniques. An important application of these techniques will be the identification of very early stages of dementia in order to quantify the subclinical expression of early brain pathology. ERPs show promise in providing this to clinicians, who may choose to include such measures along with neuropsychological tests when evaluating the presence of early dementia. Electrophysiological tests may also prove to be valuable in understanding late stages of disease, when cognitive impairment prohibits the use of neuropsychological test batteries. As such, the field of psychophysiology will be important in our ongoing efforts to understand and ultimately slow the progressive decline of dementia.

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XV-8

Neuropsychology of Cognitive Disorders

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INTRODUCTION

Defining Cognitive Dysfunction

Cognitive disorders can be subdivided broadly into (1) congenital or childhood-onset disorders that disrupt development, and (2) primary or acquired secondary dementias with typically adult and especially late-life occurrence. Regardless of onset, cognitive dysfunction is a clinical syndrome, not a specific diagnosis. As such, it can be produced by a wide variety of causes, including genetic, intrauterine, perinatal, nutritional, environmental and/or degenerative causes. Since pathogenic processes can differentially affect functional neuroanatomy underlying different cognitive domains, a large variety of cognitive disorders exists with varying permutations of intellectual dysfunction. We will describe briefly the major cognitive disorder syndromes, with emphasis on their neuropsychological profiles.

Neuropsychological Assessment and Cognitive Disorders

Mental Status Examination

The mini-mental state examination (MMSE) is currently the most widely used quantitative mental status scale (Folstein *et al.*, 1975). The MMSE is reliable on repeat testing, and has a detection range useful for evaluating and monitoring progression of moderate to severe cognitive dysfunction. Further, it has been found to correlate with brain pathology. Its major disadvantage is its insufficient sensitivity to detect mild impairment. Also, the MMSE is insensitive to certain symptom clusters (e.g., as seen in frontotemporal lobar dementia (FTLD)). The most sensitive components of the MMSE are its attentional (e.g., spelling 'world' backwards) and delayed recall tasks. Experienced testers usually supplement the MMSE with more sensitive memory tasks, constructions (e.g., clock drawing), and executive function tests (e.g., word fluency); these increase the sensitivity of mental status testing to early dementia (Bondi *et al.*, 1996).

Early-stage cognitive impairment can be subtle (Bondi *et al.*, 1996). Highly functioning individuals can mask signs of impairment for a protracted time, and most routine mental status examinations (MSEs) have a ceiling effect. Normal results can persist, despite significant deterioration, especially for patients with above-average baseline cognitive performance. When history strongly suggests cognitive impairment, but the MSE is normal, more detailed psychometric testing is necessary to resolve the discrepancy.

Neuropsychological Evaluation

Although brief MSEs (e.g. MMSE) have been shown to be effective in screening for cognitive deficits in large-scale community-based studies, they cannot substitute for comprehensive neuropsychological assessment. Formal neuropsychological testing, with far greater diagnostic sophistication and standardized norms, is more quantitatively precise and qualitatively informative than brief cognitive evaluation procedures. Because neuropsychological tests offer psychometrically acceptable reliability and construct validity, they provide more accurate procedures for describing cognitive strengths and weaknesses. Test findings can be used to detect the presence and degree of cognitive dysfunction, or to delineate specific impairment profiles, thereby aiding identification of underlying pathologies (Bozeat *et al.*, 2000). Testing also helps to clarify possible contributions of normal ageing, encephalopathy and/or comorbid psychiatric disease. See Chapter X for a more detailed review of neuropsychological principles.

In addition to detecting subtle cognitive impairment, neuropsychological testing is essential for accurately tracking progression of cognitive decline, especially when it is necessary to monitor changes in specific cognitive domains or to evaluate efficacy of treatment. For patients suffering effects of acute trauma (who may recover function), or those with progressive neurological disorders (who ultimately experience functional decline), serial neuropsychological evaluations can be used to characterize cognitive changes over time. Ongoing neuropsychological evaluation is also useful in assessing progress (or lack thereof) of children with developmental delays. Perhaps most importantly, neuropsychological assessment techniques are useful in pinpointing relative areas of strength that can inform strategies for remediation and treatment planning.

Individual neuropsychological tests are frequently designed to assay specific areas of cognition. However, popular distinctions drawn between cognitive domains are, in fact, somewhat arbitrary, since no cognitive function exists purely in isolation. For example, 'pure' measures of verbal memory require patients to both process language and sustain attention to test stimuli. A deficit in either attention or language will consequently obfuscate performance on measures of 'memory'. Because most neuropsychological instruments de facto test more than one cognitive skill, assessment batteries typically rely on a combination of measures within and across cognitive domains to systematically eliminate confounds. In addition, qualitative behavioural observations throughout assessment can offer valuable data, such as test anxiety or use of compensatory strategies, which can negatively impact test validity.

Although measures of learning and retention are the most effective neuropsychological indices for differentiating between mildly demented and normal elderly patients, assays of language, executive function and constructional ability also have diagnostic value (Bondi *et al.*, 1996). For example, differential performance

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Table XV-8.1 Cognitive domain dysfunction, associated disease states, and neuropsychological assessment

Cognitive domain	Relevant clinical syndromes	Commonly used assessment tools (adults)	Commonly used assessment tools (children)
Premorbid intelligence/academic achievement	Neurodegenerative disorders	Vocabulary subtest (WAIS-III)	Woodcock–Johnson Tests of Achievement
	Acquired brain injury	NAART	WIAT
	Toxic or metabolic encephalopathies	Barona/Wilson Method	WRAT
General intellectual ability	Learning disabilities	WAIS-III	Bayley Scales of Infant Development
	Pervasive developmental disabilities	WASI	Wechsler Preschool and Primary Scale of Intelligence
	Mental retardation	Stanford–Binet Intelligence Scale Raven's Progressive Matrices Woodcock–Johnson Test of Cognitive Ability Folstein MMSE	WISC-III Stanford–Binet Intelligence Scale Woodcock–Johnson Test of Cognitive Ability
Attention	Attention deficit disorder	Trails A Boston Cancellation Test Digit-span (WMS-III)	Trails A (intermediate) Denckla Cancellation Test Digit-span (WISC-III)
	Delirium	Spatial span (WMS-III) Continuous Performance Test	Arithmetic (WISC-III) Continuous Performance Test
	Neglect syndromes	Letter–number sequencing (WMS-III) PASAT	
	Affective disturbance	Dichotic listening	
Memory	Dementia syndromes	WMS-III Logical memory I & II, visual reproduction I & II	Wide range achievement of memory and learning
	Isolated amnestic syndromes	California Verbal Learning Test Rey–Osterrieth complex figure test Benton Visual Retention Test Famous Faces Test 7 Minute Screen	Rey Auditory Verbal Learning Test California Verbal Learning Test (Children's Version) Test of memory and learning
Language	Aphasia syndromes	Boston Naming Test Sentence repetition	PPVT
	Dementia syndromes	Boston diagnostic aphasia Examination Fluency tasks	TOLD-II, primary or intermediate CELF-III Vocabulary (WISC-III)
Visuospatial	Huntington's disease Stroke Nonverbal learning disability	Hooper Visual Organization Test Benton judgement of line orientation Block design (WAIS-III) Object assembly (WAIS-III)	TVPS-R Block design (WISC-III) Object assembly (WISC-III)
	Dementia syndromes	Cancellation tasks	
Visuomotor	Parkinson's disease Developmental delay	Grooved pegboard Fingertapping Gripstrength	Visual motor integration test Rey–Oesterrieth complex figure
Executive functioning	Acquired brain injury	Trails B Wisconsin Card Sort Test	Trails B (Intermediate) Wisconsin Card Sort Test
	Dementia syndromes	Stroop Color–Word Test Controlled Oral Word Association Test	Stroop Color–Word Test Rey–Osterrieth complex figure
	Attention deficit disorder	Animal naming Design fluency Go/no-go tasks	
	Psychiatric disorders		

CELF, clinical evaluation of language fundamentals; NAART, North American adult reading test; PASAT, paced serial addition test; PPVT, Peabody picture vocabulary test; TOLD, test of language development; TVPS, test of visuoperceptual skills; WAIS, Wechsler adult intelligence scale; WASI, Wechsler abbreviated scale of intelligence; WIAT, Wechsler individual achievement test; WISC, Wechsler intelligence scale for children; WMS, Wechsler memory scales; WRAT, wide range achievement test

of mildly demented patients with probable Alzheimer's disease and normal elderly control subjects on several types of verbal fluency tasks has greater than 90% sensitivity and specificity for the diagnosis of dementia (Bondi *et al.*, 1996). Similarly high sensitivity and specificity for dementia detection are demonstrated for category number achieved on a modified version of the Wisconsin card sorting task in a study comparing performances of mildly demented probable Alzheimer's disease patients versus normal elderly subjects (Bondi *et al.*, 1993).

Neuropsychological testing provides a systematic means for assessing individual cognitive performance relative to a normal reference population. Neuropsychological assessment tools are typically normed on the basis of age and education, yielding a standardized score range. Given the age-associated prevalence of most cognitive disorders, most patients referred for neuropsychological assessment of cognitive function are older adults (Bondi *et al.*, 1996). Normal age-related perceptual and motor performance changes influence both test material selection and performance interpretation, since such changes can impact neuropsychological performance independent of cognitive function (Bondi *et al.*, 1996). Norm-referenced scoring procedures for neuropsychological assessment are generally age matched. Consequently, normal age-related changes are incorporated into evaluation. Normative data up to age 90 years old are available for many of the more commonly used neuropsychological tests (Bondi *et al.*, 1996).

Following administration and scoring of a neuropsychological battery, an individual's performance is compared with that of the population at large and examined for population-based discrepancies. In addition, the individual's test profile is assessed for widely discrepant or outlying scores that may represent a significant relative weakness in the context of the individual's overall performance. Finally, the patient's cognitive profile must be evaluated in the context of both qualitative behavioural observations and thorough patient history, which includes information regarding physical, educational, psychiatric, social and occupational functioning.

Table XV-8.1 illustrates specific domains of cognitive function, gives examples of relevant clinical syndromes, and lists neuropsychological assessment tools commonly used to measure performance in each domain. It should be noted, however, that most neurological syndromes involve more than one area of cognitive dysfunction. As a result, Table XV-8.1 represents a descriptive but far from exhaustive list of cognitive challenges faced by people with these diagnoses. By extension, diminished performance on any one of these neuropsychological assessment tools is not necessarily pathognomonic for any specific diagnosis. Pattern recognition in the differential diagnosis of cognitive impairment is essential (Patterson *et al.*, 1999).

Aetiologically and neuropathologically distinct cognitive disorders are associated with different patterns of relatively preserved and impaired neuropsychological function. One major conceptual distinction is between degenerative syndromes affecting primarily cortical tissue (e.g. Alzheimer's disease, FTLD) and those affecting subcortical structures (e.g. Parkinson's disease, Huntington's disease, progressive supranuclear palsy (PSP)). For example, Alzheimer's disease, a prototypical cortical dementia, is classically characterized by prominent amnesia with additional deficits in language, abstract reasoning, visuospatial processing and executive function. In contrast, Huntington's disease, a prototypical subcortical dementia syndrome, usually has only moderate memory disturbance but prominent attentional dysfunction, problem-solving deficits, and arithmetic impairment (see below).

Evolving models of dementia pathogenesis have contributed to its conceptualization as a disease state with preclinical stages during which pathology exists but has not yet reached sufficient magnitude for clinical expression (e.g. mild cognitive impairment, preclinical Alzheimer's disease). This has prompted desire to develop ways to detect such states when symptom threshold for conventional clinical diagnosis has not yet been reached but some subtle functional deficit exists that potentially can be assayed. That is, as pathogenesis gradually progresses, so does cognitive impairment, which might be identified before neuronal degeneration reaches the level necessary to produce overtly detectable dementia. Although the precise length of this preclinical phase varies among neurodegenerative cognitive disorders, and between individuals, increasing evidence exists that subtle cognitive impairment can be detected several years before clinically evident dementia. Extensive efforts are under way to improve sensitivity and specificity of neuropsychological predictors of incipient dementia, and several studies have used a variety of neuropsychological measures to retrospectively examine performance of non-demented elderly subjects who subsequently became demented. For example, one study indicated that initial performance on the delayed recall measure of the selective reminding test, a recall measure from the Fuld object memory test, the digit symbol substitution subtest from the WAIS, and measures of verbal fluency are all significant predictors of subsequent dementia development (Masur *et al.*, 1994).

Investigators have attempted to improve the detection of incipient dementia with neuropsychological testing by limiting assessment to elderly patients who carry increased dementia risk (e.g. positive family history of Alzheimer's disease and/or genotype positive for apolipoprotein E (APOE) $\varepsilon 4$). For example, normal elderly with a positive family history of dementia perform worse than family-history-negative subjects on tests of learning and memory (Hom *et al.*, 1994). Similarly, non-demented elderly APOE $\varepsilon 4$-positive men exhibit poorer mean performance on visual memory testing than their APOE $\varepsilon 4$-negative dizygotic twins (Bondi *et al.*, 1996). Another study demonstrated that verbal learning and memory performance of non-demented APOE $\varepsilon 4$-positive subjects was qualitatively (though not quantitatively) similar to that of early-stage Alzheimer's disease patients (Bondi *et al.*, 1995).

DEVELOPMENTAL COGNITIVE DISORDERS

Mental Retardation

Mental retardation is a heterogeneous disorder of multiple potential aetiologies all involving subaverage cognitive function and skill performance with onset before age 18 years. Many cases of mental retardation, particularly mild retardation, have undetermined or idiopathic aetiologies.

Table XV-8.2 outlines the current diagnostic criteria for mental retardation, and Table XV-8.3 summarizes developmental deficits characterizing mental retardation syndromes of varying severities.

Table XV-8.2 Diagnostic criteria of mental retardation

A	Significantly subaverage intellectual functioning: an IQ of approximately 70 or below on an individually administered IQ test	
B	Concurrent deficits or impairments in present adaptive functioning in at least two of the following areas: communication, self-care, home living, interpersonal skills, use of community resources, self-direction, functional academic skills, work, leisure, health, safety	
C	Onset before age 18 years	
Severity based on IQ score:	50–55 to 70	Mild
	35–40 to 50–55	Moderate
	20–25 to 35–40	Severe
	<20–25	Profound

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Mental Disorders*, 4th edition. American Psychiatric Press, Washington, DC

Table XV-8.3 Developmental characteristics of mental retardation syndromes

Severity	Preschool age (0–5 years): maturation and development	School age (5–20 years): training and education	Adult (21 years and over): social and vocational adequacy
Profound	Gross retardation; minimal capacity for functioning in sensorimotor areas; needs nursing care; constant supervision required	Some motor development present; may respond to minimal or limited training in self-help	Some motor and speech development; may achieve very limited self-care; needs nursing care
Severe	Poor motor development; minimal speech; generally unable to profit from training in self-help; little or no communication skill	Can talk or learn to communicate; can be trained in elemental health habits; benefits from systematic habit training; unable to benefit from vocational training	May contribute partially to self-maintenance under complete supervision; can develop self-protection skills to a minimally useful level
Moderate	Can talk or learn to communicate; poor social awareness; fair motor development; benefits from training in self-help; requires moderate supervision	Can benefit from training in social and occupational skills; unlikely to progress beyond second-grade academic level; may learn to travel alone in familiar places	May achieve self-maintenance in unskilled or semiskilled work under sheltered conditions; needs supervision and guidance when under mild social or economic stress
Mild	Can develop social and communication skills; minimal retardation in sensorimotor areas; often not distinguished from normal until later age	Can learn academic skills up to approximately sixth-grade level by late teens; can be guided toward social conformity	Can usually achieve social and vocational skills adequate to minimum self-support, but may need guidance/assistance under stress

Adapted from *Mental Retardation Activities*, US Department of Health, Education, and Welfare, 1983

Table XV-8.4 Diagnostic criteria for autistic disorder

A Total of six or more items from (1), (2) and (3)	(1) Qualitative impairment in social interaction, as manifested by at least two of the following: (a) marked impairment in use of multiple nonverbal behaviours, such as eye-to-eye contact, facial expressions, bodily postures and gestures to regulate social interaction (b) failure to develop peer relationships appropriate to developmental level (c) lack of spontaneous seeking to share enjoyment, interests or achievements with other people (e.g. by a lack of showing, bringing or pointing out objects of interest) (d) lack of social or emotional reciprocity. (2) Qualitative impairments in communication, as manifested by at least one of the following: (a) delay in, or total lack of, development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication, such as gesture or mime) (b) in individuals with adequate speech, marked impairment in ability to initiate or sustain a conversation with others (c) stereotyped or repetitive use of language or idiosyncratic language (d) lack of varied spontaneous make-believe play or social imitative play appropriate to developmental level. (3) Restrictive repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following: (a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus (b) apparently inflexible adherence to specific nonfunctional routines or rituals (c) stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole-body movements) (d) persistent preoccupation with parts of objects.
B Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years	(1) Social interaction (2) Social language communication (3) Symbolic or imaginative play

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

The neuropsychological profile of mental retardation varies widely, as the term does not reflect a discrete condition but the common functional impact of numerous aetiological entities. Even within individual conditions (such as Down's syndrome), phenotypic expression can vary widely. Regardless of aetiology, however, a diagnosis of mental retardation assumes a diminished level of cognitive performance that measures at least two standard deviations below the mean on standardized measures of intellectual performance. The diagnosis is generally made on the basis of formal assessments of overall intellectual functioning (IQ), such as the Wechsler or Stanford–Binet intelligence scales. Although children with mental retardation may gain cognitive abilities over time, the rate of acquisition generally lags far behind that of same-age peers.

Pervasive Developmental Disorders

Pervasive development disorders represent a heterogeneous set of disorders in which communication, adaptive skills and social interaction develop improperly or are lost in early childhood. The disorders manifest in early life, afflict multiple developmental domains, and cause persistent dysfunction. There are four recognized types: autistic disorder, Asperger's disorder, Rett's syndrome, and pervasive developmental delay; we briefly describe two of the more common of these, autism and Asperger's.

Autistic Disorder

Autistic disorder is characterized by severely impaired social communication and restricted stereotypical behavioural patterns

presenting by age 3 years. Table XV-8.4 summarizes the current diagnostic criteria.

Autism is not diagnosed primarily on the basis of cognitive findings but on qualitative deficits in social interaction. Cognitive functions can vary widely from individual to individual, although all patients with autism exhibit deficits in metacognitive executive skills and demonstrate some degree of language impairment. About half of children with autism never develop any functional language. Almost 75% of autistic children meet criteria for mental retardation, with about 40% having IQ scores below 55. In general, IQ scores in autism tend to reflect deficiencies in verbal sequencing and abstraction rather than deficiencies in visuospatial and memory skills, reflecting the primacy of language-based dysfunction. Interestingly, some autistic children demonstrate unusual or precocious cognitive abilities, even within a context of overall retarded function. Included among these cases are the 'savants', who have prodigious memory function, extraordinary calculating abilities, hyperlexia (early ability to read well) and/or musical abilities.

Asperger's Syndrome

Austrian physician Hans Asperger described a syndrome in which patients with normal intelligence exhibited qualitative impairment in reciprocal social interaction and behavioural oddities without developmental language deficits. Table XV-8.5 summarizes the current diagnostic criteria.

As with autism, Asperger's disorder is diagnosed primarily on the basis of anomalous social behaviour rather than on conventional cognitive dysfunction. Although children with Asperger's disorder perform within the normal range on formal cognitive measures and lack the severe expressive language deficits of autistic children, metacognitive conceptual abilities and receptive language function are impaired. There are notable abnormalities of affective speech expression and comprehension (understanding and use of prosody). Rote memory skills can sometimes be extraordinary, as with autism.

Attention Deficit Hyperactivity Disorder

Attention deficit hyperactivity disorder (ADHD) is characterized by developmentally inappropriate deficiency of attention span and/or age-inappropriate features of hyperactivity and/or impulsivity. Diagnostic criteria are outlined in Table XV-8.6.

ADHD characteristics include hyperactivity, perceptual-motor impairment, emotional lability, general coordination deficit, attentional deficits, impulsivity, memory deficit, specific learning disabilities, disorders of speech and hearing, and equivocal neurological signs and electroencephalogram (EEG) abnormalities. About 75% of children with ADHD manifest behavioural symptoms of aggression and defiance. A conduct disorder is a frequent comorbidity (Kaplan *et al.*, 1994).

Diagnostic criteria for ADHD are generally more behavioural rather than neuropsychological. As a result, diagnosis can sometimes be assigned on the basis of undesirable behaviour alone in the absence of any true measure of attentional functioning. Consequently, before a diagnosis of ADHD is conferred, careful consideration should be paid to other factors that can result in behavioural dyscontrol (e.g. childhood trauma, depression, anxiety disorders). In addition, classroom behaviours associated with ADHD (e.g. academic task avoidance) may stem from academic difficulties related to learning disabilities, which make academic work intolerably frustrating. Conferring a diagnosis of ADHD without considering other mediating factors can result in oversight of significant psychosocial, psychiatric or learning difficulties.

'Attention' is a broad term encompassing numerous self-regulatory functions, ranging from basic capacity to maintain optimal arousal to far more complex functions, such as capacity to preferentially attend to some stimuli while simultaneously ignoring irrelevant stimuli (requiring ancillary executive functions). These children can have difficulty filtering distractions, maintaining sustained vigilance, focusing on multiple targets simultaneously, efficiently shifting focus, and/or inhibiting impulses. Not surprisingly, children with ADHD perform poorly on neuropsychological measures of attention (such as the continuous performance test, or the Wechsler digit-span subtest) and executive function (such as the Wisconsin card sort or the Stroop color word test). In addition, because attentional focus is critically important to acquisition of information, children with ADHD can fail to make adequate academic progress overall.

Table XV-8.5 Diagnostic criteria for Asperger's disorder

A	Qualitative impairment in social interaction, as manifested by at least two of the following:	(1) Marked impairment in use of multiple nonverbal behaviours, such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction (2) Failure to develop peer relationships appropriate to developmental level (3) Lack of spontaneous seeking to share enjoyment, interests or achievements with others (4) Lack of social or emotional reciprocity
B	Restricted, repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following:	(1) Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal in intensity or focus (2) Apparently inflexible adherence to specific, nonfunctional routines or rituals (3) Stereotyped and repetitive motor mannerisms (4) Persistent preoccupation with parts of objects
C	Disturbance causes clinically significant impairment in social, occupational or other important areas of functioning	
D	There is no clinically significant general delay in language (e.g. single word use by age 2 years, communicative phrase use by age 3 years)	
E	There is no clinically significant delay in cognitive development or in the development of age-appropriate self-help skills, adaptive behaviour (other than in social interaction) and curiosity about environment during childhood	

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

Table XV-8.6 Diagnostic criteria for ADHD

A Either (1) or (2)	(1) Inattention: six or more of the following symptoms of inattention have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level		(a) Often fails to give close attention to details, or makes careless mistakes in schoolwork, work or other activities (b) Often has difficulty sustaining attention in tasks or play activities (c) Often does not seem to listen when spoken to directly (d) Often does not follow through on instructions, and fails to finish schoolwork, chores or duties in the workplace (e) Often has difficulties organizing tasks and activities (f) Often avoids, dislikes, or is reluctant to engage in tasks requiring sustained mental effort (g) Often loses things necessary for tasks or activities (h) Is often distracted easily by extraneous stimuli (i) Is often forgetful in daily activities
	(2) Hyperactivity–impulsivity: six or more of the following symptoms of hyperactivity–impulsivity have persisted for at least 6 months to a degree that is maladaptive and inconsistent with developmental level	Hyperactivity	(a) Often fidgets or squirms (b) Often leaves seat in classroom or in other situations in which remaining seated is expected (c) Often runs about or climbs excessively in situations in which it is inappropriate (d) Often has difficulty playing or engaging in leisure activities quietly (e) Is often 'on the go' or often acts as if 'driven by a motor' (f) Often talks excessively
		Impulsivity	(a) Often blurts out answers to questions before questions have been completed (b) Often has difficulty awaiting turn (c) Often interrupts or intrudes on others
B Some hyperactive–impulsive or inattentive symptoms that caused impairment were present before age 7 years			
C Some impairment from the symptoms is present in two or more settings (e.g. school, work)			
D There must be clear evidence of clinically significant impairment in social, academic or occupational functioning			

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

Learning Disorders

Learning disorders are characterized by discrete impairments of academic skill acquisition within the context of normal overall intellectual ability. These disorders are diagnosed only when a significant discrepancy exists between scholastic achievement and overall cognitive function. The following sections outline current nosology.

Reading Disorders

Reading disorders are characterized by impaired ability to recognize words, slow and inaccurate reading, or poor comprehension in the absence of low intelligence or sensory deficits. Children may experience reading difficulties for a variety of reasons, including but not limited to difficulties with sound–symbol association and phonemic discrimination, difficulties with sound blending and word segmentation, and difficulties mastering automatic sight-recognition skills. Table XV-8.7 outlines *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association, 1994) criteria for reading disorder.

A variety of terms have been used in the past to describe reading disabilities, including dyslexia. Since reading disorders are frequently accompanied by other deficits (e.g. disorder of written expression, mathematics disorder, and/or one of the communication disorders), the term dyslexia was replaced by more general terms, such as learning disorder (Kaplan *et al.*, 1994).

Reading disorders are usually apparent by age 7 years (second grade). Children with ADHD have a higher risk of developing reading disorders. Psychoeducational diagnostic batteries should include standardized reading achievement tests (e.g. Standardized Reading Inventory (SRI), Woodcock Reading Mastery Test (Kaplan *et al.*, 1994).

Table XV-8.7 Diagnostic criteria for reading disorders

A	Reading achievement, as measured by individually administered standardized tests of reading accuracy or comprehension, is substantially below that expected given chronological age, measured intelligence and age-appropriate education
B	The disturbance interferes significantly with academic achievement or activities of daily living that require reading skills
C	If a sensory deficit is present, the reading difficulties are in excess of those usually associated with it

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

Disorders of Written Expression

Impaired written expression is often associated with other learning disorders, but it can appear in isolation. Current criteria are summarized in Table XV-8.8.

Children typically present in early grades with difficulties spelling words and expressing thoughts according to age-appropriate grammatical norms. Spoken and written language contain excessive grammatical errors and poor organization. Initially, children make simple spelling and grammatical errors in writing short sentences. Later, language dysfunction extends to improper diction and disorganized paragraph construction (Kaplan *et al.*, 1994).

Evaluation should begin with a standardized intelligence test, such as the Wechsler intelligence scale for children (WISC-R) or the revised Wechsler adult intelligence scale (WAIS-R) to gauge intellectual function before administering a standardized expressive writing test. Instruments commonly used to assess written

Table XV-8.8 Diagnostic criteria for disorder of written expression

A	Writing skills, as measured by individually administered standardized tests (or functional assessments of writing skills), are substantially below those expected for chronological age, measured intelligence and age-appropriate education
B	Disturbance interferes significantly with academic achievement or activities of daily living that require composition of written text (e.g. writing grammatically correct sentences and organized paragraphs)
C	If a sensory deficit is present, writing skill impairment is in excess of that associated with it

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

Table XV-8.9 Diagnostic criteria for mathematics disorder

A	Mathematical ability, as measured by individually administered tests, is substantially below that expected given chronological age, measured intelligence and age-appropriate education
B	The disturbance interferes significantly with academic achievement or activities of daily living that require mathematics ability
C	If a sensory deficit is present, the difficulties in mathematical ability are in excess of those usually associated with it

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

expression abilities include the Test of Written Language (Hammill and Larsen, 1995) and the Wide Range Achievement Test (WRAT).

Mathematics Disorder

Mathematics disorder is a disability in performing arithmetic skills expected for intellectual capacity and educational level. Past terms have included dyscalculia and the disorder has previously been classified under the rubric of Gerstmann syndrome (a syndrome typically associated with dominant parietal lobe dysfunction manifesting as left–right confusion, finger agnosia, dyscalculia and dysgraphia). Table XV-8.9 outlines the current criteria for mathematics disorder (Kaplan *et al.*, 1994).

Definitive diagnosis can be made only after demonstration of average functioning on measures of general intellectual ability and markedly below-expected performance on standardized measures of arithmetic skills, including the WRAT, the Wechsler Individual Achievement Test (WIAT), and the Woodcock–Johnson Test of Achievement.

ADULT COGNITIVE DISORDERS

Defining Cognitive Dysfunction

Cognitive dysfunction can involve single or multiple domains. When an isolated deficit exists, memory impairment is the most frequent; amnestic disorders comprise this diagnostic category. More commonly, multiple cognitive domains are affected; such disorders of adult onset involve primarily the vast and heterogeneous syndrome category collectively known as dementia. Dementia can be defined as sustained or progressive decline in cognition and/or comportment caused by chronic brain dysfunction. In contrast to the conditions described above, which impede intellectual development, dementia involves loss of previously developed cognitive abilities. Dementia can occur by acute or, more commonly, insidious onset, can be reversible or irreversible, and can have an indolent or progressive course.

Impairments can occur in any combination of comportmental, cognitive, affective and perceptual domains, potentially affecting personal, social and/or vocational function(s). Many authors specify that impairment involve at least three of the following functional capacities: attentional matrix, language, memory, visuospatial skills, executive abilities and personality. Although memory loss is sometimes considered prerequisite to diagnosis, amnesia is neither ubiquitous nor necessarily prominent in all forms of dementia. Cognitive and social abilities can be affected differently by dementia: patients with severe but focal cognitive deficits can retain functional independence, while those with relatively mild impairments affecting socially critical functions may require extensive supervision.

Table XV-8.10 summarizes core components of the widely used DSM-IV dementia diagnostic criteria.

Dementia severity can be gauged in social terms as well as by the nature and degree of cognitive impairments. Dementia is considered mild when the patient continues to conduct basic activities of daily living (e.g. dressing, grooming, eating, toileting) without assistance. Patients with moderate dementia depend partly on others for these activities. In severe dementia, these functions must be provided by caregivers. The same terms are used to describe neuropsychological deficit severity (e.g. mild, moderate or severe memory loss).

To identify an underlying pathogenic disease process, all aspects of a patient's medical history, family history and clinical examination (medical, neurological, psychiatric) must be considered along with well-reasoned laboratory investigations (van Crevel *et al.*, 1999). Dementia features such as age at symptom onset, nature and severity of cognitive and behavioural deficits, and pattern of progression are considered in evaluating the differential diagnosis of a dementia (Patterson *et al.*, 1999). Neuropathological examination usually establishes the underlying disorder and constitutes the gold standard against which ante-mortem diagnostic accuracy is measured.

Since cognitive impairment must be measured against baseline performance, highest level of education, life achievements and premorbid personality traits should be established first. Since premorbid neuropsychological data are rarely available, the clinician must often rely on a history of cognitive deterioration provided by an informant. Several studies have demonstrated that informants (usually a close relative) are able to provide valid reports of progression in dementia patients. Family assessments of patient memory deficits have been found to correlate well with objective cognitive scores. Furthermore, informant judgements of patient cognitive status have been found to be reasonably accurate when compared with formal monitoring of patient progression. However, certain caveats need to be maintained. For example, informant report accuracy can be influenced by the relationship between the patient and the informant (e.g. spouses of memory-impaired patients report lower levels of impairment than do younger relatives) (McGlone *et al.*, 1990; Bondi *et al.*, 1996). Consequently, accuracy of reports of cognitive decline can be enhanced by interviewing multiple family members.

Different dementing processes often selectively involve different brain nuclei and/or neurotransmitter systems. Consequently, not all cognitive faculties are affected simultaneously. Neuropsychological profile is often dictated more by neuroanatomical lesion site than by specific histopathology. Several dementias reflect direct damage to cortical association areas (e.g. asymmetric cortical degeneration); in others, dysfunction is secondary to impairment of interconnected subcortical regions, such as basal ganglia, thalamus and limbic structures. Disruption of white-matter pathways, whether multifocal (as in multiple sclerosis) or confined to single areas such as the internal capsule genu (as in strategic infarct dementia), can critically slow or disconnect neocortical areas. Degeneration of brainstem and/or basal forebrain nuclei, which normally provide ascending cholinergic and monoaminergic neurotransmitter supply to neocortical association areas, can cause profound dementia.

Table XV-8.10 Selected DSM-IV dementia criteria

Dementia of the Alzheimer's type	Development of multiple cognitive deficits manifested by: • memory impairment • one or more of the following: – aphasia – apraxia – agnosia – executive dysfunction Cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning The course is characterized by gradual onset and continuing cognitive decline The cognitive deficits in criteria A1 and A2 are not due to any of the following: • other CNS conditions • systemic conditions • substance-induced conditions The deficits do not occur exclusively during the course of a delirium
Vascular dementia	Development of multiple cognitive deficits, manifested by: • memory impairment • one or more of the following: – aphasia – apraxia – agnosia – executive dysfunction The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning Focal neurological signs and/or symptoms or laboratory evidence indicative of cerebrovascular disease, which are judged aetiologically related to the disturbance The deficits do not occur exclusively during the course of a delirium

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition. American Psychiatric Association, Washington, DC

Table XV-8.11 Classifying cognitive dysfunction

Cognitive impairment	Primary or secondary process	Potential reversibility	Example
Encephalopathy	Secondary	Usually reversible	Toxic/metabolic
Dementias	Secondary	Sometimes reversible	Vitamin B_{12} deficiency
		Irreversible	Creutzfeldt–Jakob disease
	Primary	Irreversible	Alzheimer's disease

Differentiating Cognitive Dysfunction from Other Mental Disorders

Although there can be considerable phenomenological overlap of cognitive impairment aetiologies (e.g. subdural haematoma can manifest as delirium or reversible dementia), knowledge of the conceptual distinctions shown in Table XV-8.11 facilitates the approach.

Only 10–15% of dementias are reversible, and only a fraction of these are completely reversible (Freter *et al.*, 1998).

Delirium/Encephalopathy/Acute Confusional State

Several terms are applied to acute or subacute sensorium disturbance. The terms delirium, encephalopathy, and acute or subacute confusional state are clinically equivalent and can be used interchangeably. Since almost any systemic or primary central nervous system (CNS) syndrome can cause an encephalopathic derangement, potential delirium aetiologies are multiple.

The sine qua non clinical feature of delirium is clouded sensorium. Although dementia involves cognitive impairment within the context of an awake patient with clear sensorium, acute confusional states are frequently misdiagnosed as dementia. When a delirium is superimposed on an underlying dementia, cognitive abilities can decline precipitously. Although dementia and delirium can be comorbid, delirium is differentiated by an acute or subacute onset occurring within hours or days. While demented patients without delirium frequently demonstrate preserved attentional capacities, patients with delirium experience fluctuations in levels of consciousness, with consequent gross attentional impairments. Patients may have difficulty focusing, sustaining and shifting attention in a productive or goal-directed manner, with consequent disorientation and secondary memory deficiency. Hallucinations are frequent and can involve any sensory modality. Dysarthria is a common correlate.

Because delirium involves a fluctuating course, a thorough assessment should seek to identify variations in cognitive performance by either history or direct observation. However, patients with delirium are typically compromised to the extent that they are unable to tolerate extensive testing. Brief, time-limited measures are therefore useful in identifying fluctuating cognition over time. The digit span and orientation subtests from the Wechsler Memory Scales (WMS) III prove useful in this regard. When variable performance on these tasks is accompanied by dysarthria or hallucinations, a diagnosis of delirium should be considered and an acute physiological aetiology sought.

Amnestic Syndromes

Amnesia, a disorder of memory, can occur as a component of a multifaceted mental status change, such as delirium or dementia, or as an isolated abnormality. When occurring as an isolated cognitive

deficit, it is useful to consider amnesia as a distinct nosologic category.

'Memory' is not a discrete entity, but rather a complex amalgamation of cognitive processes underlying the acquisition, consolidation, accession and mutation of neural representations of past experience. As such, memory represents the end-product of multilayered cognitive operations performed by multiple interdependent neural substrates. While medial-temporal, diencephalic and frontal systems figure prominently in memory functioning, the pathways connecting these regions are equally important. Hence, amnesia can result from disruption of any one of many cognitive circuits. Table XV-8.12 summarizes the neural substrates of specific memory processes and associated anatomic regions (see Chapter X for a more detailed discussion of normal memory function).

True amnesia, i.e. distinct memory impairment isolated from any other cognitive deficit, is relatively rare. Patients with true amnesia typically experience deficits in explicit memory, usually anterograde (see below). Implicit memory systems frequently remain intact, such that procedures, habits, skills and classically conditioned responses may still be acquired in the absence of conscious awareness (O'Connor and Morin 1998). As a consequence, patients may modify responses to environmental stimuli while lacking awareness of events leading to the behavioural change.

Damage to the medial-temporal lobe, including the hippocampus, is classically associated with memory consolidation dysfunction and rapid forgetting. Retrieval problems are often associated with diencephalic lesions, particularly those involving the mammillary bodies and thalamic nuclei (Lezak, 1995). Retrieval deficiency is also commonly associated with disruption of white-matter tracts, as seen in small-vessel ischaemia, diffuse axonal injury or autoimmune deficiency syndrome (AIDS) dementia complex (Filley, 2001).

Regardless of aetiology, memory deficits exist on a multidimensional continuum, such that memory dysfunction may be either mild or severe, global or circumscribed, and anterograde or retrograde, depending on extent and location of neurolopathology. Retrograde amnesia is characterized by the inability to recall information before disease onset, while anterograde amnesia is marked by impaired capacity to form new memories after a precipitating event. Although pure anterograde amnesia (i.e. in the absence of any significant retrograde component) is a common form of amnesia, retrograde amnesia almost never occurs in the absence of an accompanying anterograde amnesia of equivalent or greater severity (Kopelman, 1987; Lezak, 1995).

Patients with primarily anterograde amnesia (e.g. Korsakoff syndrome) attempt to fill in memory gaps with false recollections (confabulations), which can take the form of elaborate contrivances or genuine memories misplaced in time, while distant long-term memory is relatively preserved, perhaps because well-established memories are stored more diffusely. The most deeply ingrained memories, such as one's own name, are almost always spared in neurological memory disturbances. In contrast, personal memories may be impaired prominently or exclusively in psychogenic amnesias.

A comprehensive neuropsychological assessment addressing multiple aspects of cognition is required in order to establish whether memory deficits are circumscribed (as in amnesia) or part of a broader cognitive syndrome such as dementia. Measures of retrograde (or remote) memory tend to be less objective and have norms that are less precise than measures of new learning, as autobiographical memory requires independent verification by the examiner, and patient knowledge of historical events lends itself to idiosyncratic variation. Despite this, tools such as the Autobiographical Memory Interview, the Famous Faces Test (which assesses knowledge of well-known historical figures), and the Transient Events Test (which measures knowledge of high-profile news items and historical events) have been developed to yield a broad overview of remote memory functioning. One advantage of these structured assessment tools is that they allow for temporal grading of remote memory loss. Neuropsychological measures addressing both retrograde and anterograde memory functioning can help characterize memory dysfunction, so that optimal rehabilitation strategies and environmental supports can be implemented.

Memory-specific neuropsychological assessment tasks designed to address new learning (which are included in most neuropsychological assessments regardless of the referral question) probe encoding, storage and retrieval skills. Tests such as the WMS-III, the California Verbal Learning Test (CVLT), Rey–Osterreith Complex Figure, and Warrington Recognition Memory Test are frequently used to gauge both verbal and nonverbal aspects of memory. These neuropsychological tasks present the patient with verbal and/or nonverbal material and require the patient immediately to reproduce information from memory (to assess initial perceptual and encoding capacities). After a circumscribed period of time, the patient is asked to recall information independently, assessing memory storage and retrieval capabilities. Following the independent recall (or 'free-recall') condition, the patient is presented with multiple-choice cues to assess overall retention rates in the absence of retrieval demands. If the patient performs significantly better with cueing, then it can be assumed that they have recorded and stored the information over time but are having relative difficulty retrieving the information as needed (similar to the 'tip of the tongue' phenomenon that most of us experience from time to time). In all, neuropsychological tasks assessing memory attempt to pinpoint whether a memory deficit is present, identify the type and degree of memory impairment, and determine whether the memory deficit is isolated (as in true amnestic syndromes) or occurring in conjunction with other cognitive anomalies.

Imaging and autopsy studies frequently reveal bilateral hippocampal and/or related structure (e.g. dorsomedial thalamus) abnormalities.

Table XV-8.12 Functional neuroanatomy and memory system classification

Length of storage	Long-term memory					Short-term memory
Awareness	Explicit		Implicit			Explicit
System	Episodic memory	Semantic memory	Simple classical conditioning	Procedural memory	Priming	Working memory
Anatomy	Medial-temporal lobe, diencephalon	Inferior and lateral temporal lobe	Amygdala, limbic system (autonomic) Cerebellum (motoric)	Basal ganglia, cerebellum	Cortical sensory association areas	Prefrontal cortex

Adapted from Budson and Price (2001)

Normal Ageing

One of the most controversial challenges in clinical neuroscience is where to demarcate normal from pathologic age-related cognitive changes. Potential contamination of some normative ageing study populations with subjects in the earliest stages of dementia highlights the difficulty (Goldman *et al.*, 2001). Although interpretation of normal cognitive ageing is complicated by daunting methodological issues, cognitive functions that should remain relatively preserved over the lifespan include temporal orientation, immediate attention, vocabulary and most visuospatial skills. Delayed recall in healthy elderly people is generally preserved into the tenth decade. Mild age-related decrements involve difficulties with sustained attention, greater visual than verbal memory recall, confrontation naming, mental flexibility and response speed (Quinn and Kaye 2001). Episodic memory declines with age, but semantic, implicit, procedural, recognition and working memory remain relatively intact (see Chapter X for detailed definitions of these constructs). Further, mild age-related cognitive decrements may be counterbalanced by improvements in vocabulary, judgement, insight and wisdom. In sum, normal cognitive ageing, even into the ninth decade, is compatible with independent living. It is therefore invalid to attribute significant memory complaints or other cognitive impairment merely to the consequences of normal ageing.

Various terms, such as age-associated memory impairment, benign senescent forgetfulness, and isolated amnestic disorder, have been used to define a category intermediate between cognitive changes associated with normal ageing and dementia. This issue is addressed below. For now, we summarize consensus opinion regarding normal age-related cognitive changes in Table XV-8.13 Neuropsychological tests demonstrating greatest age-related sensitivity are listed in Table XV-8.14 and Table XV-8.15 provides quantitative data from the Oregon Brain Aging Study.

A general slowing in processing time spans all cognitive domains. Not surprisingly, the most significant age-related declines in neuropsychological test performance are on timed tests (Quinn and Kaye, 2001).

In addition to functional parameters, certain neuroanatomical indices, obtainable *in vivo* by neuroimaging, can also distinguish normal from pathological ageing. For example, hippocampal and temporal volume are significantly greater in subjects who remain cognitively stable compared with those developing dementia, and rate of change of temporal (but not hippocampal) volume is greater

Table XV-8.13 Cognitive changes associated with normal ageing

Functional domain		Finding with normal ageing
Attention	Sustained	Preserved into eighth decade
	Selective	Preserved into ninth decade
Memory	Registration	Preserved
	Short-term	Declines
	Long-term	Preserved
	Working	Impaired
Language	Syntax	Preserved
	Naming	Gradual decline
Executive function	Integration	Declines
	Planning	Declines
	Response-inhibition	Declines
Visuospatial function	Perceptual	Declines
	Constructional	Declines
Processing speed		Gradually progressive slowing

Adapted from Quinn and Kaye (2001).

Table XV-8.14 Neuropsychological tests showing greatest age sensitivity

WAIS-III subtests	Object assembly
	Digit symbol
	Picture arrangement
Hallstead–Reitan battery	Finger tapping
	Sensory-perceptual
	Rhythm
	Tactile form recognition
	Tactile performance test-memory
	Trails B
	Category
	Tactile performance test-time/block
	Tactile performance test-location
	Grooved pegboard

Adapted from Quinn and Kaye (2001)

Table XV-8.15 Cognitive signs in Oregon Brain Aging Study

		Age 65–85 years Mean score (SD)	Age >85 years Mean score (SD)	*P* value
WMS-R	Logical memory I (max. = 50)	26.7 (7.9)	22.4 (7.2)	0.06
	Logical memory II (max. = 50)	22.2 (7.7)	16.8 (7.7)	0.03
	Visual reproduction I (max. = 41)	33.2 (3.8)	27.3 (6.3)	0.0001
	Visual reproduction II (max. = 41)	27.9	19.5	0.002
WAIS digit span	Digits forward (max. = 9)	6.1 (1.2)	5.7 (1)	0.28
	Digits backward (max. = 8)	4.7 (0.9)	4.5 (1)	0.56
WAIS-R	Picture completion	15 (2.1)	10.8 (3.9)	0.0001
	Block design	29.8 (11.1)	18.6 (7.3)	0.001
Boston naming test (max. = 60)		54.4 (4.6)	50.8 (5.2)	0.02

SD, standard deviation
Adapted from Quinn and Kaye (2001)

Table XV-8.16 Distinguishing characteristics of cortical and subcortical patterns of dementia

Function			Cortical dementia	Subcortical dementia
Psychomotor speed			Normal	Slowed
Language			Dysnomia Variable aphasias	Spared
Executive function			Poor insight	Poor problem solving, global slowing
			Poor judgement Reduced abstraction	
Memory	Recent	Retrieval/recall	Impaired	Impaired
		Recognition	Impaired	Preserved
	Remote		Anterograde amnesia Agnosia	No temporal gradient
Motor			Spared until late	Early involvement
Visuospatial			Impaired	Normal
Agnosia			Present	Less significant or absent
Depression			Less common	More common
Comportment			Disinhibition Apathy/abulia	Apathy
Neuroanatomy			Cerebral cortex	Subcortical structures Dorsolateral prefrontal cortex
Prototypic examples			Alzheimer's disease	Huntington's disease Wilson's disease

Adapted from Cummings JL, Trimble MR, *Concise Guide to Neuropsychiatry and Behavioral Neurology*, American Psychiatric Press, 1995.

in subjects developing dementia than cognitively stable subjects (Bozoki *et al.*, 2001).

Functional brain imaging can also help to distinguish normal from pathological ageing. For example, functional neuroimaging has been used to compare task-related brain network activation in Alzheimer's disease patients with age-matched controls. Positron emission tomography (PET) scans of symptomatic Alzheimer's disease patients demonstrate task-difficulty-dependent activation of brain networks distinct from those recruited by healthy elderly people. Such alternative network activation may indicate compensation for processing deficits, and transition from normal to alternative brain networks may indicate a point at which ageing has functionally transgressed from normal to pathological (Stern *et al.*, 2000; Bookheimer *et al.*, 2000).

COGNITIVE DISORDER SUBTYPES

Differential Diagnosis of Cognitive Disorders

Alzheimer's disease is the most common cause of dementia, being responsible for up to two-thirds of cases. The remaining one-third are caused by a wide variety of aetiologies.

Differentiation between cortical and subcortical dementia profiles can be clinically useful, although it is often anatomically incorrect as pathology is rarely limited to cortical or subcortical regions. Deficits caused by subcortical disease include slowed and inefficient cognitive processing, as well as alterations in personality, mood and behaviour. Other disorders (e.g. Creutzfeldt–Jakob disease (CJD), dementia with Lewy bodies (DLB), neurosyphilis) can involve both cortical and subcortical structures. Amnesia, cognitive disorganization and impaired visuospatial skills can be caused by either cortical or subcortical lesions. We will consider neuropsychological comparison of cortical and subcortical dementias in more detail when reviewing Huntington's disease below. For now, Table XV-8.16 contrasts the clinical features of cortical and subcortical dementias.

Primary Cognitive Disorders

Mild Cognitive Impairment

Some elderly people exhibit age-related memory deficits but are not considered to have dementia because general intellect is preserved and there is no significant impairment in activities of daily living. The concept of so-called mild cognitive impairment (MCI) has been the subject of increasing clinical and empiric attention. Patients with MCI can have memory dysfunction similar to that of mild Alzheimer's disease patients, while the balance of cognitive functions is similar to that of to healthy age-matched controls (Small, 2001). MCI is a consideration in the differential diagnosis of cognitive impairment. When diagnosed, it carries significant implications for long-term patient monitoring. Table XV-8.17 summarizes the current diagnostic criteria for MCI.

The significance of MCI diagnosis is amplified by meta-analyses indicating that MCI patients have increased risk of progressing to dementia in general and Alzheimer's disease in particular.

There is converging evidence (epidemiological, psychometric, neuroimaging, neurogenetic, etc.) that MCI is characterized by a symptomatological pattern and prognosis that, while remaining relatively benign in many cases, increasingly appear to exist within the same disease spectrum as other neurodegenerative cognitive disorders, such as Alzheimer's disease (Bozoki *et al.*, 2001; Morris *et al.*, 2001). Hippocampal atrophy demonstrated on structural neuroimaging has been shown to predict conversion to dementia in MCI patients (Bozoki *et al.*, 2001). PET scans of MCI patients reveal a pattern of hypometabolism resembling that

Table XV-8.17 Mild cognitive impairment criteria

Memory complaint, preferably corroborated by an informant
Objective memory impairment
Normal general cognitive function
Not demented

Adapted from Petersen *et al.* (2001)

of Alzheimer's disease patients more than that of healthy elderly controls (Bozoki *et al.*, 2001). There is also some preliminary evidence that cerebrospinal fluid (CSF) measures of Alzheimer's disease-related pathology, e.g. CSF tau and β-amyloid, might predict dementia conversion among MCI patients (Diaz-Arrastia and Baskin, 2001). Not unexpectedly, age is a strong predictor of conversion from MCI to dementia (Celsis, 2000).

The annual conversion rate of MCI to frank dementia is estimated at 12–15% (Morris, *et al.*, 2001).

Neurodegenerative Dementias

Alzheimer's Disease

Although Alzheimer's disease was previously considered a diagnosis of exclusion, it can in fact be diagnosed confidently on the basis of several inclusion and exclusion criteria. Table XV-8.18 outlines current National Institute of Neurologic, Communicative Disorders and Stroke Alzheimer's disease diagnostic criteria; Table XV-8.19 summarizes the DSM-IV criteria.

Preclinical Alzheimer's Disease There is evidence for a preclinical stage of Alzheimer's disease (distinct from MCI), i.e. Alzheimer's disease may have a long stage of neuropathological change that precedes symptom appearance (Goldman *et al.*, 2001). Cognitive decline before conventional Alzheimer's disease diagnosis is made can be marked by deficits in multiple domains and is associated with subtle interferences with social and occupational function. The presence of such cognitive deficits, even when minimal, is highly correlated with neuropathological Alzheimer's disease (Goldman *et al.*, 2001).

However, although the term 'preclinical Alzheimer's disease' has been used to characterize the period from mild cognitive symptom onset (usually marked by mild deficits in episodic memory and abstract reasoning, thereby perhaps better characterized as MCI) to the time of Alzheimer's disease diagnosis, data suggest that the term should refer solely to a condition of histopathological Alzheimer's disease unaccompanied by any cognitive impairment (Goldman *et al.*, 2001). Careful comparison of psychometric measures of patients with healthy brains (characterized by absence of senile plaques or by only patchy neocortical plaque deposition), non-demented patients with autopsy-confirmed neuropathological Alzheimer's disease, and patients with early symptomatic Alzheimer's disease reveals that non-demented patients with neuropathological Alzheimer's disease do not differ significantly in cognitive performance from individuals with healthy brains (Celsis, 2000; Goldman *et al.*, 2001). Hence, pathologically confirmed preclinical Alzheimer's disease is not necessarily associated with significant cognitive impairment, even on measures shown to be sensitive to very mild Alzheimer's disease. Since neuritic/senile plaques and neurofibrillary tangles, the neuropathological hallmarks of Alzheimer's disease, can be found in adults without any significant cognitive impairment, neuronal pathology leading to Alzheimer's disease likely begins years before any clinical changes occur (Goldman *et al.*, 2001).

Functional neuroimaging studies seem to support the nosologic validity of the concept of a truly preclinical Alzheimer's disease state. Functional neuroimaging employing neuropsychological activation tasks (enabling subtraction comparison of regional brain activities during activation versus resting states) can reveal more subtle alterations in brain function before emergence of even mild cognitive impairment. PET and functional magnetic resonance imaging (fMRI) studies reveal that neural activity magnitude and spatial extent often increase with cognitive task demand. Brain activity in Alzheimer's disease-affected regions observed during memory task performance is greater in APOEε4 allele carriers than in APOEε3 allele carriers. Such differing brain activation patterns suggest the need for greater cognitive effort among those at higher genetic risk for Alzheimer's disease than lower Alzheimer's disease risk counterparts performing the same task (Stern *et al.*, 2000). Further, PET studies have identified parietal, temporal and prefrontal

Table XV-8.18 National Institute of Neurologic, Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for diagnosis of Alzheimer's disease

Criteria	• Dementia established by clinical examination and standardized brief MSE and confirmed by neuropsychological tests • Deficits in two or more areas of cognition • Progressive worsening of memory and other cognitive function • No disturbance of consciousness • Onset between age 40 and 90 years • Absence of other systemic or neurological disorders sufficient to account for the progressive cognitive deficits
Features supporting diagnosis	• Progressive deterioration of specific cognitive functions, such as language (aphasia), motor skills (apraxia) and perception (agnosia) • Impaired activities of daily living and altered pattern of behaviour • Family history of a similar disorder, especially if confirmed neuropathologically • Normal lumbar puncture • Normal pattern or nonspecific changes in EEG • Evidence of cerebral atrophy on CT, with progression on serial observation
Features against diagnosis	• Sudden onset • Focal neurological findings, such as hemiparesis, sensory loss, visual field deficits, and incoordination early in the course of the illness • Seizures or gait disturbance at the onset or very early on in the course of the illness

Adapted from McKhann *et al.* (1984)

Table XV-8.19 DSM-IV criteria for diagnosis of Alzheimer-related dementia

A Development of multiple cognitive deficits manifested by both:
  (1) Memory impairment (impaired ability to learn new information or to recall previously learned information)
  (2) One or more of the following cognitive disturbances:
    (a) Aphasia
    (b) Apraxia
    (c) Agnosia
    (d) Disturbance of executive function

B The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning

C The course is characterized by gradual onset and continuing cognitive decline

D The cognitive deficits in criteria A1 and A2 are not due to any of the following:
  (1) Other CNS conditions that cause progressive deficits in memory and cognition
  (2) Systemic conditions that are known to cause dementia
  (3) Substance-induced conditions

E The deficits do not occur exclusively during the course of delirium

F The disturbance is not accounted for better by another axis I disorder

Adapted from American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association, Washington, DC

deficits in glucose metabolism in cognitively normal APOEε4 allele-positive middle-aged individuals in whom Alzheimer's disease is unlikely to develop for decades (Bozoki *et al.*, 2001).

Clinical Alzheimer's Disease Although mild memory dysfunction is likely to be the first evidence of Alzheimer's disease, Alzheimer's disease can present in a variety of ways. Language disturbances (e.g. word-finding difficulty), behavioural changes (e.g. decreased self-care) and/or personality changes (e.g. increased irritability) can characterize initial presentation, thereby making incipient Alzheimer's disease more difficult to identify. Moreover, years can elapse between onset of such changes and even a probable diagnosis of Alzheimer's disease. Nevertheless, typical Alzheimer's disease presentation involves gradual memory impairment and related functional ineffectiveness over 1–2 years. Anterograde amnesia with rapid attrition of recall is the clinical hallmark of Alzheimer's disease. Although Alzheimer's disease can begin with deficits in many possible domains, episodic memory impairment (wherein important conversations or events are entirely forgotten) is usually the heralding sign, followed by increasingly obvious declines in judgement, reasoning and word-finding ability (Salmon and Bondi, 1997). Disease progression is continuous, although the rate can vary both intraindividually and interindividually. This pattern of continuous deterioration generalizes across Alzheimer's disease aetiology (familial or sporadic), clinical onset (early or late), sample composition (hospital or community) and assessment method.

Delusions are present in up to 75% of cases, and hallucinations are reported in up to half of Alzheimer's disease patients (Lyketsos *et al.*, 2001). Hallucinations tend to appear as Alzheimer's disease progresses into its middle phases (Wilson *et al.*, 2000). Functional imaging studies have demonstrated that Alzheimer's disease patients who manifest psychosis may have disproportionate dysfunction of bilateral dorsolateral frontal, left anterior cingulate, left ventrostriatal, left pulvinar and dorsolateral parietal structures (Mega *et al.*, 2000). Longitudinal studies have found the presence of psychosis to be associated with more rapid cognitive decline (Gormley and Rizwan, 1998). Depression is a common comorbidity, and patients with a family history of major depression are at increased risk (Butt and Strauss, 2001).

Late Alzheimer's disease produces nearly global cognitive and behavioural deficits, reflecting massive neocortical dysfunction. Although Alzheimer's disease usually begins insidiously, progressive intellectual decline and behavioural dysfunction can end in death within 5–10 years of onset.

Early-Onset Alzheimer's Disease Alzheimer's disease beginning before the age of 60 years is usually associated with more than cognitive dysfunction. Early Alzheimer's disease patients more frequently manifest comportmental changes, gait instability and extrapyramidal signs. Early-onset Alzheimer's disease is more often familial: having a first-degree relative with Alzheimer's disease, or being positive for the APOE4 allele, has greater prognostic significance in patients with memory loss at a young age (see below) (Hardy, 2001). Patients with trisomy 21 (Down's syndrome) have a high incidence of Alzheimer's disease beginning in the fourth decade.

Neuropsychological Assessment

The neuropsychological profile of Alzheimer's disease patients (as confirmed by post-mortem examination) is well established. Attentional functioning is generally unimpaired and remains so well into the course of the disease (Fuld, 1982; Schachter *et al.*, 1989). However, while attention and memory encoding capacities are relatively spared, Alzheimer's disease patients manifest notable memory storage deficits with rapid decay of new learning. Unlike patients with subcortical or small-vessel ischaemic dementias, who perform well on delayed memory tasks when presented with multiple-choice cues, Alzheimer's disease patients fail to effectively consolidate new information secondary to hippocampal degeneration. Alzheimer's disease patients generally demonstrate gross deficits on confrontation naming tasks (e.g. Boston naming test) early in the disease course (Cummings and Benson, 1989; Huff, 1990). Patients frequently make semantic paraphasic errors on naming tasks (e.g. calling a cactus a 'tree'), or make circumlocutious responses (calling a hammock a 'swing-chair' or a pen an 'ink-spreader'). Despite this, Alzheimer's disease patients typically benefit from phonemic cuing. Performance on timed verbal fluency tasks is also diminished early in the course of the disease, with decreased performance on semantic (categorical) tasks such as animal naming, while performance on phonemic (letter-specific) fluency measures may be relatively preserved. In all, this profile of performance (decreased memory storage, naming abilities, and semantic fluency in the context of average attentional abilities and letter fluency) distinguishes early Alzheimer's disease from other dementing processes with a relatively high degree of accuracy.

In addition to memory storage and naming difficulties, Alzheimer's disease patients frequently demonstrate cognitive dysfunction broadly associated with other types of dementing illnesses. Executive dysfunction may be present even in early stages of the disease, with decreased judgement, insight, reasoning and abstraction capabilities (McGlynn and Kaszniak, 1991; Schacter, 1991). In middle and late stages of the disease, cognitive dysfunction becomes increasingly severe and diffuse. Visuospatial problem-solving abilities diminish, and patients frequently become apraxic. Language continues to deteriorate, and patients gradually lose the capacity to recall even remote information. Retrograde memory loss tends to follow a temporal grading, with memory for relatively recent events deteriorating first, followed by a deterioration in memory of temporally distant events (Kopelman *et al.*, 1989; Nebes, 1992a; Nebes, 1992b). In late stages, patients are frequently inattentive, minimally responsive, and unable to recognize or communicate their needs.

Several clinical inventories and scales are available for evaluating Alzheimer's disease and monitoring progression (Knopman *et al.*, 2001). The Alzheimer's disease assessment scale–cognitive subscale (ADAS-Cog) is an objective, clinically validated, 11-item cognitive assessment instrument used routinely in clinical trails of dementia. It examines selected aspects of cognitive domains, including memory, language, orientation and praxis. Score ranges from 0 to 70, with 70 representing the worst degree of impairment; the average score of Alzheimer's disease patients with mild to moderately severe Alzheimer's disease is 15–25.

Many studies evaluating the diagnostic utility of neuropsychological tests have analysed patients with clinically diagnosed probable Alzheimer's disease (of mild to moderate dementia severity) and compared their performance with that of carefully screened healthy older adults. Although Alzheimer's disease patients are impaired in a wide variety of cognitive functions, the most effective neuropsychological measures for distinguishing between mild to moderate Alzheimer's disease patients and healthy elderly people are those that assess the ability to learn new information and to retain it over time. Comparisons of usefulness of measures of learning, retention, confrontation naming, verbal fluency and constructional ability for differentiating between very mildly demented patients with probable Alzheimer's disease and normal elderly adults have demonstrated that the highest diagnostic accuracy (up to 90% as confirmed by post-mortem histopathological analysis) is achieved with a cued recall measure on verbal memory tasks (Tierney *et al.*, 1988; Morris *et al.*, 1988).

Comparison of cognitive performance of probable Alzheimer's disease patients on various dementia rating scales has suggested that although most scales are adequate for tracking progression through

early and middle stages, the Mattis dementia rating scale (DMS) is especially useful for tracking cognitive decline in advanced stages, likely secondary to its inclusion of a wider difficulty range of test items (Bondi *et al.*, 1996).

Studies of the relationship between Alzheimer's disease neuropathological indices and cognitive function have also been performed. For example, quantitative measures of synaptic loss, senile plaques and neurofibrillary tangles in cortical association areas of patients with autopsy-proven Alzheimer's disease have been correlated with standardized cognitive examination performance before death (Cummings *et al.*, 1996). Of neuropathological variables, midfrontal synaptic density appears to have the greatest correlation with global cognitive impairment measures. Similarly, midfrontal synaptic density seems to account for the greatest amount of variance in cognitive scores in regression analyses, including all neuropathological variables (Cummings *et al.*, 1996; Cummings and Cotman, 1995).

Neuroimaging

Structural imaging in Alzheimer's disease can reveal medial-temporal atrophy; more generalized cerebral atrophy is seen only after a substantial amount of neuronal death. Coronal magnetic resonance imaging (MRI) is particularly useful for detecting hippocampal atrophy. Possible findings in early-onset or familial Alzheimer's disease are atrophy disproportionate to age, ventricular enlargement, and asymmetric medial-temporal atrophy.

Functional brain imaging by PET or single position emission computerized tomography (SPECT) reveals characteristic (but nonspecific) bilateral temporoparietal hypoperfusion and/or hypometabolism in up to 85% of cases, a finding that can predate symptom onset (Celsis, 2000). PET may be superior to MRI measures of hippocampal atrophy since cerebral glucose metabolism changes antedate onset of memory decline while structural MRI hippocampal changes do not (Scheltens, 1999).

Frontotemporal Lobar Dementia

Certain forms of degenerative disease disproportionately affect circumscribed areas of the frontal and/or frontotemporal cortex, producing a variety of clinical syndromes grouped diagnostically under the term 'frontotemporal lobar dementia'. Although it is the third most frequent cause of degenerative cortical dementia after Alzheimer's disease and DLB (some epidemiological studies place FTLD second), FTLD is much less common than Alzheimer's disease, DLB and vascular dementia, especially in very early dementia patient populations. Nevertheless, FTLDs are important to recognize because (1) although insidious in onset, they usually progress to severe disability and sometimes fatality; (2) their symptomatology can mimic other disease states (particularly primary affective psychiatric disturbance); and (3) management requirements are very different from those of other dementias.

While the generic term FTLD refers to progressive circumscribed frontotemporal degeneration, distinct clinical syndrome phenomenology is determined primarily by neuropathological distribution. Based on such differential neuroanatomic involvement, three prototypic neurobehavioural syndromes are recognized: frontal variant FTLD (fvFTLD) (predominantly frontal pathology), semantic dementia (predominant anterior temporal pathology), and progressive nonfluent aphasia (mixed frontal, superior temporal), (Hodges, 2001). We briefly review each of these below.

Because of the complexity of the symptom and pathological spectra of FTLDs, optimal evaluation and management require cooperation across four disciplines: neurology, psychiatry, neuropsychology and neuroimaging.

Frontal Variant Fronto Temporal Lobar Dementia

fvFTLD is the most common FTLD (Hodges, 2001). Functional neuroanatomical division of the frontal lobe into three separate regions (medial, orbitobasal, dorsolateral) provides a conceptual basis for organizing fvFTLD symptomatology. For example, abulia can be associated with medial frontal–anterior cingulate involvement, social disinhibition and impulse dyscontrol with orbitobasal involvement, and executive dysfunction with dorsolateral prefrontal cortex involvement. Symptom admixtures commonly occur with disease progression (Hodges, 2001).

Personality alteration is prominent but highly variable, ranging from reduced volition and affective blunting to aggression and gross social inappropriateness. Such marked behavioural disturbances can be attributed erroneously to primary psychiatric disease, often prompting initial patient presentation to a psychiatrist. For example, progressive apathy can be mistaken for depression. Alternatively, contextually inappropriate ebullience and impaired judgement can mimic the main phase of bipolar disorder (Hodges, 2001).

Multiple potential behavioural changes can manifest. Activity often becomes stereotyped. Rituals (e.g. eating the same food at the same time every day), verbal/ideational perseveration (e.g. repeated use of the same catchphrase), and behavioural perseveration (e.g. watching the same video over and over) are common. Speech output frequently becomes abridged, sometimes culminating in mutism (often associated with an amotivational/hypokinetic state), although episodic speech excess/pressure can occur in some fvFTLD patients with prominent disinhibition. Patients often display a food preference change (increased sweets), and behavioural features of Kluver–Bucy syndrome (e.g. hypersexuality, placidity, hyperorality) can occur (Hodges, 2001).

The term 'dysexecutive syndrome' has been applied to the constellation of cognitive deficits in fvFTLD. Dysfunction routinely involves cognitive domains, including attention, abstraction, planning, organization, problem solving, judgement and mental flexibility. Primary elements of language, perception and spatial function are well preserved. Typically not significantly amnesic, patients are usually well oriented. Memory deficits tend to be a secondary epiphenomenon of frontal regulatory disturbances (i.e. inattention with consequent encoding deficiency, defective strategies for learning and retrieval) rather than manifestations of a primary anterograde amnesia. Executive deficits are typically more evident in inert, avolitional patients than in overactive, disinhibited patients. Poor insight regarding these impairments sadly complicates patient management (Hodges, 2001).

Pick's disease is the primary form of fvFTLD. A distinctive but inconsistent neuropathological correlate of Pick's disease is the Pick body, a basophilic intraneuronal inclusion, affecting neocortical temporal and hippocampal neurons. Atrophy involving the frontotemporal cortex and subjacent white matter is severe, ultimately producing 'knife-edge' gyri (ulegyria) in these regions, with abrupt transitions between mildly and severely involved sectors (e.g. between the anterior and posterior segments of the superior temporal gyrus).

Neuropsychological testing plays an essential role in fvFTLD evaluation (Hodges *et al.*, 1999). Simple cognitive screening tests, such as the MMSE lack sensitivity for early signs of executive dysfunction and are therefore unreliable for detection and monitoring of fvFTLD. Useful neuropsychological tests are the Wisconsin card sorting test, the Stroop test and the verbal fluency challenge. Test profiles typically reveal frontal system deficits, including attention, verbal fluency, abstraction and executive function. Quantifiable tests involving decision making and risk taking, which are better able to detect orbitobasal frontal function, have been developed (Gregory *et al.*, 1999).

Anterograde memory performance is variable; fvFTD patients tend to do worse on recall than recognition tasks. Nontemporally graded remote memory loss can occur late in the disease course. The most striking neuropsychological finding is how well subjects perform on tests of visuospatial ability, particularly when organization aspects are minimized (Gregory *et al.*, 1999).

Structural neuroimaging usually demonstrates bilateral and relatively symmetrical frontal atrophy. However, this is not universal, especially early in the disease process. Functional imaging may be more sensitive for early diagnosis of fvFTLD. T_{99}SPECT is probably the most sensitive and can detect frontal hypoperfusion before atrophy is evident (Miller and Gearhart, 1999).

Frontotemporal Lobar Dementias with Primarily Language Disturbance

Like other FTLD diagnostic subdivisions, controversy exists regarding nosological classification of FTLDs whose primary symptomatology involves language disturbance. A syndrome initially suggested by Pick, Mesulam's initial report of patients with gradually progressive dysphasia within the context of preserved intellect and insight sparked contemporary interest in the phenomenon commonly called primary progressive aphasia (PPA). Although language disturbances in such FTLD-related dysphasias are heterogeneous, two distinct clinical prototypes can be identified: progressive fluent aphasia, also known as semantic dementia, and progressive nonfluent aphasia.

It is important to recognize patients with progressive aphasia since, because of the 'focal' nature of their deficit (i.e. isolated language impairment), patients can be mistaken for stroke, neoplastic or even Alzheimer's disease cases. In general, language impairment remains an isolated but increasingly severe aphasia in about half of cases; the balance eventually progresses to a more global dementia.

Temporal Lobe Variant Frontotemporal Dementia Semantic dementia (Table XV-8.20) involves progressive dysnomia and word comprehension impairment in the context of fluent, grammatical speech (it is therefore grossly similar to Wernicke's aphasia). There is relative preservation of repetition, as well as ability to read aloud and write orthographically regular words (i.e. words whose phonemes follow regular rules of spelling representation). Patients can also display associative agnosia (impaired understanding of visual percepts); thus there is loss of meaning for both verbal and nonverbal elements (Hodges *et al.*, 1999).

Patients with semantic dementia typically present complaining of word-finding difficulty. Though often painfully aware of their worsening expressive vocabulary, patients are frequently unaware of their impaired comprehension. Since syntactic and phonological language structures remain intact, clinical signs are relatively subtle in early stages (Hodges *et al.*, 1999).

Although behavioural symptoms are generally absent or minimal at presentation, fvFTLD-like features can emerge at later stages.

Semantic dementia patients are globally impaired on semantic memory tests. This is most apparent on tasks requiring verbal output, such as category fluency tests, picture naming and verbal definition tasks. Although able to read and write words with regular spelling-to-sound correspondence, semantic dementia patients have difficulty reading and writing irregular words. This pattern, known as surface dyslexia/dysgraphia, has been attributed to loss of semantic support for pronunciation and/or spelling of irregular words. Episodic memory is relatively spared, although quantitative assessment reveals impaired recall of more distant life events (a reversal of the usual temporal gradient of Alzheimer's disease) (Gregory *et al.*, 1999).

Structural neuroimaging reveals anterior temporal neocortical atrophy, with inferior and middle temporal gyri predominantly affected. Asymmetries of temporal involvement reflect relative severity of impairment for verbal versus visual concepts (word meaning versus object recognition). Functional changes on SPECT usually precede detectable structural alterations, demonstrating dominant greater than nondominant temporal hypoperfusion (Miller *et al.*, 1999).

Nonfluent Progressive Aphasia Progressive aphasia (Table XV-8.21) is a disorder of expressive language characterized by effortful speech production with phonological and grammatical errors. Comprehension is relatively preserved. The language disorder occurs in the absence of impairment of other cognitive domains, although behavioural changes of fvFTD may emerge late in the disease course. Patients present with complaints of speech dysfluency and distortion or word-finding difficulty.

The pattern of cognitive deficits in progressive nonfluent aphasia is, in many ways, the complement of that in semantic dementia. Patients perform well on tests of semantic memory, except those requiring phonological competence. Although conversational speech is severely disrupted, anomia is mild.

Table XV-8.20 Clinical features of semantic dementia

Core diagnostic features	Insidious onset and gradual progression		
	Language disorder characterized by:		
	– Content-impoverished but fluent speech		
	– Impaired naming and comprehension		
	– Semantic paraphasias		
	Perceptual disorder characterized by associative agnosia (impaired recognition of object identity)		
	Preserved perceptual matching and drawing reproduction		
	Preserved single-word repetition		
	Preserved ability to read aloud and write orthographically regular words		
supportive diagnostic features	Speech and language	– Idiosyncratic word usage	
		– Surface dyslexia/dysgraphia	
		– Speech pressure	
		– Absence of phonemic paraphasias	
	Behaviour	– Loss of sympathy and empathy	
		– Narrow preoccupations	
		– Parsimony	
	Physical signs	– Akinesia, rigidity, tremor	
		– Absence or late primitive reflexes	
	Investigations	Neuropsychology	– Profound semantic loss
			– Preserved phonology, syntax
		EEG	Normal
		neuroimaging	Anterior temporal abnormalities

Adapted from Neary *et al.* (1998)

Table XV-8.21 Diagnostic features of progressive nonfluent aphasia

Core diagnostic features	Insidious onset and gradual progression Nonfluent spontaneous speech with at least one of the following: – Agrammatism – Phonemic paraphasias – Anomia		
Supportive diagnostic features	Speech and language		– Stuttering or oral apraxia – impaired repetition – Alexia, agraphia – Late mutism – Preservation of word meaning
	Behaviour		– Early preservation of social skills – Late behavioural changes similar to FTD
	Physical signs		– Late contralateral primitive reflexes – Akinesia, rigidity, tremor
	Investigations	Neuropsychology	– Nonfluent aphasia – Absence of severe amnesia
		EEG	Normal or minor asymmetric slowing
		Neuroimaging	Dominant hemisphere frontotemporal abnormality

Adapted from Neary *et al.* (1998)

Structural neuroimaging shows Sylvian fissure widening with atrophy of the insula, inferior frontal and superior temporal lobes (dominant greater than nondominant) (Miller and Gearhart, 1999).

Posterior Cortical Atrophy

Posterior cortical atrophy (PCA) is a lobar dementia characterized by initial disturbances of visual perception and integration. Involvement of the occipitoparietal region produces visuospatial and integrated attentional disturbances with relative sparing of personality, insight and memory until late in the disease. PCA is sometimes referred to as the visual variant of Alzheimer's disease. Progression to a global dementia occurs in most cases.

Parkinson's Disease

Nearly all Parkinson's disease patients develop cognitive deficits over the course of the disease, while up to 40% of patients go on to develop impairments sufficiently severe to comprise a true dementia (Brown and Mardsen, 1984). Cognitive impairment in Parkinson's disease is often under-recognized because of the overwhelming phenomenological prominence of motor disturbances. Parkinson's disease-related cognitive dysfunction can be considered a component of the spectrum of neuropsychiatric disturbances attendant to basal ganglia disorders (Rosenblatt and Leroi, 2000). McHugh has described such basal ganglia disease-related disturbances as marked by a 'triadic syndrome' comprising depression, dementia and dyskinesia (McHugh, 1989).

Parkinson's disease-related cognitive impairment is characterized by psychomotor slowing, executive dysfunction, memory impairment and visuospatial disturbances, as well as increased propensity for depression. Parkinson's disease-related cognitive dysfunction is reportedly more common in individuals with asymmetric onset of parkinsonism affecting the nondominant side and accentuated gait disturbance relative to tremor (McPherson and Cummings, 1996).

Parkinson's disease-related cognitive deficits tend to exist on a continuum, with subtle dysfunction emerging early and progressive worsening over time. As subcortical projections to the frontal cortex deteriorate, patients demonstrate decreased cognitive functioning associated with loss of both subcortical and frontal functions (Freedman, 1990). Cognitive slowing is ubiquitous, and patients demonstrate many significant response latencies, even in response to simple questions. In addition, they typically exhibit diminished attentional capacities, including decreased mental tracking, divided attention, and sustained attentional capacities. Executive difficulties are also prominent. Parkinson's disease patients tend to perform poorly on measures of cognitive flexibility, such as tasks requiring establishment, maintenance or shifting of mental sets (e.g. Trails B, Wisconsin card sort test). The ability to conceptualize and solve problems can be impaired early in the disease course (Levin *et al.*, 1989), while judgement is often preserved. Visuoperceptual difficulties may also emerge as measured by performance on tasks that require patients to judge and match line orientation or construct designs from a model (Hovestadt *et al.*, 1987).

Although attentional difficulties can impact memory acquisition, patients tend to consolidate and retain new information until late in the disease course. Despite this, Parkinson's disease patients often demonstrate profound difficulties with memory retrieval, such that they are unable to independently access new learning unless presented with external cues (Pillon *et al.*, 1993). For example, on neuropsychological measures of memory (such as the logical memory subtest of the WMS), patients with Parkinson's disease tend to perform poorly on delayed recall tasks in which they are required to independently retrieve information (as when asked to spontaneously relate a story heard 20 minutes ago). However, when provided with a recognition format, such as multiple choice, these same patients (unlike Alzheimer's disease patients) may demonstrate unimpaired performance compared with normals. In contrast to Alzheimer's disease, language function remains relatively unimpaired until late in the disease course, with relatively preserved performance on confrontation naming. Parkinson's disease-related hypophonia should not be allowed to confound careful language function assessment.

Cognitive symptoms may respond partially to dopaminergic pharmacotherapy in early stages of the illnesses, but they tend to become refractory as the disease progresses and dementia becomes more severe. As elsewhere, treatment of comorbid depression is imperative for optimizing cognitive function.

Dementia with Lewy Bodies

DLB is probably the second most common type of degenerative dementia after Alzheimer's disease (Campbell *et al.*, 2001). DLB is defined clinically by the presence of dementia, gait/balance disorder, prominent hallucinations and delusions, sensitivity to traditional antipsychotics, and fluctuations in alertness (Papka *et al.*, 1998). DLB has clinical features that overlap diagnostically with Alzheimer's disease and Parkinson's disease.

DLB-related cognitive impairment can range from transient dysfunction to sustained dementia (Gomez-Tortosa *et al.*, 1998).

A superimposed fluctuation in alertness can sometimes mimic a delirium-like component. DLB-associated depressive features can include neurovegetative derangement, dysthymia and psychomotor agitation (Ballard *et al.*, 2001). Delusions, if present, are typically depression-congruent. The characteristic motor disturbance is parkinsonism, often with prominent bradykinesia; tremor is variable. Frequent falling is a common feature. Hallucinations tend to be visual and complex in nature (Fields, 1998). REM sleep is often disturbed. In contrast to the decrease in muscle tone normally accompanying REM sleep, DLB patients do not become atonic when attaining REM sleep. The result is that patients sometimes have a worrisome capacity to act out their dreams (i.e. develop REM sleep disorder).

Universally accepted diagnostic criteria still require validation (Lund and Manchester Groups, 1994; Serby and Samuels, 2001).

Prominent deficits in attention, language, executive function, memory retrieval and visuoconstruction skills are neuropsychological features of DLB (Fields, 1998; McKeith *et al.*, 1994). In general, DLB patients have greater visuospatial impairment and executive dysfunction than Alzheimer's disease patients. In contrast, sparing of memory storage in DLB distinguishes DLB memory impairment from that of Alzheimer's disease (McKeith *et al.*, 1994). Language dysfunction in DLB distinguishes its cognitive profile from Parkinson's disease.

Compared with Alzheimer's disease, DLB patients show less temporal lobe atrophy on structural MRI and more occipital lobe hypoperfusion on SPECT (Lobetesis *et al.*, 2001), but neuroimaging has not proven successful in differentiating DLB from Alzheimer's disease.

Lewy bodies are eosinophilic intracytoplasmic inclusions originally described in the brainstem (substantia nigra) of patients with Parkinson's disease; they occur with slight ultrastructural differences in neocortical and paralimbic neurons in several disorders. Lewy bodies are widely distributed in both the cerebral cortex and substantia nigra in DLB (Lewy bodies are usually confined to the substantia nigra in Parkinson's disease) (Lowe and Dickson, 1997).

As neuroleptic use becomes a complicated task in any dementia with comorbid parkinsonism (since neuroleptic-related dopamine blockade can worsen parkinsonian motor disturbance), this caution becomes imperative in DLB. DLB patients are often exquisitely sensitive to neuroleptics, which can precipitously worsen overall cognitive function. Cholinesterase inhibitors have shown some efficacy for DLB-related cognitive and behavioural symptoms.

Progressive Supranuclear Palsy

About 7% of patients with parkinsonian motor abnormalities suffer from PSP a disorder affecting volitional eye movements early in its course. Typical findings are impaired vertical (primarily downward) eye movements, axial/truncal postural disturbance and bulbar dysfunction. Unlike Parkinson's disease, PSP-associated resting tremor is minimal, symptom onset is symmetrical, and disease progression is rapid (Litvan and Hutton, 1998).

PSP causes a dementia similar to that of PD in 50–75% of affected individuals. However, the neuropsychological profile of PSP has not been documented as consistently as that of Parkinson's disease, and there appears to be significant variation among patients. Despite this, the general pattern of performance on neuropsychological tasks includes cognitive slowing, attentional deficits, executive dysfunction, impaired visual scanning (due to oculomotor deficits), motor dysfunction and sequencing difficulties (Grafman *et al.*, 1990; Grafman *et al.*, 1995; Litvan *et al.*, 1989).

Although memory dysfunction can occur at nearly every stage of memory processing, deficits in memory storage tend to be not as severe as those seen in Alzheimer's disease (Pillon *et al.*, 1994). Mild word-finding difficulties and naming deficits are seen frequently, and executive deficits such as impaired cognitive flexibility, abstraction and reasoning capabilities may emerge early in the disease course. As in all subcortical dementias, cognitive slowing, executive dysfunction and memory retrieval are prominent features.

Dementia Pugilistica

Although a 'secondary' dementia by virtue of its association with boxing or other modes of repeated head trauma, this syndrome is described here because of its neurohistopathological similarities with Alzheimer's disease and Parkinson's disease. Dementia pugilistica, or punch drunk syndrome, was originally described in boxers and is characterized by the combination of dementia and parkinsonism occurring as a late consequence of repeated head trauma. Brain imaging often reveals diffuse cerebral atrophy and cavum septum pellucidum. Histopathological hallmarks include neuronal loss and diffuse neurofibrillary tangles. Diffuse beta-amyloid immunoreactivity within senile plaques and blood vessels has recently been identified in dementia pugilistica brains; along with other evidence, this suggests an important association between head trauma and Alzheimer's disease.

Huntington's Disease

Huntington's disease is a progressive, eventually fatal neuropsychiatric disease with a prevalence of 5–10 per 100 000 in the general population. A prototypical genetically based neurodegenerative disorder, Huntington's disease is inherited as an autosomal dominant trait with complete penetrance.

One-third of Huntington's disease patients have psychiatric changes at onset, and almost all suffer progressive cognitive decline (Squitieri *et al.*, 2001). Patients display multiple deficits attributable to frontostriatal circuit dysfunction. Meta-analyses of Huntington's disease cognitive studies demonstrate deficiencies of delayed recall, memory acquisition, cognitive flexibility, abstraction, manual dexterity, attention and verbal skill. In general, cognitive symptoms are associated with the severity of motor impairment (Zakzanis, 1998).

The neuropsychological profile is consistent with other subcortical dementias, with prominent executive and memory retrieval deficits (Pillon *et al.*, 1993). Huntington's disease patients can also exhibit impaired concentration and memory encoding, however, and they are especially sensitive to interference effects (Folstein *et al.*, 1990). For example, on memory tasks such as the CVLT, Huntington's disease patients are likely to show rapid decay of established new learning if asked to immediately encode additional information. Executive deficits include impulsivity, disinhibition and difficulty with planning and organization. Visuoperceptual difficulties occur early in the course of the disease and may include problems with left–right orientation and perceptual fragmentation (in which patients may focus on small details of an object while failing to appreciate the gestalt) (Josiassen *et al.*, 1983). Language functioning tends to be preserved until the late stages of the disease. Ultimately, Huntington's dementia evolves into severe, global cognitive deficits, which progress throughout the course of the illness.

Huntington's Disease as a Prototypical Subcortical Cognitive Disorder

Huntington's disease serves as a useful prototype for comparing neuropsychological differences between cortical and subcortical dementias. For example, Huntington's disease and Alzheimer's disease patients can be subdivided by pattern of respective memory deficits. Alzheimer's disease patients demonstrate severe episodic memory deficit seemingly resulting from ineffective consolidation of new information. In contrast, the memory disorder of Huntington's disease is thought to be secondary to difficulty initiating a systematic retrieval strategy for recalling information from either episodic or semantic memory. Although, like Alzheimer's disease patients, Huntington's disease patients have difficulty learning and recalling information in a free-recall task, they exhibit marked improvement when switched to a recognition task (Bondi *et al.*, 1996).

Cortical and subcortical dementias can also be differentiated by remote memory testing. Whereas Alzheimer's disease patients display severe retrograde amnesia with temporal grading in early stages (i.e. distant memories are recalled better than more recent memories), Huntington's disease patients have relatively preserved retention and, like other subcortical dementias, manifest only mild retrograde amnesia without temporal grading (Bondi *et al.*, 1996).

Based on such different memory impairment mechanisms, Huntington's disease and Alzheimer's disease patients can be differentiated fairly accurately by certain memory tests (e.g. CVLT, which can discriminate component memory functions and consequently disorders affecting them differentially) (Bondi *et al.*, 1996).

Another major distinction between cortical and subcortical dementias can be appreciated in semantic function: while cortical dementias are often marked by progressive deficits, there is little or no impairment in subcortical dementias. This has been studied by the use of multidimensional scaling, which provides a method for generating a spatial representation of the degree of conceptual association among semantic memory elements. Semantic maps can be created of cognitive spatial representation clusters according to the degree of relatedness in the patient's semantic network along specific conceptual dimensions, such that the distance between concepts reflects association strength. A study comparing such cognitive maps of Huntington's disease, Alzheimer's disease and normal elderly control subjects demonstrated that while Alzheimer's disease patients reveal a breakdown in the structure and organization of semantic memory, resulting in greater reliance on concrete perceptual characteristics, Huntington's disease patients do not differ significantly from normal controls (Chan *et al.*, 1993; Bondi *et al.*, 1996).

Qualitative analysis of other neuropsychological testing errors can further reveal differential performance between cortical and subcortical dementias. For example, whereas Huntington's disease patients make graphic, visuospatial and planning errors in both the command and copy conditions of a constructional task (e.g. clock drawing), Alzheimer's disease patients make conceptual errors (e.g. misrepresenting a clock by drawing a face without numbers or with incorrect numbers) in the command condition but not in the copy condition (Freedman *et al.*, 1994; Bondi *et al.*, 1996).

Cortical-Basal Ganglionic Degeneration

Cortical-basal ganglionic degeneration (CGBD) is a relatively rare neurodegenerative disease beginning in the sixth decade or later, marked by asymmetric rigidity and postural disturbances predisposing to falls. Focal myoclonus and tremors can be associated features.

The neuropsychological profile of CGBD-related dementia has not been well validated, although features of both cortical and subcortical dementias are generally present. CBGD patients demonstrate executive dysfunction similar to PSP (Pillon *et al.*, 1995). Apraxias are characteristic of the dementia of CBGD and are usually apparent before cognitive dysfunction in other domains (Troster, 1998). Approximately 60% of patients experience 'alien limb syndrome', a phenomenon in which the affected limb assumes positions or carries out actions disconnected from the patient's awareness.

CBGD is often associated with asymmetric cortical atrophy affecting the frontoparietal neocortex, with attendant reductions of cerebral metabolism in that region. Neuropathological findings include patchy neuronal loss and gliosis in the cerebral cortex and substantia nigra, as well as a characteristic intraneuronal nigral inclusion body.

CBGD typically progresses to global dementia, and death usually ensues within 5–10 years. There is no known treatment.

Secondary Cognitive Disorders

Potentially Reversible Secondary Dementias

Few processes that damage the brain sufficiently to cause dementia are readily reversible. Even when a potentially reversible cause of a dementia is identified, actual reversal is often elusive. Nevertheless, certain structural, nutritional, metabolic, infectious and psychiatric disorders can produce cognitive decline lasting weeks or months that potentially can be reversed or at least ameliorated if detected and treated. Underscoring the importance of prompt consideration and investigation of reversible aetiologies are studies demonstrating that the earlier intervention is initiated, the greater the likelihood of actual clinical improvement (Freter *et al.*, 1998; Steffens-and Morgenlander, 1999).

Meta-analyses of dementia aetiology studies have revealed at least potential reversibility in approximately 13% of patients (Freter *et al.*, 1998). The most frequent causes of reversible dementias are depression and iatrogenic (e.g. adverse drug effect), which together constitute about 50% of reversible dementias (Freter *et al.*, 1998).

Primary Psychiatric Disturbances

Depression

Dementia and depression are not only often comorbid but can also appear remarkably similar. Up to 55% of elderly with depression develop cognitive impairment phenomenologically consistent with a dementia syndrome; this has commonly been called pseudodementia, but it is called more correctly the dementia of depression or depression-related cognitive impairment (DRCI). Meta-analyses demonstrate that new antidepressants not only treat depression comorbid with dementia but can also improve cognitive function, suggesting a high prevalence of superimposed DRCI among dementia patients in general (Meyers, 1998).

Patients with DRCI are more likely to complain of memory and concentration problems than patients with degenerative dementia. Also, in contrast to patients with degenerative dementia, those with depression can do reasonably well on formal mental status testing when confounds such as motivation and attention are minimized. Careful bedside mental status or neuropsychological assessment is necessary to establish dementia in a patient with both affective symptomatology and cognitive deficits.

While recall is impaired in both dementia and DRCI, DRCI patients usually have preserved recognition while degenerative (cortical) dementia patients show impaired recognition and more intrusion errors. Cognitive testing in DRCI reveals performance variability partly dependent on variations of attention and effort during memory encoding. Examiner tenacity at ensuring successful embedding of short-term memory items is essential to distinguish the memory epiphenomenon of depression-related attention impairment from a primary memory disturbance.

Key differences can often be elicited in language functions, which are generally preserved in DRCI but frequently become impaired in degenerative dementia. Similarly, apraxias, visuospatial deficits and gross executive dysfunction, common features of degenerative dementias, are seen in only the most severe forms of DRCI.

Clues suggesting depression include prior history of mood disorder in the patient or family, onset over days to weeks, marked psychomotor retardation or agitation, mood-congruent delusions (e.g. paranoia), precipitation by an identifiable stressor, and explicit patient complaints of concentration or memory difficulty. However, geriatric depression can be extremely protean in its manifestations. Symptoms and signs of depression in the elderly can be much more subtle than typical adult presentations. Sometimes, the only sign can be disturbance of neurovegetative function, e.g. deranged sleeping pattern or decreased appetite. A low threshold to consider diagnosis of depression and initiate an empirical treatment trial should

be maintained. DRCI is potentially reversible with antidepressants or electroconvulsive therapy (ECT). Highly anticholinergic antidepressants can worsen cognitive deficits.

Of note, depression can itself be an early symptom of several degenerative dementing processes (Meyers, 1998). Despite initial improvement via antidepressant therapy, almost half of elderly patients with depression-related cognitive dysfunction develop irreversible degenerative dementia within 5 years. Those patients with depression unaccompanied by cognitive impairment develop dementia at rates more comparable to those of the general population. Therefore, once an episode of depression complicated by cognitive impairment is documented in an elderly patient, that patient should be monitored closely for signs of relapse and incipient dementia thereafter.

Regrettably, neither clinical features nor currently available biological markers reliably differentiate elderly patients with a primary dementia from those with DRCI.

Structural Psychiatric Disturbances

Normal-Pressure Hydrocephalus

Normal-pressure hydrocephalus (NPH) can be a structural cause of dementia potentially treatable by surgery. NPH classically presents as a triad of gait apraxia, urinary urgency/incontinence, and mental status changes. Usually developing in late-middle or old age, psychomotor slowing combines with cognitive findings suggestive of a mixed cortical and subcortical dementia. Importantly, parkinsonism, sensory complaints and depression can be associated findings.

NPH usually develops over weeks to months. A gait disorder is often the initial manifestation, typically in the form of a gait apraxia characterized by apprehension and unsteadiness on standing and difficulty initiating ambulation despite absence of weakness or ataxia on isolated testing. The patient can perform leg movements associated with walking, bicycling or kicking, and can trace figures with the feet while lying or sitting, but is unable to do so while legs are bearing weight. Often described as broad-based and 'magnetic', as if glue-footed, NPH-related gait dysfunction is also notable for impaired turning marked by the need for multiple small steps in order to effect a 180° turn. Pyramidal signs, including spasticity, hyper-reflexia and extensor plantar responses, are sometimes present. Extrapyramidal and parkinsonian features (hypokinesia, bradykinesia, cogwheel rigidity) can occur. Motor perseveration and frontal release signs (e.g. grasp reflexes) can sometimes be seen. Urinary incontinence is usually a later development.

NPH-related cognitive impairment is usually insidious in onset. Initially characterized by generalized slowing and apathy, global cognitive dysfunction ensues if untreated (Pinner *et al.*, 1997). Memory deficiency is common; other focal cognitive signs such as aphasia and agnosia are rare. NPH presenting solely as a psychiatric disturbance (e.g. paranoid psychosis) without the classic neurological triad has been reported. Comorbid Alzheimer's disease can often occur, with consequent admixtures of clinical symptomatology (Savolainen *et al.*, 1999).

NPH can be idiopathic or secondary to conditions that interfere with CSF absorption. Communicating hydrocephalus is most likely the result of alterations in clearance and/or flow of CSF, causing ventricular enlargement and secondary susceptibility of brain parenchyma to mechanical and/or ischaemic injury. Impaired CSF absorption via arachnoid granulations of hemisphere convexity subarachnoid space can result from the meningeal fibrosis, which can be a complication constituting the final common pathohistological outcome of multiple meningo-encephalitic and cerebrovascular processes. Predisposing factors such as subarachnoid haemorrhage, meningitis or head trauma can sometimes be identified. Ventricular enlargement can stretch frontocerebellar nerve fibres as they circumnavigate the ventricles, disturbing gait and bladder function. Deranged ventricular CSF flow on adjacent basal ganglia structures might be responsible for the development of parkinsonian features.

Neuroradiological correlates include communicating/nonconstructive hydrocephalus (enlarged ventricles out of proportion to prominence of cortical sulci), anterior third ventricle enlargement, downward extension of mammillary bodies, bowing of corpus callosum, and a flow-void in the fourth ventricle on T2-weighted MRI. Radionuclide cisternography can show ventricular isotope accumulation, delayed clearance, and failure of ascent over the cerebral convexities. SPECT can reveal frontal or diffuse hypoperfusion.

If a specific cognitive impairment profile can be identified in NPH, then earlier differentiation from other dementia types, and thereby earlier intervention initiation, can be facilitated (Golomb *et al.*, 2000). Based on previous neuropsychological evidence of apparently greater effect of NPH on executive function, attempts to identify an NPH-specific cognitive impairment profile have utilized tests relatively sensitive to frontal lobe function to examine whether such tasks are particularly susceptible to NPH-induced damage. Results of such studies reveal that there are two distinctive patterns of cognitive change in NPH: (1) predominantly executive (only later becoming more global), frequently not relieved significantly by shunting, and (2) generalized dementia detectable by gross measures (e.g. MMSE), in which shunting can reverse dementia (Iddon *et al.*, 1999).

Some patients, especially those with hydrocephalus from meningitis or subarachnoid haemorrhage, can improve following ventriculoatrial, ventriculoperitoneal or lumboperitoneal shunting.

Subdural Haematoma

Subdural haematoma (SDH) is the most readily treatable intracranial mass lesion that can cause cognitive dysfunction. Patients aged 50–70 years are most commonly affected. The most frequently identified precipitating factor is head trauma. SDH is bilateral in about one-sixth of cases.

SDH can be clinically quiescent without overt symptoms evident for months. Headache is the most common initial symptom. Cognitive dysfunction (ranging from mild, nonspecific confusion to significant dementia) and contralateral hemiparesis are the most frequent signs, followed by papilloedema and extensor plantar responses.

SDH can usually be visualized on noncontrast computerized tomography (CT) as an extra-axial crescent-shaped hypodensity. Usually involving a portion of or, less commonly, an entire cerebral convexity, SDH can also occur above and below the tentorial dural regions. Neuroimaging should be reviewed carefully for evidence of bilateral subdural collections. SDH age can be estimated on CT by evolution of subdural defect from hyperdense (bright), representing recently accumulated blood, progressing through successive shades of grey, culminating in isodense collections that sometimes require contrast infusion to be visualized.

Neoplastic Psychiatric Disturbances

Cancer can cause cognitive impairment in many ways. Although rarely reversible, the potential for partial reversibility or even cure exists, hence our inclusion of this subclass here.

Brain tumours manifesting solely by progressive cognitive impairment are relatively infrequent. Nevertheless, certain slow-growing intracranial neoplasms occurring in strategic locations (e.g. frontal or temporal meningioma) represent a curable cause of secondary dementia. When otherwise clinically covert (i.e. absence of noncognitive neurological signs), the threshold for performing screening neuroimaging becomes key. Related cognitive dysfunction can range from subtle focal dysfunction (e.g. anterograde explicit memory) to severe diffuse (e.g. stupor) dysfunction.

Metabolic Psychiatric Disturbances

Almost any metabolic abnormality can potentially impact cerebral function to such an extent as to cause cognitive dysfunction. Here, we summarize a prototypical example of a metabolic disturbance associated with chronic cognitive dysfunction.

Wilson's Disease

Wilson's Disease, also known as hepatolenticular degeneration, is an autosomal recessive disorder of copper metabolism producing neurological and hepatic dysfunction. Prevalence is approximately 30 per million. A gene defect has been localized to the long arm of chromosome 13. Pathogenesis involves decreased copper binding to the transport protein ceruloplasmin, resulting in excessive circulating unbound copper, eventually depositing in a variety of tissues, including the brain, liver, kidney and cornea. Although the average age of onset is 11 years for patients presenting with hepatic dysfunction and 19 years for those with neurological manifestations, the disease can remain clinically covert until the sixth decade.

Ocular and hepatic abnormalities are the most prominent non-neurological complications of the disease. Kayser–Fleischer rings, pathognomonic for Wilson's disease, are bilateral brown corneal rings resulting from copper deposition present in virtually all patients with neurological involvement. Hepatic involvement can take the form of an asymptomatic rise in liver enzymes, hepatitis or jaundice; if chronic, cirrhosis can ensue with portal hypertension-related complications.

Neurolopsychiatric manifestations reflect disproportionate involvement of the caudate, putamen, cerebellum and cortex. Signs can include facial grimacing, dysarthria, dysphagia, hypokinesia, abnormal posture, rigidity, resting and/or postural tremor, choreiform movements and ataxia. There is a tendency for dystonia or parkinsonism with hyper-reflexia and extensor plantar responses to predominate with disease onset before age 20 years; older patients tend to exhibit tremor, chorea and/or ballismus. A wing-beating tremor is a frequent late manifestation.

The cognitive impairment of Wilson's disease is consistent with a subcortical dementia profile marked by generalized thought process slowing, concentration impairment, and memory deficits in the absence of aphasia, apraxia or agnosia (Lang *et al.*, 1990; Lang, 1989). Research investigating whether visuomotor slowing in Wilson's disease is more a product of motor involvement than cognitive deficiency has been performed using specially designed neuropsychological batteries controlling for manual dexterity effects: tests confirm motor defect-independent slowing. Psychomotor impairment in Wilson's disease may result from impaired corticobasal ganglionic interaction. Associated psychiatric signs include affective and personality changes.

Diagnostic testing reveals decreased serum copper, low ceruloplasmin levels, elevated 24-hour urinary copper excretion, and abnormal liver function tests. Neuroimaging often demonstrates bilateral basal ganglia atrophy with compensatory ventricular dilatation. Inconsistently present but relatively unique characteristics visualizable on structural neuroimaging are putaminal cystic degeneration and cavitary necrosis. Liver biopsy reveals marked excess copper as well as cirrhosis. The principal differential diagnosis based on clinical symptomatology is Huntington's disease.

Management includes copper chelation via penicillamine and restriction of dietary copper. Treatment response can take months. Treatment is lifelong. Most patients treated early can expect a complete or nearly complete recovery.

Endocrine Disorders

Multiple endocrinological disturbances can potentially contribute to cognitive dysfunction. We focus on two prototypic endocrinopathies known to be able to cause reversible dementias.

Hypothyroidism

In addition to being a cause of an acute confusional state, hypothyroidism (myxoedema) can cause a reversible global dementia characterized by generalized slowing and nonspecific memory impairment. Psychiatric signs include depression sometimes complicated by psychotic features including paranoia and hallucinations. Associated neurological manifestations can be helpful in suggesting hypothyroidism as an underlying cause of dementia. Symptoms can include headache, tinnitus, paraesthesias and generalized weakness. Signs include delayed relaxation of deep tendon reflexes, hearing impairment, and typical stigmata of hypothyroidism (e.g. dry skin, pretibial myxoedema). Thyroid function tests usually confirm diagnosis. Cognitive dysfunction usually reverses significantly with thyroid hormone replacement.

Hypocortisolism

Patients with severe or chronic adrenal insufficiency can present with mild to moderate cognitive impairment (5–20%), depression (20–40%) and/or psychosis (20–40%). At least partial reversibility of hypocortisolism-related dementia via exogenous steroid repletion is the rule.

Nutritional Disorders

Vitamin B12 Deficiency

Vitamin B12 (cyanocobalamin) deficiency can produce many neurological complications, including cognitive dysfunction ranging from a mild confusional state to severe dementia and/or psychosis (megaloblastic madness).

Although presenting symptoms are most commonly due to anaemia, neurological abnormalities can precede development of macrocytic anaemia. Potential signs include peripheral neuropathy, subacute combined degeneration of the spinal cord, nutritional amblyopia, distal paraesthesias, gait ataxia, bandlike tightness around the trunk or limbs, and Lhermitte's sign (electric shock-like sensation along spine precipitated by rapid neck flexion). Associated leukopoenia or thrombocytopoenia can signal the disease presence via infection and or bleeding, respectively.

Like the acute confusional state associated with vitamin B12 deficiency, the cognitive impairment of B12 deficiency can occur with or without haematological manifestations. Vitamin B12-related cognitive impairment consists of global dysfunction with generalized slowing, impaired concentration and memory deficits (Larner *et al.*, 1999). Psychiatric disturbances are often prominent, including depression, mania and psychosis with paranoia and hallucinations (Zucker *et al.*, 1981).

Diagnostic clues include glossitis, yellow skin discoloration and/or cutaneous hyperpigmentation. Spinal cord involvement is manifested by impaired vibratory and joint position sense, sensory ataxia, spastic paraparesis, extensor plantar responses, decreased deep tendon reflexes (especially in the legs), and urinary retention. Laboratory investigations reveal low vitamin B12 levels (important to check since folate deficiency can cause the same set of haematological abnormalities), macrocytic anaemia, leukopoenia with hypersegmented neutrophils, and thrombocytopoenia with giant platelets. Schilling's test helps identify the origin of vitamin B12 deficiency (the most frequent cause is pernicious anaemia). Serum methylmalonic acid and homocysteine can also be useful measures. They are elevated in both absolute vitamin B12 deficiency and in relative deficiency (when tissue vitamin B12 stores are low but the serum vitamin B12 level is still normal). Some patients, particularly the elderly, with normal vitamin B12 levels but elevated serum methylmalonic acid can manifest neuropsychiatric abnormalities, including cognitive impairment. Treatment of patients with such covert vitamin B12 deficiency can often produce at least partial improvement.

Wernicke Encephalopathy, Korsakoff's Syndrome, and Alcohol-related Cognitive Impairment

Wernicke encephalopathy is produced by thiamine (vitamin B1) deficiency, a condition associated most commonly with alcoholism-related nutritional deficiency, but also a component of many other syndromes involving nutritional compromise (e.g. hyperemesis gravidarum, cancer).

Pathologically, Wernicke encephalopathy is characterized by neuronal loss, demyelination and gliosis in periventricular grey matter regions. Regions most commonly involved include the medial thalamus, mammillary bodies, periaqueductal grey matter and cerebellar vermis, as well as oculomotor, abducens and vestibular nuclei. MRI can demonstrate corresponding regional anormalities (Rourke and Loberg 1996).

Onset of Wernicke encephalopathy is typically abrupt but can be insidious. The classic syndrome comprises the triad of ophthalmoplegia, ataxia and encephalopathy. However, in one post-mortem study, the complete triad was present in only-one-third of patients with Wernicke-related lesions. The most common ocular abnormalities are nystagmus, VI nerve palsy, III nerve palsy, horizontal gaze palsy, and/or vertical gaze palsy. Ataxia is typically cerebellar, primarily affecting gait; this can be aggravated by peripheral neuropathy-related sensory ataxia. Most patients have peripheral neuropathy (often a multifactorial amalgam of Wernicke encephalopathy- and alcohol-related) with absent ankle jerks. Hypothermia and hypotension can occur, presumably secondary to hypothalamic involvement. Pupillary abnormalities, including mild anisocoria, and/or sluggish light reactivity are seen occasionally. Cognitive examination reveals global confusion consistent with a subacute delirium (Halliday *et al.*, 1994). Untreated, Wernicke encephalopathy can progress to stupor and coma.

Treatment requires prompt thiamine administration. Parenteral thiamine is continued for several days to ensure repletion of tissue stores. Ocular abnormalities usually begin to improve within days, while ataxia and confusion improve within weeks. Opthalmoplegia, vertical nystagmus and acute confusion are generally reversible, usually within 1 month. Horizontal nystagmus and ataxia, however, resolve completely in only about 40% of cases.

The major long-term complication of Wernicke encephalopathy is a residual alcohol amnestic disorder commonly known as Korsakoff's syndrome. Korsakoff's syndrome is primarily a memory disorder of moderate to severe anterograde greater capacity, with marked increased sensitivity to interference (Butters 1988). A temporally graded retrograde amnesia is less prominent. Other cognitive deficits (e.g. visuoperceptual, perceptual-motor) are variably present and likely represent other comorbid long-term, alcohol-related impairments. Disorientation when present is usually secondary to recent memory impairment. Confabulation and impaired insight are common (Rourke and Loberg, 1996). Executive deficits suggesting frontal lobe dysfunction sometimes occur. Attention and concentration are usually normal, and language is usually unaffected (Salmon and Butters, 1987). Consequently, a large disparity between general IQ and memory performance (e.g. MQ from the WMS) is common.

Although Korsakoff's syndrome is the expected outcome of Wernicke encephalopathy, yielding the diagnostic rubric Wernicke–Korsakoff syndrome (WKS), either component can occur without the other (Blansjaar and van Dijk, 1992; Torvik *et al.*, 1982).

Neuropsychological deficits resulting more directly from alcohol use (separate from those comprising WKS secondary to thiamine deficiency) represent a much more heterogeneous set of impairments (Rourke and Loberg, 1996). Deficits involving attention, visuospatial, perceptual-motor, memory and executive function have all been described. Pathogenic mechanisms remain to be elucidated, along with better characterization of cognitive impairment constellations (Rourke and Loberg, 1996).

Inflammatory Disorders

A variety of inflammatory disorders can affect cognitive function. Primary (e.g. primary CNS angiitis) and systemic vasculitides (e.g. Churg–Strauss vasculitis), granulomatous disorders (e.g. neurosarcoidosis), and other autoimmune syndromes (e.g. Hashimoto's encephalitis) can contribute to cognitive impairment. These are especially important to identify since associated dementias can be at least partially reversible via anti-inflammatory or immunosuppression therapy.

Secondary Dementias: Usually Irreversible

Vascular Dementias

Strokes, small and large, ischaemic and haemorrhagic, cortical and subcortical, together represent the second most common cause of dementia, accounting for up to 10% of late-life cognitive impairment (Leys *et al.*, 1998) Up to 25% of stroke patients are demented after stroke (Leys *et al.*, 1998; van Kooten and Koudstaal, 1998).

Diagnostic criteria for vascular dementia are more controversial than those for other dementia aetiological subtypes and still require validation by systematic clinicopathological study (Lopez *et al.*, 1999). Vascular dementia criteria currently in use include the State of California AD Diagnostic and Treatment Centers Criteria (the California criteria), the National Institute of Neurologic Disorders and Stroke, the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria, the Hachinski Ischaemic Score (HIS), and those found in DSM-IV (Nyenhuis and Gorelick, 1998; Scheltens and Hijdra, 1998).

Recent neuropathological analyses offer perspective on the difficulty in correctly diagnosing cerebrovascular pathology in dementia. Rather than considering vascular dementia as present or absent, these studies distinguish between any vascular lesions versus pure vascular pathology (where vascular pathology was both sufficient to account for cognitive symptoms and unaccompanied by other pathology). In one study, although at least some vascular pathology existed in up to 41% of dementia cases coming to autopsy, pure vascular pathology accounted for dementia in only approximately 10% (Nolan *et al.*, 1998).

A diagnosis of vascular dementia is supported by sudden onset of cognitive impairment in two or more domains, step-wise deterioration, asymmetries on neurological examination, neuroimaging evidence of stroke, and presence of cerebrovascular risk factors. Vascular dementia progresses characteristically in a step-wise fashion, with new episodes of minor or major deterioration reflecting additional vascular events.

Vascular dementia can take several forms, as outlined in Table XV-8.22

Table XV-8.22 Categories of vascular dementia

Category	Clinical presentation
Lacunar infarctions	Progressive dementia, focal deficits, apathy, frontal lobe syndrome, possibly absent history of clinically recognized strokes
Single strategic infarctions	Sudden-onset aphasia, agnosia, anterograde amnesia, frontal lobe syndrome
Multiple infarctions	Step-wise appearance of cognitive and motor deficits
Mixed Alzheimer's disease–vascular dementia	Progressive dementia with remote or concurrent history of stroke
White mater infarctions, Binswanger's disease	Dementia, apathy, agitation, bilateral corticospinal/bulbar signs

Marked differences in the clinical presentation of these variants make it inappropriate to regard vascular dementia as a unitary diagnostic entity (Nyenhuis and Gorelick, 1998). Rather, vascular dementia represents a group of dementia aetiologies sharing the general common pathogenesis of brain injury secondary to derangement of cerebrovascular functional and/or structure. A pleomorphic nature thereby arises from variations in type and location of cerebrovascular compromise (Looi and Sachdev, 1999).

Multiple small infarctions of subcortical white-matter pathways, disconnecting circuitry among cognitively important cortical centers, causes a leukoencephalopathy previously called Binswanger's disease. The resulting clinical situation has been analogized to a highway damaged by potholes: destinations remain intact but travel between destinations is slowed.

In the majority of patients assessed for dementia, an exclusive and highly certain diagnosis of vascular dementia can rarely be made. Instead, clinicians are often confronted with patients having some vascular pathology features together with a history that can otherwise seem more compatible with a diagnosis of AD. Such clinical and pathological admixtures are a common presentation for cerebrovascular disease (Nyenhuis and Gorelick, 1998).

SPECT frequently reveals a scattered/patchy pattern, reflecting the variable localization of vascular-based insults.

Treatment of vascular dementia consists primarily of identifying and correcting predisposing cerebrovascular risk factors. The overall rate of progression can be slow compared with other causes of dementia, but some patients suffer an accelerated decline.

Cerebral Autosomal Dominant Arteriopathy Subacute Infarcts and Leukoencephalopathy

Cerebral autosomal dominant arteriopathy subacute infarcts and leukoencephalopathy (CADASIL) is an increasingly recognized clinicopathological syndrome. CADASIL causes subcortical lacunar infarction and dementia in over 80% of cases (Salloway and Hong, 1998). CADASIL can appear very similar to hypertensive microvascular disease (Binswanger's disease). Signs and symptoms of CADASIL tend to appear between the ages of 40 and 60 years, but changes are apparent on MRI much earlier. Approximately 30% of patients have migraines with aura; mood disorders are common. Neuropathological findings include smooth muscle hypertrophy in small arteries, demyelination, gliosis in subcortical white matter and basal ganglia, and corpus callosum involvement. Given the autosomal dominant hereditary basis of this disease, family history is key.

Neoplastic Disease

Dementia secondary to cancer was briefly mentioned above under potentially reversible secondary dementias. Irreversible cancer-related dementias are unfortunately much more common. Direct mechanisms of cognitive disruption include primary and metastatic brain tumours and leptomeningeal disease. Indirect/systemically mediated mechanisms include neoplasm-related metabolic, haematological and/or endocrinological derangements causing secondary CNS dysfunction. Although oncological disease affecting the CNS is usually heralded by focal neurological signs, a nonspecific dementia can sometimes be the sole presenting manifestation.

Paraneoplastic limbic encephalitis (PLE) is a rare disorder characterized by variably progressive cognitive and affective changes; less often, focal motor and/or sensory signs can occur. PLE is both an inflammatory and a degenerative disorder of certain CNS grey matter regions occurring as a rare complication of a non-CNS cancer (Gultekin *et al.*, 2000). Symptoms typically precede diagnosis of the underlying cancer (Posner, 1995).

Symptoms usually develop over the course of several weeks. The disorder is characterized by profound impairment of recent memory corresponding to an inability to learn new information. Attention and registration are unaffected, and remote memory is less impaired. Confabulation occurs in some cases. Affective symptoms, especially anxiety and/or depression, are common early features. Hallucinations are variable. The primarily amnestic syndrome sometimes progresses to a global dementia. Depending on the extent to which grey matter regions outside the limbic system are involved, cerebellar, pyramidal, bulbar and peripheral nerve disturbances can coexist with cognitive/affective/behavioural symptomatology (Gultekin *et al.*, 2000; Posner, 1995).

MRI can reveal abnormal signal intensity in the medial temporal lobes. Diffuse slowing or bitemporal slow waves and spikes are sometimes seen on EEG. CSF often demonstrates a modest mononuclear pleocytosis and mildly elevated protein. Serological tests for detection of paraneoplastic antineuronal antibodies have become increasingly available. Excluding other potentially treatable disorders is of primary importance before settling on a diagnosis of PLE.

PLE can be static, progressive or even remitting. No specific treatment is available except management of the underlying malignancy.

Radiation

The effects of radiation therapy on the brain can be extremely variable in type and time of onset; late effects, in the order of 10 years after treatment, are seen frequently. Clinical expression depends in part on radiation type (e.g. focal bean or whole brain) and amount. MRI is diagnostic.

Extensive white-matter disease is the most common type of complication, with neuropsychological deficits predictably consistent with leukoencephalopathy.

Infectious Dementias

Chronic CNS infectious processes can sometimes cause dementia as a primary manifestation; we briefly describe some major types.

Viral Encephalitides

Viral encephalitis is caused by viral infection of the brain parenchyma, producing neuronal and glial degeneration, inflammatory infiltration, oedema and tissue necrosis. A specific pathogen remains unidentified in up to 75% of presumed viral CNS infection cases. Indeed, the confusing term 'aseptic meningitis' is a testament to the historic difficulty of isolating definitively an aetiological agent in presumed viral meningitides.

Amplification of viral nucleic acids from CSF, as done by the polymerase chain reaction (PCR) for example, has improved diagnosis considerably of several acute, subacute and chronic viral CNS infections. In autoimmune deficiency syndrome (AIDS), PCR can help differentiate lesions due to human immunodeficiency virus (HIV) itself or to opportunistic infections such as progressive multifocal leukoencephalopathy (PML) caused by JC virus or cytomegalovirus (CMV)-related complications.

Herpes Encephalitis Herpes simplex virus (HSV) causes the most common form of sporadic potentially fatal encephalitis in children older than 6 months and adults worldwide. CNS HSV infection is divided into two groups: neonatal HSV infection, caused by HSV-2, and herpes simplex encephalitis (HSE), which occurs in children outside the neonatal period and adults and is caused by HSV-1. HSE in older children and adults is caused by both primary and recurrent HSV infection. Approximately two-thirds of cases are caused by virus reactivation (Whitley, 1997).

HSE is characterized pathologically by intense meningitis and destructive changes in the brain parenchyma. Inflammation with

accompanying necrosis and haemorrhage occur, often maximally involving the frontal and temporal lobes. Temporal brain regions are most involved quantitatively in autopsy studies (Whitley, 1997).

HSE initially presents as a focal encephalitis. Neurolopsychiatric signs found early in the disease course (e.g. aphasia, confusion, behavioural changes) can progress to a fulminant illness characterized by recurrent focal or generalized seizures, coma and death. Hemiparesis is evident at the time of presentation in approximately one-third of cases. Although variable, the most common residual neuropsychological sequela is memory impairment. Owing to HSV's anterior temporal tropism, anterograde amnesia, frequently severe, is the usual manifestation (Whitley, 1997).

Routine blood tests reveal peripheral leukocytosis. Lumbar puncture demonstrates elevated pressure. CSF studies show normal or mildly decreased glucose, mildly elevated protein and pleocytosis. Red blood cells and xanthochromia are frequently seen. Virus is rarely recovered from the CSF, and detection of HSV antibodies in the CSF is not sensitive or specific enough to make a treatment decision. PCR has become the mainstay of non-invasive diagnosis, while isolation of HSV from brain biopsy specimens remains the diagnostic gold standard.

CT can demonstrate temporal hypodensity and contrast enhancement but is often normal during the first week of disease. MRI can reveal temporal lobe pathology, including loss of grey–white differentiation, oedema and haemorrhagic components during the first week of disease. HSE produces dramatic EEG changes (e.g. paroxysmal lateralizing epileptiform discharges (PLEDs)) that, while not pathognomonic, are highly suggestive (80% of biopsy-proven cases).

Treatment with antiviral therapy (e.g. acyclovir) can be life-saving.

Human Immunodeficiency Virus Up to 70% of HIV patients develop neurological complications, including cognitive disturbances. Although neuropsychiatric disease typically occurs within the context of other AIDS-defining illnesses, neuropsychiatric signs can herald AIDS in up to 20% of HIV patients (Berger and Simpson, 1997). The spectrum of neuropsychiatric disorders complicating HIV-1 infection is extremely diverse. CNS complications can be divided into those due to HIV infection itself and those due to HIV-related secondary aetiologies, e.g. opportunistic infections.

HIV-associated dementia has been reported in up to 15% of HIV-infected adults. Although pathogenesis remains unclear, there is a correlation between CSF HIV load and neuropsychological function. Magnetic resonance spectroscopy has demonstrated abnormalities in cognitively normal HIV patients. AIDS dementia complex (ADC) is an increasingly frequent infectious cause of dementia. Estimated to occur in at least 25% of AIDS patients, ADC produces psychomotor slowing, attention decrement and personality changes (Berger and Simpson, 1997).

Scales demonstrated to have enhanced specificity and sensitivity for detecting cognitive impairment in HIV/AIDS patients include the HIV dementia scale (HDS) and executive interview (EXIT) (Beghuis *et al.*, 1999).

Creutzfeldt–Jakob Disease CJD is one of the slow virus infections, also known as prion diseases after the presumed infectious agent, and transmissible spongiform encephalopathies (TSE) after the histopathological changes associated with these infections.

Prion diseases are categorized into three groups: sporadic, inherited and acquired. Most slow virus infections — approximately 85% — are sporadic. CJD is the most common human TSE. A new variant of sporadic CJD likely caused by the same agent as bovine spongiform encephalopathy (BSE) was first described in 1996. CJD occurs primarily as a sporadic illness with an annual incidence of about one per million in the general population (Poser *et al.*, 1999).

CJD produces a rapidly developing progressive dementia, which can be clinically variable. Initial complaints in approximately one-third of patients are constitutional, consisting of fatigue, disordered sleep or decreased appetite. Another third have non-focal neuropsychiatric features at onset, e.g. confusion or atypical behaviours. The remainder can present with prominent signs of corticospinal and extrapyramidal dysfunction, including gait disturbance and rigidity, as well as focal neurological features, including ataxia, visual loss, aphasia, hemiparesis or focal amyotrophy, sometimes leading to an erroneous initial clinical impression of stroke or motor neuron disease. Diagnosis becomes clearer with the onset of cognitive decline and emergence of and startle myoclonus to abrupt sound or touch. Pyramidal, extrapyramidal and cerebellar signs eventually occur in the majority of patients. Seizures, especially myoclonic, occur in up to 20% of patients. In all reports of new variant CJD, psychiatric symptoms (e.g. depression, anxiety, personality changes) were followed within a median of 6 months by neurological symptoms such as dysaesthesias and paraesthesias. Terminal stages of CJD are characterized by progressive loss of neuropsychological function leading to akinetic mutism (Asher, 1997).

There can be characteristic EEG changes. Early in the disease course, EEG may show nonspecific slowing. Later, periodic, biphasic or triphasic synchronous sharp-wave complexes are superimposed on a slow background rhythm. Periodic triphasic complexes have shown a high sensitivity and specificity for CJD detection (Asher, 1997).

Until recently, CJD diagnosis relied on clinical symptoms, characteristic EEG pattern, and brain biopsy assessment. Introduction of 14-3-3 CSF protein Western blot immunodetection has greatly improved the diagnostic accuracy (sensitivity up to 99% and specificity up to 96%) (Collins *et al.*, 2000). MRI often demonstrates a characteristic 'cortical ribboning' on diffusion-weighted imaging (Poser *et al.*, 1999). Definitive diagnosis still requires identification of abnormal prion protein and/or related neuropathology in brain tissue. Brain pathology consists of a vacuolar (spongiform) degeneration.

CJD is uniformly fatal, with progression to death within 12–18 months of symptom onset. Management is supportive, and there is no cure.

COGNITIVE DISORDERS, NEUROPSYCHOLOGICAL FUNCTION, AND NEUROIMAGING

New neuroimaging modalities provide extraordinary capacity for *in vivo* exploration of the correlation of neuropsychological function and dysfunction with corresponding neurocircuitry. Data derived from such investigations are appropriately informing our knowledge of the neuropsychology of cognitive disorders and relevant functional neuroanatomy.

Structural Neuroimaging

Structural neuroimaging can be an essential aid in determining the structural neuroanatomy underlying cognitive impairment.

CT generally provides adequate data for evaluation of atrophy, ventricular system integrity, most haemorrhages (epidural, subdural, subarachnoid, intraparenchymal), and mass effects (of tumours, abscesses, etc.). Consequently, CT is usually adequate to exclude most structural causes of cognitive dysfunction.

Although CT usually yields adequate screening data, MRI's capacity for multiplanar views and multiple imaging sequence modalities provides vastly more information about potential vascular, neoplastic, inflammatory and infectious aetiologies of cognitive impairment. Sagittal MRI is especially useful for demonstrating selective lobar atrophies (e.g. the focal atrophy attendant

to frontotemporal dementias, e.g. 'knife-like' gyri of Pick's disease). Only gadolinium-enhanced magnetic resonance images are truly adequate for evaluating certain serious and potentially treatable conditions (e.g. leptomeningeal disease).

Presymptomatic hippocampal atrophy on MRI has been demonstrated in asymptomatic individuals at risk of autosomal dominant AD (De Leon *et al.*, 1997). In clinically diagnosed AD of moderate severity, MRI-based volumetric measurements show a reduction of up to 40% in the size of the hippocampus, amygdala, thalamus and anterior temporal lobe (Pantel *et al.*, 1997). Neuropsychological assessments of recent memory are highly correlated with visually rated hippocampal atrophy on MRI, which in turn is associated strongly with neurofibrillary pathology in AD (Scheltens, 1999).

Despite the promising utility of structural neuroimaging in the assessment of cognitive disorders, problems with interpretation are common: (1) some patients can have completely normal structural scans; (2) sulcal widening and ventricular dilatation are found in many elderly cognitively intact individuals; (3) some non-demented patients can have multiple lesions without cognitive impact; (4) the degree of atrophy is not a reliable predictor of dementia severity; (5) hippocampal atrophy is nonspecific; and (6) common T2 MRI sequence abnormalities are extremely nonspecific and bear an unclear relationship to the type and severity of cognitive impairment.

Functional Neuroimaging

Single-Photon Emission Computed Tomography

Perfusion abnormalities in certain cognitive disorders frequently reflect pathological changes underlying cognitive demise. SPECT has been employed widely in the investigation of dementia. Some studies combining assessment of hippocampal atrophy on structural neuroimaging with cerebral blood flow studies on SPECT have yielded high sensitivity and specificity rates in discriminating AD patients from normal controls. Although encouraging, these figures are not consistently better than those obtained by diagnosis with established clinical criteria. The added value of SPECT is greatest for a positive test among patients with mild dementia in whom there is doubt regarding diagnosis (Scheltens, 1999).

Positron Emission Tomography

Like SPECT, PET is able to demonstrate functional abnormalities correlating with structural changes. However, PET has the added potential value of quantifying neurotransmitter (e.g. cholinergic) and other neurochemical changes. For example, a direct comparison of PET and SPECT in their ability to differentiate AD from vascular dementia revealed higher diagnostic accuracy for PET regardless of dementia severity (Scheltens, 1999).

Functional Magnetic Resonance Imaging

fMRI offers the advantage of functional neuroimaging without the need for intravenous administration of radioactive isotopes. More powerful machines (providing finer structural resolution) and advanced methodologies involving neuropsychological probes (providing event-related temporal resolution) are being employed to better characterize the neuropsychology of cognitive disorders.

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XV-9

Functional Neuroanatomy

P. Cras

NEUROANATOMY OF COGNITION

Introduction

Amnesia is the lack or failure of memory, which can be of organic or psychic origin, and can be either transient or permanent. Diseases that cause amnestic syndromes are rarely focal and very often simultaneously affect several regions of the brain. Dementia is defined as a syndrome characterized by memory loss and the presence of at least one of agnosia, apraxia and aphasia, resulting in a decline from a previously higher level of intellectual function of sufficient severity to interfere with social or occupational performance or both. The intellectual deterioration should involve more than a single cognitive function, and while memory is typically involved in an early stage, attention, language, visuospatial skills, perception and problem-solving skills are also affected.

Neurodegenerative disorders, such as Alzheimer's disease, can serve as models for the dissection of the neuroanatomy of memory. In other instances, neurotrauma or surgical interventions will cause localized defects, resulting in amnesia or other cognitive disturbances. The unravelling of the anatomical basis of memory should involve the study of the brain regions involved, their interconnections, and their functions in terms of neurotransmitter use.

In what follows, the anatomical structures involved in memory processing will be introduced, and several neurodegenerative disorders that perturb memory and other cognitive functions will be described.

Anatomical Substrate of Memory

Limbic System

The limbic system is the anatomical substrate of behaviours, including social behaviours that assure the survival of the individual and the species. Within the limbic system, there are more or less specialized functional systems that are involved with particular aspects of behaviour.

Historically, the limbic system has taken an important place in the neuroanatomy of memory (Shaw and Alvord, 1997). The concept of the 'limbic lobe' was first put forward by Paul Broca in 1878 to designate the structures on the edge (the 'limbus') of the neocortex (Broca, 1878). Broca defined the limbic lobe as the hippocampal and the cingulate gyri. In addition to the limbic cortex, a number of subcortical structures, including the hippocampus, the amygdala and the septal nuclei, form part of the limbic system. Depending on the authors, other structures, such as the posterior frontal cortex, raphe nuclei, ventral tegmental nucleus and nucleus basalis of Meynert, are also considered to be part of the limbic system (Braak and Braak, 1992). Since the work of Papez (1937), numerous refinements have been made to the limbic circuit model, which is now referred to by some authors as the 'limbic forebrain model'. The original 'limbic circuit of Papez' involved the mammillary bodies, fasciculus mamillothalamicus, cingulate gyrus, anterior thalamic nucleus, amygdala and hippocampus. Kornhuber (1988) added the thalamic mediodorsal nucleus to the Papez circuit. Markowitsch (1985) proposed a second limbic circuit, which could act complementarily or in parallel in information processing: the basolateral limbic circuit.

The hippocampal system and related structures are involved intimately in sensory processing and memory. It has been established that episodic memory is based on the transfer of information through so-called bottleneck structures that form part of this limbic system (Pritzel and Markowitsch, 1983). Amnesia results from disconnection of this intricate brain circuitry. The paramount role of the amygdala is illustrated by numerous case reports and by the study of amygdaloid lesions in neurodegenerative disorders, such as Alzheimer's disease (Sarter and Markowitsch, 1985a; Sarter and Markowitsch, 1985b).

Prefrontal Cortex

The prefrontal cortex is divided into the dorsolateral, medial and orbital regions. All three regions receive input from the mediodorsal thalamic nucleus, which relays information from the temporal cortex, the pyriform cortex and the amygdala. The traditional view is that the human prefrontal cortex is involved in emotion and performance more than concrete actions (Fuster, 1989). Any disturbances in perception, memory and thinking are considered secondary to emotional deficits. A major issue in the description of these patients was that some appeared joyful and unconcerned, while others appeared depressed (Mesulam, 1998). This led to the distinction of orbitofrontal regions, damage of which would result in disinhibited behaviour and emotional flatness, while injury of the dorsolateral regions resulted in an inhibited, depressed and even compulsive personality (Mesulam, 1998).

Seminal work revealing the role of the prefrontal cortex in memory and behaviour is found in the case description of Phineas Gage, who suffered massive damage to the prefrontal cortex due to a blow from a crowbar (Harlow, 1848), and in the description of numerous soldiers suffering from shrapnel wounds (Harlow, 1848). Markowitsch and Kessler (2000) described a young woman with massive impairment in executive functions and partial preservation of other cognitive functions, apparently caused by severe degeneration of the prefrontal cortex. This patient showed not only the typical cognitive and behavioural alterations due to combined orbitofrontal and dorsal frontal lesions, but also major disturbances of executive functions. She was unable to perform even the simplest task that demanded continued attention, and she was impressively deficient in visuoconstructional tasks.

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Five frontal-subcortical circuits have been identified and named according to their function or cortical site of origin. The motor circuit, originating in the supplementary motor area, and the oculomotor circuit, originating in the frontal eye fields, are dedicated to motor function (Markowitsch, 1984). The dorsolateral prefrontal, lateral orbital, and anterior cingulate circuits subserve executive cognitive functions and aspects of personality and motivation, respectively (Mesulam, 1999). Each of the five circuits has the same member structures, including the frontal lobe, neostriatum, globus pallidus, substantia nigra and thalamus (Figure XV-9.1).

Within each of the circuits, there are two pathways: a direct pathway links the striatum with the globus pallidus interna/substantia nigra complex, and an indirect pathway projects from the striatum to the globus pallidus externa, and then to the subthalamic nucleus and back to the globus pallidus interna/substantia nigra (Alexander and Crutcher, 1990). Both direct and indirect circuits project from the globus pallidus interna/substantia nigra to the thalamus. Each circuit uses the same transmitters at each anatomic site. The circuits remain discrete throughout their courses. The relative anatomic positions of each circuit are preserved in each circuit structure; thus, the dorsolateral prefrontal cortex projects to the dorsolateral region of the caudate nucleus, the orbitofrontal region projects to the ventral caudate area, and the anterior cingulate cortex connects to the medial striatal-nucleus accumbens region. Similar anatomic arrangements are maintained in the globus pallidus and thalamus. There is progressive spatial constraint of the circuits at subcortical levels, with a reduction in the volume occupied by the circuits in subcortical compared with cortical regions.

The dorsolateral prefrontal-subcortical circuit originates in Brodmann's areas 9 and 10 on the lateral surface of the anterior frontal lobe. Neurons in these regions project to the dorsolateral head of the caudate nucleus (Selemon and Goldman-Rakic, 1985). Fibres from this region of the caudate project to the lateral aspect of the mediodorsal globus pallidus interna and rostrolateral substantia nigra pars reticulata via the direct pathway. The indirect pathway sends fibres to the dorsal globus pallidus externa, which in turn projects to the lateral subthalamic nucleus; fibres from the lateral subthalamic nucleus then terminate in the globus pallidus interna/substantia nigra pars reticulata (Goldman-Rakic and Selemon, 1990). Output from the basal ganglia projects to parvocellular portions of the ventral anterior and mediodorsal thalamus. The mediodorsal thalamus closes the circuit by projecting back to the circuit's origin in areas 9 and 10 of the dorsolateral frontal lobe (Goldman-Rakic and Selemon, 1990).

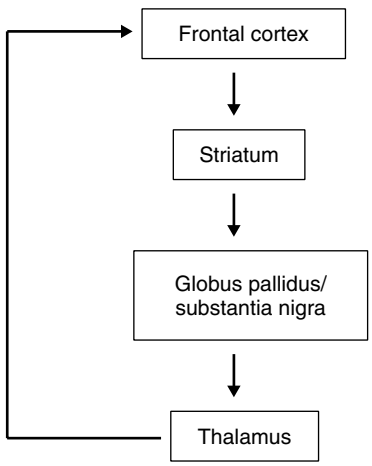


Figure XV-9.1 Frontal-subcortical circuits

The inferolateral prefrontal cortex originates in Brodmann's areas 10 and 11, and sends projections to the ventromedial caudate. This portion of the caudate projects directly to the most medial portion of the mediodorsal globus pallidus interna and to the rostromedial substantia nigra pars reticulata. The ventromedial caudate also sends an indirect loop through the dorsal globus pallidus externa to the lateral subthalamic nucleus, which then projects to the globus pallidus interna and substantia nigra pars reticulata. Neurites are sent from the globus pallidus and substantia nigra to the medial section of the magnocellular division of the ventral anterior thalamus as well as an inferomedial sector of the magnocellular division of the mediodorsal thalamus (Selemon and Goldman-Rakic, 1985). The circuit then closes with projections from this thalamic region to the lateral orbitofrontal cortex (Goldman-Rakic and Selemon, 1990).

Neurons of the anterior cingulate serve as the origin of the anterior cingulate-subcortical circuit. From Brodmann's area 24, they provide input to the ventral striatum (Goldman-Rakic and Selemon, 1990), which includes the ventromedial caudate, ventral putamen, nucleus accumbens and olfactory tubercle. This area is termed the limbic striatum. Projections from the ventral striatum innervate the rostromedial globus pallidus interna and ventral pallidum (the region of the globus pallidus inferior to the anterior commissure), as well as the rostrodorsal substantia nigra. There may also be a less well defined indirect loop projecting from the ventral striatum to the rostral pole of the globus pallidus externa (Haber *et al.*, 1990). The external pallidum in turn connects to the medial subthalamic nucleus, which returns projections to the ventral pallidum. The ventral pallidum provides limited input to the magnocellular mediodorsal thalamus. The anterior cingulate circuit is closed with projections from the dorsal portion of the magnocellular mediodorsal thalamus to the anterior cingulate (Goldman-Rakic and Selemon, 1990).

There are approximately three signature behavioural/cognitive syndromes that occur after damage to prefrontal cortex. Damage to the dorsolateral prefrontal circuit causes executive dysfunction. These patients have difficulty generating hypotheses and flexibly maintaining and shifting sets as required by changing task demands. Damage to the orbitofrontal circuit causes disinhibited, irritable and labile behaviour. This type of lesion also results in imitation and utilization behaviour. Unlike patients with dorsolateral damage, these patients may not have executive impairments. Damage to the medial frontal/anterior cingulate circuit causes akinesia, apathy and unconcern. Of course, patients may have damage to more than one of these frontal circuits, which can lead to a mixture of clinical deficits.

Hippocampus

The temporal lobe can be divided into a lateral part, involved in audition and speech, and a medial part, considered to be a part of the limbic system and involved in memory. The ventromedial temporal lobe consists of the parahippocampal gyrus, entorhinal cortex (ERC), hippocampal formation, uncus, amygdala and the cortex of the temporal pole. All sensory information passes through the unimodal and multimodal association areas, before converging on the amygdala and hippocampus (Figure XV-9.2).

Integrative neuroscience approaches have revealed that the dorsolateral prefrontal cortex, hippocampus and cingulate cortex are engaged in novelty detection and attention. The hippocampus occupies a central position in the limbic system. The hippocampal formation consists of the subiculum, the hippocampus proper and the dentate gyrus. The ERC is the gateway to the hippocampal formation and consists of a special six-layered cortex. The hippocampus is subdivided into different regions denominated by CA1, CA2, CA3 and CA4. The hippocampal gyrus consists of three layers, with the pyramidal cell being the most distinctive cell type. In addition to the

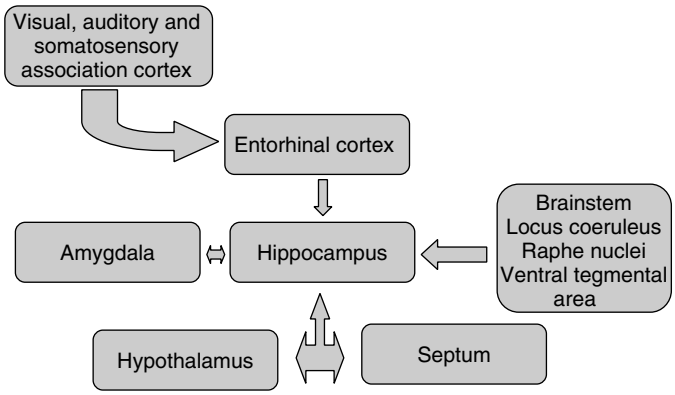


Figure XV-9.2 Connections of the human hippocampus

ERC, other inputs to the hippocampal formation are provided by the septal nuclei, hypothalamus and thalamus, as well as the brainstem.

Imaging studies have indicated that atrophy of the hippocampus correlates with impaired memory function and may be an early indicator of a subsequent cognitive decline. The hippocampus is associated with declarative memory, which is the memory of facts, experiences and information about events. While the hippocampus is involved in memory proper, the nearby amygdala is involved in emotion. The amygdala consists of a nuclear complex located inside the temporal lobe and consisting of basolateral nuclei, a central nucleus and corticomedial nuclei. The role of the amygdala is to associate a particular emotion to a current experience.

Neuropathological data concerning the progression of Alzheimer's disease indicate the hippocampus as an early focus of anatomical change. In addition, studies of subjects who are enrolled as being normal but subsequently decline have indicated that magnetic resonance imaging (MRI)-based hippocampal volumes can be predictive. Jack and colleagues have demonstrated the utility of volumetric measurements of the hippocampus as being useful in discriminating normal elderly subjects from a group of mild Alzheimer's disease patients (Jack *et al.*, 1999). Preliminary data on cross-sectional hippocampal volumes indicate that subjects with mild cognitive impairment have hippocampal atrophy beyond what we would expect for normal control subjects yet less than subjects with very mild Alzheimer's disease. Measures of the ERC have also been suggested as being useful at discriminating normal ageing from early cognitive impairment. While structural studies of the hippocampal formation are likely to be useful, functional imaging studies also show promise at assessing patients with very mild impairment.

Stimulus novelty facilitates memory encoding, suggesting that the hippocampus is likely to be involved in novelty processing (Izquierdo *et al.*, 2000). Animal research has linked the hippocampus with novelty or mismatch detection (Shaw and Alvord, 1997). The study of late evoked potentials in patients with posterior hippocampal region damage has shown normal parietal P3b activity but marked reductions of the frontocentral P3a response associated with novelty. A crucial question is whether the dorsolateral prefrontal cortex or the hippocampal region performs the initial novelty detection (Tulving and Markowitsch, 1998). Some studies indicate that a neocortical system controlled by the prefrontal cortex performs the initial deviancy/novelty detection (Eichenbaum, 1999). A prefrontal signal may then alert the hippocampal cortex to the fact that a potentially significant event requiring memory processing has occurred. Corticolimbic connections through the ERC or fornix-mammilothalamic-cortical pathways may also contribute to the novelty detection.

NEUROPATHOLOGY OF AMNESTIC DISORDERS

The Ageing Process and Mild Cognitive Impairment

Clinical Findings

With the advent of therapeutic agents for Alzheimer's disease, there has been increasing clinical emphasis on the early detection of mental decline in order that treatment strategies can be initiated as soon as possible. The emphasis on early detection in turn has led to attempts to characterize transitional states between normal ageing and dementia. Such states have also been viewed as part of the ageing process (e.g. age-associated memory impairment, minimal cognitive impairment) or alternatively as incipient or early-stage Alzheimer's disease. The term 'minimal cognitive impairment' (MCI) is intended to identify individuals with measurable, abnormal memory loss who lack decline in other cognitive abilities. There is general consensus that some aspects of cognition decline with age, whereas others may be preserved. In the absence of disease, however, it may be possible to compensate for the cognitive changes associated with age by using cues and other memory strategies, such that overall performance may be 'normal', albeit slower. However, some studies suggest that much of the cognitive decline previously attributed to age alone may instead reflect the effect of unrecognized very mild dementia. This point is supported by a recent population study of people aged 85 years, wherein decline in cognitive performance over 3 years was associated with individuals who had Alzheimer's disease at baseline or developed it during the study, whereas those individuals who remained free of Alzheimer's disease had little evidence of cognitive decline (Aevarsson and Skoog, 1996). Patients with MCI are at increased risk of developing Alzheimer's disease. Conversion rates to Alzheimer's disease vary between 10% and 15% per year, while in normal aged adults, this conversion rate lies between 1% and 2% (Morris *et al.*, 2001; Petersen *et al.*, 1997). The heterogeneity of this nosologic entity is well recognized, and its predictive value for Alzheimer's disease is limited (Shah *et al.*, 2000).

Neuropathology

The neuropathological substrate of patients with MCI is not known, as affected individuals do not normally die at this early level of cognitive dysfunctioning. A few case reports that have been published have been due to accidental deaths of patients who had previously been examined neuropsychologically. Autopsy studies have shown that even cognitively normal elderly individuals may sometimes meet the neuropathologic criteria for Alzheimer's disease. In a study of patients with a clinical dementia rating CDR (scale) of 0.5, corresponding to the level of dysfunction found in MCI, it was shown that most of the patients met the neuropathological diagnostic criteria of Alzheimer's disease (Morris *et al.*, 2001). All individuals had changes in the medial temporal cortex, and approximately half of them showed neocortical plaques and tangles consistent with Alzheimer's disease. Neuropathological data in early Alzheimer's disease have shown alterations in the ERC and hippocampus that may be involved in the disturbances of episodic memory found in the early stages (Braak and Braak, 1992). Currently, it is not clear what remains an acceptable number of Alzheimer's disease-type lesions in an elderly brain. As such, it appears that MCI patients span a spectrum of neuropathological findings, from those who are felt to be associated with ageing, such as neurofibrillary tangles and senile plaques in the medial temporal regions, to patients who would otherwise meet most neuropathological criteria for Alzheimer's disease. Inherent in this discussion is a characterization of the neuropathology of normal ageing.

Delirium

Clinical Findings

Delirium is characterized by a disturbance of consciousness with a change in cognition developing over a short period of time; it is often accompanied by the presence of visual and auditory hallucinations. Delirium is a common mental disorder in the elderly, with old age being a major risk factor; another major risk factor is dementia (Robertsson *et al.*, 1998). The presence of visual hallucinations, but not delusions or auditory hallucinations, was associated significantly with more systemic disorders and often multiple aetiologies causing the delirium. Psychotic symptoms are not uncommon in delirium, but specific psychotic symptoms may have different factors contributing to their development. In a recent study, the prevalence of psychotic symptoms in delirium was 42.7% ($n = 97$), with 27% of patients ($n = 61$) having visual hallucinations, 12.4% ($n = 28$) having auditory hallucinations, 2.7% ($n = 6$) having tactile hallucinations, and 25.6% ($n = 58$) having delusions (Webster and Holroyd, 2000). Visual hallucinations appear to be associated with a greater number of systemic disorders, but presumably other unknown factors are also associated with the development of psychotic symptoms in delirium (Webster and Holroyd, 2000).

Neuropathology

Delirium has been considered a syndrome of generalized dysfunction of higher cortical functions due to its breadth of symptoms. No particular neuropathological abnormalities have been associated with delirium. The prefrontal cortices, anterior and right thalamus, and right basilar mesial temporoparietal cortex may play a significant role in subserving delirium symptoms, and may be the final common pathway from a variety of aetiologies. This final common pathway may be responsible for certain core symptoms (disorientation, cognitive deficits, sleep–wake cycle disturbance, disorganized thinking, language abnormalities), while other symptoms (delusions, hallucinations, illusions, affective lability) may occur, depending on the aetiology. An imbalance in the cholinergic and dopaminergic neurotransmitter systems is most commonly implicated in causing delirium (Trzepacz, 1999).

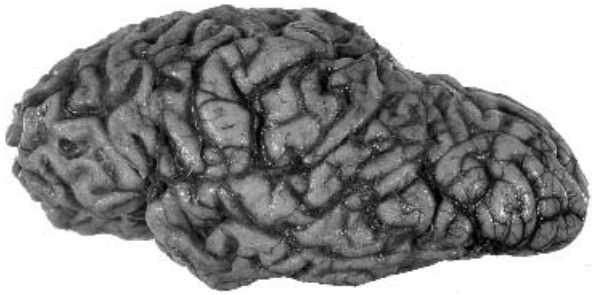
Alzheimer's Disease

Clinical Findings

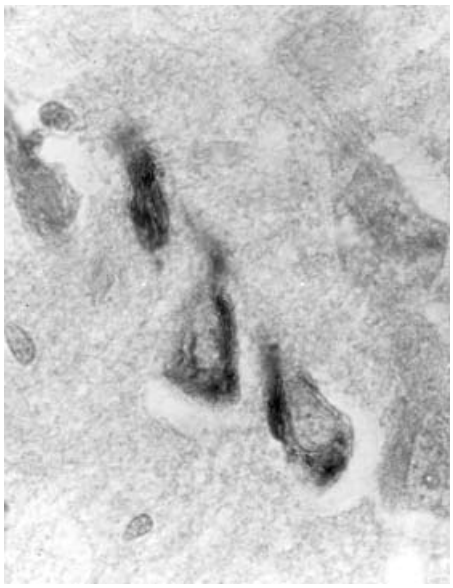
Alzheimer's disease is the commonest cause of dementia. Word-finding difficulty often parallels the onset of memory problems, and impaired reading comprehension and written language may also be seen. Changes in visual perception and praxis can also result in difficulties with activities of daily living, such as dressing or grooming. Except for the mental state, the neurological examination is usually normal. Extrapyramidal features, including rigidity, bradykinesia, shuffling gait and postural change, are relatively common, but primary motor and sensory functions are otherwise spared. Analysis of 100 autopsy-confirmed cases with Alzheimer's disease indicated that the average duration of symptoms until death may be 10 years, with a range of 4–16 years. Women are affected more commonly than men, and disease duration tends to be longer for women. The decline in intellectual ability generally starts between age 50 and 65 years, although onset at a younger age may occur, especially with some of the familial autosomal dominantly inherited forms.

Neuropathology

Grossly visible changes in the brains of Alzheimer's disease patients are similar to the changes observed in normal ageing, although the brain weight of Alzheimer's disease patients is often less than 1000 g. Examination of the brain discloses frontal and temporal cortical and subcortical atrophy, with thinning of the cortex and widening of the sulci (Figure XV-9.3). The ventricles are enlarged, and atrophy is most pronounced in the medial



(a)



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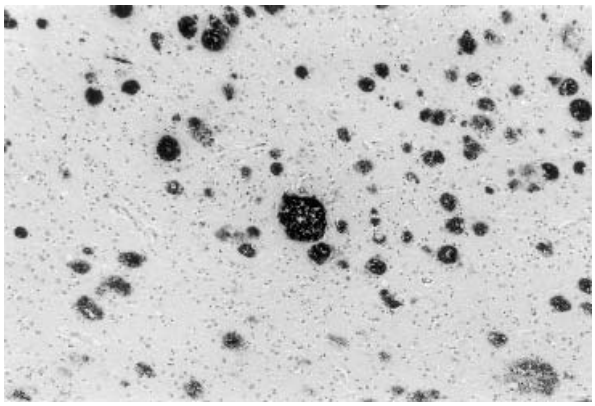


(c)

Figure XV-9.3 (a) Cortical atrophy in Alzheimer's disease; (b) neurofibrillary tangles immunoreactive with a monoclonal antibody to phosphorylated protein tau (AT8)(magnification 720×); (c) senile plaque amyloid immunoreactive with an antibody to amyloid-β (4G8) (magnification 78×)

Table XV-9.1 Components of intraneuronal inclusions

Structural protein	Stress protein	Classic name	Disease
Neurofilament	Ubiquitin, β-crystallin	Lewy body	Parkinson's disease
		Cortical Lewy body	Diffuse Lewy body disease
		Dystrophic neurites	Pick's disease
		Ballooned neurons	CBGD
		Lewy body-like inclusions in ALS	ALS
Tau	Ubiquitin	Neurofibrillary tangles	Alzheimer's disease
		Neuropil threads	Progressive supranuclear palsy
		Pick's bodies	Pick's disease
		Neuropil grains	CBGD
			Dementia with grains
		Gliofibrillary tangles	CBGD, PSP
		Glial cytoplasmic inclusions	MSA
Unknown	Ubiquitin	Marinesco body	Ageing, nonspecific
Actin	None	Hirano body	Ageing, nonspecific

ALS, amyotrophic lateral sclerosis; CBGD, corticobasal ganglionic degeneration; MSA, multiple system atrophy; PSP, progressive supranuclear palsy.

temporal lobes, with the hippocampus being disproportionately small. Microscopically, one observes neurofibrillary tangles, senile plaques and granulovacuolar degeneration. Additionally, amyloid angiopathy is often observed.

Neurofibrillary Tangles

The cellular cytoskeleton is an intricate cytoplasmic organelle linking the cellular membrane with the nuclear envelope. As well as its obvious structural function, the cytoskeleton may play a role in transcellular signalling and axonal transport. It is composed of three major classes of proteins forming filaments: the microfilaments (5 nm), the intermediate filaments (10 nm), and the microtubules (22 nm). Microfilaments are composed of actin. The intermediate filaments are made up of relatively tissue-specific proteins. In the nervous system, neurofilament is the neuronal intermediate filament and glial fibrillary acidic protein (GFAP) is the glial intermediate filament. Multiple forms of these proteins are present in the normal brain, and post-translational modifications, such as phosphorylation, can modify their functional characteristics. The microtubules consist of cylindrical aggregates of globular tubulin molecules. In addition to tubulin, microtubules contain a variety of microtubule-associated proteins (MAPs).

The most important MAP is the protein tau, which exists in multiple isoform and phosphorylation states. When the cell is stressed, the intermediate filament network immediately collapses to form perinuclear bundles or clumps. This molecular response may reflect increased phosphorylation or proteolysis of these proteins due to the influx of calcium. If the stress experienced by the cell is not lethal, then the cell deploys an adaptive response called the heat-shock response. The cell produces several proteins that may restore functional activity of partially denatured proteins; if the denaturing is too severe to permit restoration, then the proteins are marked for proteolysis. Proteins designated for destruction are conjugated with ubiquitin. Other stress proteins may be involved, including β-crystallin and a family of other heat-shock proteins. If the proteins conjugated to these stress proteins are not degraded, then these conjugated complexes aggregate to form intracellular inclusions. The inclusions in neurodegenerative disorders are examples of such aggregates. Ubiquitin immunoreactivity is seen in many intracellular inclusions, and is not specific to any particular disease. Ubiquitin immunoreactivity is much more sensitive than classical staining methods, and is therefore increasingly the method of choice in the detection of cellular protein inclusions. Hence, neurofilament, tau and synuclein can all be discerned.

Table XV-9.1 lists the components of various inclusions.

Neurofibrillary tangles are intraneuronal cytoplasmic collections of poorly soluble 7–9-nm paired helically wound filaments with a periodicity of 80 nm. These filaments share some antigenic sites with neuronal intermediate filaments, and are best stained with classical silver methods or thioflavin S. Tangles are found predominantly in projection neurons. Hyperphosphorylated tau protein is a major component of tangles, although neurofilament protein and ubiquitin are also present in significant amounts.

Tau consists of a family of post-translationally modified proteins that are normal components of the cytoskeleton (Figure XV-9.4). In the adult brain, differential splicing of a single gene located on chromosome 17 generates six isoforms of tau (Goedert, 1999). Three of these forms carry the segment specified by exon 10 and have four repeats of a microtubule-binding domain; the other three isoforms lack this fourth domain. Pathological accumulation of hyperphosphorylated tau comprises the abnormal inclusions in neurons and glia.

Tau proteins aggregate and are chemically modified in the inclusions, such as Alzheimer's neurofibrillary tangles and Pick bodies, of which they constitute the major component. Brain homogenates from Alzheimer's disease show three bands at 55, 64 and 69 kD (Delacourte, 1994). Dementia with Pick bodies yields a doublet at 55 and 64 kD, with an additional weaker band at 69 kD (Probst *et al.*, 1996; Buee and Delacourte, 1999). Corticobasal degeneration also yields a doublet, but at 64 and 69 kD (Buee

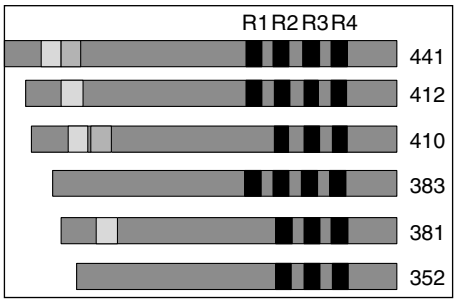


Figure XV-9.4 The microtubule-associated protein tau consists of six isoforms with three or four microtubule-binding regions (R) and one or two N-terminal inserts (light shading)

and Delacourte, 1999). Further analysis of these bands using exon-specific antibodies has shown that the pathological tau proteins in Alzheimer's disease comprise all six tau isoforms. In contrast, only the isoforms lacking the fourth microtubule-binding domain encoded by exon 10, and thus characterized as '3 repeat tau' are found in the inclusions in Pick's disease. Conversely, the inclusions of corticobasal degeneration and progressive supranuclear palsy contain the three isoforms with the fourth microtubule-binding domain encoded by exon 10 ('4 repeat tau') exclusively (Buee and Delacourte, 1999).

Senile Plaques

Senile plaques are extracellular 20–150-μm structures consisting of a central amyloid core surrounded by blunt, swollen neuritic processes; like tangles, they stain well with silver stains. Senile plaques come in different forms, amorphous and small amyloid deposits and diffuse plaques being considered an early stage of formation as they are found in normal ageing and in Down's syndrome patients. Some authors believe that the severity of dementia correlates well with the number of mature or neuritic plaques per unit area in the brain, while others hold that the neurofibrillary tangles correlate better with dementia. Terry *et al.* (1991) have shown that synaptic loss is best correlated with intellectual decline. All authors agree that diffuse plaques, which are large diffuse areas of staining devoid of central cores or swollen neuritic processes, do not correlate with the severity of dementia. The third microscopic hallmark of Alzheimer's disease is granulovacuolar degeneration, which appears as small (5 μm), clear, intraneuronal cytoplasmic vacuolation with each vacuole containing a single argyrophilic granule.

The guidelines developed by the multicentre Alzheimer's disease project CERAD (consortium to establish a registry for Alzheimer's disease) represent the most widely used diagnostic criteria. The CERAD protocol employs a semiquantitative evaluation of neuritic plaques coupled with the patient's age to reach an age-related plaque score (Mirra, 1997b). Three sections of neocortex, including the superior and middle temporal gyrus, middle frontal gyrus, and inferior parietal lobule, are examined at 100× using either the Bielschowsky silver technique or the more sensitive thioflavin-S staining technique (Mirra, 1997a). The thioflavin-S technique requires access to fluorescence microscopy and is not as widely available as silver staining. The number of plaques is designated as none, sparse, moderate or frequent. CERAD provides images illustrative of these plaque (and tangle) frequencies to help neuropathologists calibrate their estimates. Fewer plaques are tolerated in young people (age less than 50 years) than in the elderly (age greater than 75 years), and the presence or absence of a history of dementia is taken into account.

The Braak and Braak staging of Alzheimer's disease can be simplified into three tiers: involvement of the transentorhinal cortex in stages 1 and 2, the limbic system in stages 3 and 4, and finally the isocortex in stages 5 and 6 (Braak and Braak, 1996; Braak and Braak, 1997). Braak and Braak (1996) redirected attention to the neurofibrillary tangles and described the spread of tangles from the transentorhinal cortex and hippocampus (stages 1 and 2) to the limbic system (stages 3 and 4) to the isocortex, including the frontal, temporal, parietal and occipital neocortex (stages 5 and 6) as Alzheimer's disease progresses. They have provided compelling evidence that description of the distribution of neurofibrillary tangles is important in the neuropathological assessment of Alzheimer's disease. Individuals in stages 1 and 2 are generally asymptomatic, those in stages 3 and 4 have incipient or mild Alzheimer's disease characterized by episodic memory loss, and those in stages 5 and 6 have symptomatic Alzheimer's disease. There is, however, a relatively poor anatomoclinical correlation, with some individuals functioning at an adequate level despite the presence of numerous lesions in the isocortex.

Senile plaques contain beta-amyloid protein, a peptide consisting of 39–43 amino acids (Selkoe, 2000). This small amyloidogenic peptide is derived from proteolytic cleavage of a large transmembrane glycoprotein, amyloid precursor protein (APP). APP is synthesized in three major forms (695, 751 and 770 amino acid residues) by differential mRNA splicing (Selkoe, 2000). The two larger forms have regions that have homology with serine protease inhibitors. Beta-amyloid is toxic to mature neurons; this peptide may bind protease inhibitor receptors on cell surfaces, permitting the accumulation of extracellular proteases, leading to membrane and eventually cytoskeletal damage.

Mutations in the APP gene on chromosome 21 account for about 3% of autosomal dominant Alzheimer's disease, while mutations in the presenilin-1 gene are found in the majority of families (Rubinsztein, 1997; Cruts *et al.*, 1996). Mutations in the APP gene are also responsible for intracerebral haemorrhages due to amyloid angiopathy, and thereby cause dementia. We have described a novel APP mutation that results in an intermediate phenotype, with progressive dementia and stroke occurring in the same family (Cras *et al.*, 1998). Two forms of autosomal dominant familial Alzheimer's disease are linked to chromosomes 14 and 1, and result from missense mutations of integral membrane proteins (presenilin 1 and presenilin 2), which may be a receptor, membrane channel or protein involved in docking and fusion of membrane-bound vesicles. There is evidence that the presenilins may be involved in proteolytic processing at the cell surface required to release Notch and APP fragments into the extracellular space (De Strooper, 2000). *In vitro* experiments have shown that the presenilin mutations result in increased amyloid-beta protein production (Cruts and Van Broeckhoven, 1998). Familial forms of Alzheimer's disease tend to have earlier onset and a more aggressive course than sporadic or apolipoprotein E (ApoE) E4-linked Alzheimer's disease (Martin *et al.*, 1991). Homozygosity for the E4 allele of ApoE has been shown to confer increased risk for Alzheimer's disease, to advance the age of onset, and to result in a higher number of senile plaques (Hofman *et al.*, 1997). ApoE is involved in lipid transport and binds to the microtubule-associated protein tau under certain conditions, but the precise molecular basis of its relation to Alzheimer's disease pathogenesis remains unsolved.

Cholinergic Deficit

In terms of neurotransmitter alterations, Alzheimer's disease is characterized by a striking reduction in cortical acetylcholine, as reflected in the amount of choline acetyl transferase (ChAT) in the cortex (Davis *et al.*, 1999b). In biopsy cases, ChAT levels had a weak but definite correlation. This biochemical information led to the observation that the basal forebrain nucleus responsible for the majority of cortical cholinergic projections, the basal nucleus of Meynert, is depleted of cells in Alzheimer's disease (Ezrin-Waters and Resch, 1986). Early studies showed that the muscarinic cholinergic receptor was unchanged in Alzheimer's diseased brain despite losses of ChAT and accumulation of neuritic plaques. Other neurochemical abnormalities have been described in Alzheimer's disease, but cholinergic reduction remains the major alteration (Engelborghs and De Deyn, 1997; Mesulam *et al.*, 1983; Mesulam, 1986). Serotonin metabolism is decreased in regions of the cortex and some other regions in Alzheimer's disease. Biopsy studies showed that both serotonin and the metabolite 5-hydroxyindoleacetic acid (5-HIAA) were decreased in cortical biopsy samples, and that both uptake and release of serotonin were diminished in these samples.

The dorsal raphe nuclei of the brainstem are decreased in number, although not to as large an extent as either the cholinergic or neuradrenergic neurons (Aletrino *et al.*, 1992). The surviving neurons of the raphe often contain neurofibrillary tangles. While cholinergic

deficiencies have some correlations with levels of cognitive impairment, and cholinergic enhancement has been associated with cognitive improvement, serotonergic alteration does not appear to have cognitive correlates. Rather, depression and aggressive behaviour appear to be more associated with serotonergic loss (Bondareff, 1996). Tyrosine hydroxylase is the rate-limiting enzyme for noradrenaline synthesis and is diminished significantly in Alzheimer's disease (Torack and Morris, 1992). The source neurons for forebrain noradrenaline are in the locus coeruleus in the midbrain. Neurons are depleted in the locus coeruleus in Alzheimer's disease compared with controls. A hint of the anatomical connectivity that underlies the selective vulnerability in Alzheimer's disease is seen in the pattern of locus coeruleus neuron loss. Within the locus coeruleus, neurons in the anterior and medial portions of the nucleus are lost; these are the cells that project to the forebrain. The more caudal and lateral locus coeruleus regions are spared in the disease; these neurons project to the spinal cord and cerebellum, which do not show classic neurofibrillary tangles and plaques, and are not associated with clinical deficits. Parkinsonian symptoms do evolve in significant numbers of Alzheimer's disease cases, however (Ala *et al.*, 1997; Ala *et al.*, 1999). A large number of these cases have Lewy bodies and dopaminergic neuron loss in the substantia nigra (Ala *et al.*, 1997; Ala *et al.*, 1999).

Altered function or dysregulation of glutamate receptors can cause neural death through a cascade involving increased calcium entry into the cell (Blass and Gibson, 1991; Gooch and Stennett, 1996). The large pyramidal cells of the cortex and the hippocampus utilize glutamate as their neurotransmitters and have significant numbers of glutamate receptors (Jellinger *et al.*, 1991). The earliest site of neurofibrillary tangle deposition, the ERC, also utilizes glutamate as a transmitter, as do many of the affected neurons in the cortex. A number of other neurotransmitters lost in Alzheimer's disease are peptides, such as corticotrophin-releasing factor (CRF) and somatostatin (Davis *et al.*, 1999c). Loss of the small interneurons that utilize these transmitters, or downregulation of their function in response to diminished neural activity, may account for such findings. The relationship of these changes to either the sequence of pathology in the disease or the clinical behavioural changes remains unknown.

Diffuse Lewy Body Disease

Clinical Findings

Dementia with Lewy bodies, or diffuse Lewy body disease (DLBD), is a general term that describes patients with cognitive impairment related to the eosinophilic inclusions first described by F.H. Lewy (see Perry *et al.*, 1997). In autopsy series of demented patients, DLBD is a frequent finding. It is less common than Alzheimer's disease, but it occurs at least as frequently as vascular dementia. In 1995, an international workshop proposed clinical and neuropathological guidelines and nomenclature that proposed DLBD as a distinct clinical syndrome (McKeith and O'Brien, 1999). Dementia is the mandatory feature of DLBD. Furthermore, in order to meet the criteria, three core features have to be present: fluctuation of cognitive disturbances, parkinsonism and visual hallucinations. When two or three core features are present, the diagnosis of probable DLBD can be made; one core feature is consistent with possible DLBD. Many patients seem to have an insidious onset and show gradual progression (Kosaka, 1998). Age at onset overlaps with Alzheimer's disease, ranging from about 60 to 85 years. The duration from onset of dementia to death is variable and overlaps with Alzheimer's disease, but it is often shorter in DLBD. The name acknowledges the importance of Lewy's bodies in the dementia syndrome and retains flexibility about associated Alzheimer's disease pathology.

Neuropathological Findings

The neuropathology of DLBD has been documented extensively by Kosaka (2000), who drew attention to the neuropathology, describing Lewy bodies in subcortical sites such as the substantia nigra, nucleus basalis of Meynert and locus ceruleus, as well as cortical regions, especially in limbic areas. Due to their morphology, Lewy bodies are sometimes difficult to distinguish from rounded, globose-type neurofibrillary tangles. In about half of the patients, neurofibrillary tangles and senile plaques are also found in sufficient numbers to fulfil the diagnostic criteria for Alzheimer's disease. Therefore, some nosologic confusion arose when authors distinguished the Lewy body variant of Alzheimer's disease from the 'pure' form of DLBD (Brown *et al.*, 1998). However, as Alzheimer's disease lesions and Lewy bodies increase in prevalence with age, this may explain their co-occurrence by chance.

Classic Lewy bodies in the substantia nigra are brightly eosinophilic, with a hyaline core and a paler halo, while cortical Lewy bodies are faintly eosinophilic and lack a core (Figure XV-9.5). Immunostaining with antibodies against ubiquitin or alpha-synuclein facilitates the detection of all Lewy bodies and highlights Lewy neurites, degenerating neuronal processes found especially in the CA2-3 region of the hippocampus (Spillantini *et al.*, 1998). Spongiform changes in the hippocampus also occur in DLBD. Careful examination reveals Lewy bodies in the cortex of most if not all patients with idiopathic Parkinson's disease, but they are less abundant than in DLBD (Spillantini *et al.*, 1998). Counts of nigral and cortical Lewy bodies do not correlate with each other, or with the duration or age at onset of DLBD (Spillantini *et al.*, 1998). This disfavours the idea that Lewy bodies spread from the nigra to cortical regions (Perry *et al.*, 1997). In both Parkinson's disease and DLBD, the brain may show a combination of Alzheimer's disease lesions, senile plaques, neuritic changes, neurofibrillary tangles and Lewy bodies. In fact, Alzheimer's disease lesions coexist in most elderly patients with DLBD. Counts of diffuse and classic plaques do not differ significantly between Alzheimer's disease and DLBD patients who die at advanced stages of dementia, i.e. there is a similar amyloid burden. In the most common form of DLBD, associated Alzheimer's disease pathology consists mainly of senile plaques, with fewer neurofibrillary tangles than in 'pure' Alzheimer's disease, especially in the neocortex. Sufficient neuritic plaques, however, are found in many DLBD patients to meet pathology criteria for Alzheimer's disease, such as those of CERAD (Mattila *et al.*, 1998).

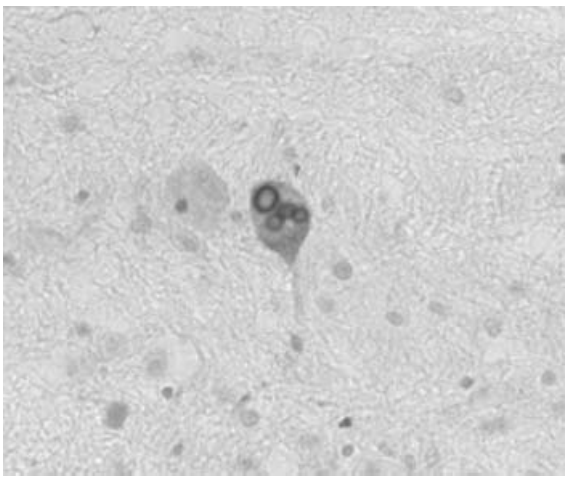


Figure XV-9.5 Cortical Lewy bodies are demonstrated with antibodies to alpha-synuclein (magnification 520×)

The basis of motor dysfunction in Parkinson's disease is well known, namely neuron loss in the substantia nigra leading to deficiency of nigrostriatal dopaminergic circuits. In DLBD, the origin of cognitive or behavioural symptoms is less clear. Neuronal dysfunction due to Lewy bodies could occur in cognitively important areas. These include the entorhinal regions, frontal, temporal or parietal association cortex, cingulate and amygdala. Neuron loss is not significant in cortical areas affected by Lewy bodies, but synaptic loss or dysfunction may be an alternative explanation (Mattila *et al.*, 2000; Goedert, 1999). Marked reduction of acetylcholine could be due to Lewy bodies in the nucleus basalis of Meynert, and decreased dopamine in the basal ganglia and cortex has also been demonstrated (Piggott *et al.*, 1999; Walker *et al.*, 1997). Additive effects of Alzheimer's disease pathology, especially in temporolimbic regions, could also have an impact on memory in DLBD (Hansen and Samuel, 1997). Lewy bodies may be clinically silent. For example, a few pathological series have examined the brains of supposedly normal elderly individuals (Davis *et al.*, 1999a): Nigral Lewy bodies were found in 5–10% of subjects over the age of 50 years. The frequency rose with age, reaching 12% by the ninth decade. The Lewy bodies were dubbed 'incidental' and thought to represent preclinical Parkinson's disease. In a later autopsy study, all available clinical records were reviewed carefully, and patients with neurological or psychiatric symptoms were rigorously excluded. The findings challenged the viewpoint that incidental Lewy bodies are relatively common: only 2.8% of 131 cases had Lewy bodies. More data are needed on Lewy bodies in well-characterized normal elderly subjects.

Vascular Dementia

Clinical

As for Alzheimer's disease, the incidence and prevalence of vascular dementia increase with age (Roman, 1999). Classically, vascular dementia is associated with the clinical picture of stroke: the sudden onset of focal neurological deficits (e.g. weakness, sensory loss, incoordination, speech disturbance). The nature of the neurological deficit depends on the location and the size of the occluded vessel and the presence of collateral circulation. Therefore, vascular dementia is a heterogeneous syndrome caused by small-vessel disease, large single or small strategically placed strokes (Roman, 1999). The natural history of vascular dementia is characterized by the sudden onset of cognitive impairment, followed, with each new ischaemic event, by further step-wise decline. Individuals with vascular brain injury are likely to be apathetic and depressed rather than agitated or psychotic (Cherrier *et al.*, 1997). Memory impairment is generally more variable and less severe than in Alzheimer's disease (Bentham *et al.*, 1997). With small-artery disease, diminished executive ability (e.g. planning, abstraction, evaluation, correction) is characteristic (Wallin, 1998; Erkinjuntti *et al.*, 1994); with large-artery stroke, aphasia, apraxia and neglect are often seen. Based on the wide variability in location and size of brain injury, there is no single characteristic pattern of behavioural and cognitive disturbance associated with vascular dementia.

MRI is exquisitely sensitive to structural changes in the periventricular and deep white matter (Barber *et al.*, 1999; Barber *et al.*, 2000; O'Brien *et al.*, 2000). White matter lesions (WML) are visualized as areas of increased signal intensity in T-2 weighted images, and as partially decreased signal intensity in T-1 weighted images. On computerized tomography (CT) scan, these lesions are also known as leukoaraiosis, or white matter hyperintensities. The frequency of WML increases with age and elevated blood pressure (Shintani *et al.*, 1998; Golomb *et al.*, 1995; Pasquier and Leys, 1998). Among patients with first lacunar stroke, the presence of leukoaraiosis is a poor prognostic sign, increasing the risk for recurrent stroke, dementia and death. The favoured, but still not well-established, explanation is that WMLs represent incomplete infarcts due to chronic or recurrent ischaemia. The regions that develop WMLs, namely the periventricular and deep white matter, receive their blood supply via long, narrow, penetrating endarterioles. A limited number of studies suggest that cognitive changes do not become apparent until WML volume reaches a threshold (Barber *et al.*, 2000).

Neuropathology

The likelihood that infarction leads to cognitive impairment is related to location, size and number of ischaemic lesions (Boston *et al.*, 1999). The key anatomical locations associated with cognitive impairment are expected to include multimodal association areas, limbic areas (e.g. hippocampus), and certain subcortical grey matter nuclei (e.g. head of the caudate, globus pallidus, and anterior and dorsomedial thalamus). The latter nuclei are key relay stations within frontal-subcortical loops. Anatomical locations less likely to cause cognitive impairment include primary motor-sensory cortex, putamen, posterior limb of the internal capsule, ventrolateral and ventroposterior nuclei of the thalamus, brainstem and cerebellum.

Large strokes are easily recognized on macroscopic examination of the brain, showing tissue loss and cavitation. Microscopically, neuronal loss and reactive gliosis can be recognized in the deeper cortical layers, while the subpial molecular layer is often spared in incomplete infarcts. Smaller cortical strokes are sometimes associated with meningeal amyloid angiopathy (Figure XV-9.6).

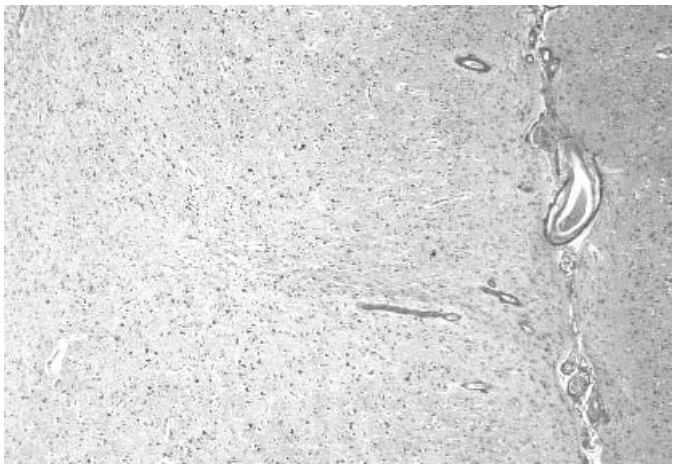
The confluent WMLs of vascular leukoencephalopathy are associated with tissue rarefaction, mild gliosis and a non-amyloid small-vessel sclerosis that occurs in the central, preferentially frontal deep white matter (Englund, 1998). Microvascular brain damage in the form of severe cribriform change and associated subcortical white matter damage and microinfarction were correlated with a history of dementia (Esiri *et al.*, 1997). Arteriolosclerosis is characterized by thickening of the media, deposition of collagen, and hyaline changes. In some families with APP mutations, vascular dementia was also observed (Haan *et al.*, 1992; Bornebroek *et al.*, 1997). Sporadic congophilic angiopathy is also frequently associated with vascular dementia (Yoshimura *et al.*, 1992; Esiri *et al.*, 1997).

Pick's Disease

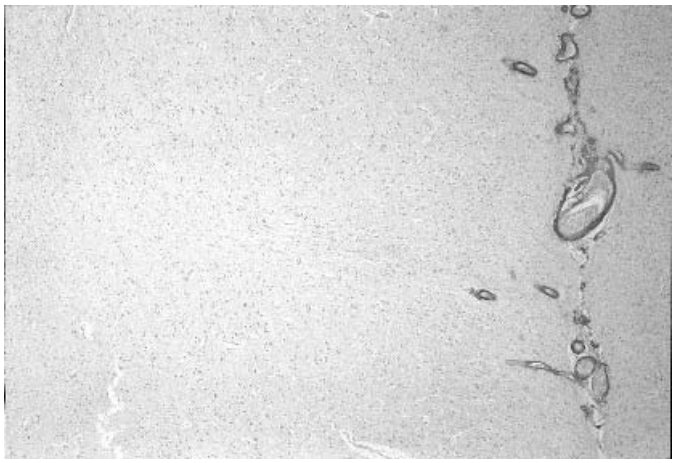
Clinical Findings

Traditionally, Pick's disease has been considered a relatively rare disorder that is characterized by an initial presentation with emotional and behavioural changes indicative of frontal lobe changes (Litvan *et al.*, 1997b). However, the existence of Pick's disease as a unique entity is in doubt, and there is sufficient neuropathological and clinical overlap of classic Pick's disease, Pick variants, and other rare and poorly characterized non-Alzheimer's dementias that a unifying concept of dementia with focal atrophy has emerged under the name of 'frontotemporal dementia' (FTD) (Neary, 1999; Jackson and Lowe, 1996; Tanabe, 2000). Some authors propose to use the term 'Pick complex' to encompass the spectrum of focal cortical atrophies, FTD, corticobasal degeneration (CBD) and Pick's disease strictu sensu.

The mean age of onset for FTD is usually between the fifth and seventh decades. The first symptoms involve changes in personality, social behaviour, language or executive function, as FTD patients lose functions associated with select regions in the prefrontal and anterior temporal cortex. The disinhibition and antisocial behaviours due to orbital-basal involvement occur in approximately one-half of the patients with FTD, and can be presenting symptoms (Lebert *et al.*, 1998; Kertesz *et al.*, 2000). Kluver and Bucy



(a)



(b)

Figure XV-9.6 Leptomeningeal amyloid angiopathy associated with cortical infarct. (a) Cresylviolet stain (magnification 254×); (b) immunostain with antibody 4G8 directed to amyloid-β shows amyloid angiopathy (magnification 254×)

(1939) showed that after bilateral anterior temporal lobectomies, caged monkeys developed hyperorality, psychic blindness, hypermetamorphosis, blunted emotional response, hypersexuality and dietary changes. Later, studies were performed on animals returned to their social environment after similar ablations. Bilateral anterior temporal lobectomy, or selective bilateral amygdalectomy, led to less social interaction and loss of social rank. Franzen and Myers (1973) noted loss of facial expression, and diminished gestures and postures. Mothering was impaired, and the temporal lobectomized group had aggression towards their own babies but preserved, sometimes increased, interest in sex. These studies predicted the types of social deficits that characterize patients with bilateral temporal lobe degeneration.

Edwards-Lee *et al.* (1997) described ten patients with the 'temporal lobe variant' of FTD, in which there was selective involvement of the anterior temporal lobes with sparing of the frontal regions. Five of these patients were predominantly left-sided, while the other five had selective right-sided involvement. Patients with left-sided involvement suffer from 'semantic dementia' and exhibit severe losses of verbal facts and words but relative sparing of visual skills.

In some patients, there was enhanced ability to paint, draw, invent or play music (Miller *et al.*, 2000). In contrast, right-sided patients presented with irritability, impulsiveness, bizarre alterations in dress, limited and fixed ideas, ideological fanaticism, decreased facial expression, loss of empathy and increased visual alertness (Miller *et al.*, 2000). Both left and right temporal lobe patients exhibited intense visual (left) or verbal (right) preoccupations and compulsions. They showed normal working memory (Waltz *et al.*, 1999) and visuoconstructive abilities (Edwards-Lee *et al.*, 1997), while semantic naming, reading and writing (Hodges *et al.*, 1999) and facial recognition have been demonstrated to be impaired (Tyrrell *et al.*, 1990).

Neuropathology

The gross appearance of the brain in dementia with Pick bodies is usually striking for the contrast of the atrophic and preserved areas (Dickson, 2001). The atrophy may preferentially affect the frontal or the temporal lobes. Tissue loss is severe and therefore sometimes referred to as knife-edge atrophy. In contrast, there is usually preservation of the precentral gyrus and the posterior third of the superior temporal gyrus. There is marked variability in the involvement of subcortical structures, ranging from grossly preserved to severe atrophy of the caudate nucleus, substantia nigra and thalamus (Litvan *et al.*, 1997b; Rossor, 1999).

Microscopically, severe cortical neuronal loss with gliosis is seen, as are intraneuronal cytoplasmic oval silver-loving filamentous inclusions (Pick bodies) (Figure XV-9.7). In addition to classic Pick bodies, ballooned neurons with achromatic cytoplasmic inclusions are commonly seen. These ballooned neurons overlap with the neuronal changes seen in CBD. Neocortical neurons in layers II and upper III are preferentially involved, contrasting with the distribution of neurofibrillary tangles in Alzheimer's disease. Pick bodies are also found in the amygdala, the neostriatum and other subcortical nuclei. Ballooned neurons are always present in the deep cortical layers. Glial inclusions are not prominent, but include the occasional thorny astrocyte and oligodendroglial coiled bodies. There is severe loss of myelin and axons in the white matter, which may reflect additional damage to that secondary to neuronal loss.

In contrast to Alzheimer's disease, several studies have shown normal cortical levels of ChAT, acetylcholinesterase and somatostatin in dementia with Pick bodies (Kanazawa *et al.*, 1988; Meier-Ruge *et al.*, 1984).

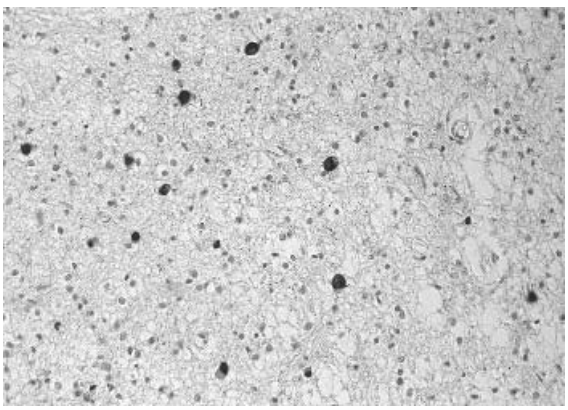


Figure XV-9.7 Pick bodies are immunoreactive with the monoclonal antibody AT8 directed to phosphorylated protein tau (magnification 186×)

Primary Progressive Aphasia

Clinical Findings

Mesulam (1982, 2001) described a series of cases of slowly progressive aphasia and subsequently named the syndrome primary progressive aphasia (PPA). Since then, the term has been used widely, although reports of similar patients have also appeared under various terminology, such as progressive aphasia without dementia, progressive nonfluent aphasia, and pure progressive aphemia (Westbury and Bub, 1997). Approximately 10% of dementias present primarily with progressive aphasia (Pasquier, 1999; Noble *et al.*, 2000). The initial presentation of PPA is often with word-finding difficulty or anomia. In this respect, PPA patients are not much different from Alzheimer's disease patients, except that they have relatively preserved memory and nonverbal cognition. However, Alzheimer's disease patients, by the time they present with word-finding difficulty, already have significant memory loss, disorientation, and constructional, visuospatial and other cognitive impairment (Greene *et al.*, 1996). Typical Alzheimer's disease presentation is loss of episodic memory with relative preservation of semantic memory and language. The initial 2 years of the disease symptomatology distinguishes the two groups of patients. This period was originally suggested by Mesulam as the operational definition of PPA, although in many publications it is not adhered to (Mesulam, 2001). The more typical clinical picture progresses from anomia to a nonfluent type of aphasia with increasing word-finding difficulty. Sometimes, patients are called 'logopoenic' yet are still considered 'fluent' when the word-finding difficulty is prominent but the overall phrase length is longer than four words and syntax is preserved. Decreasing speech output involves spontaneous speech and repetition to a lesser extent initially.

Neuropathology

In many patients with PPA, the clinical picture evolves into classical Alzheimer's disease, and corresponding neuropathological alterations are found at autopsy (Ikeda *et al.*, 1996; Li *et al.*, 2000; Mann *et al.*, 1993). There are also patients with PPA who develop motor neuron disease with a rapid course and bulbar symptoms, such as an increasing amount of dysarthria and dysphagia as indicated earlier, even though typical amyotrophy or spasticity of amyotrophic lateral sclerosis (ALS) may be absent (Tsuchiya *et al.*, 2000). Several cases of PPA with motor neuron disease have been described pathologically, with ubiquitinated, tau-negative inclusions in dentate fascia and neocortical neurons (Tsuchiya *et al.*, 2000). Patients with PPA may also develop unilateral extrapyramidal symptoms, associated with severe apraxia and 'alien hand', characteristic for CBD (Ikeda *et al.*, 1996). The pathology is indistinguishable from more typical cases of CBD. Of all the diseases of the Pick spectrum, PPA is the clinical entity that shows most overlap with the others.

Corticobasal Ganglionic Degeneration

Clinical Findings

The pathology of CBD was thought initially to be linked tightly to a distinctive asymmetric akinetic-rigid syndrome with associated involuntary movements and signs of cortical dysfunction (Litvan *et al.*, 1997a). More recently, however, a wider spectrum of clinical phenotypes has been recognized, including, but not limited to, presentations with dementia, agnosia, dyspraxia, dysphasia and altered behaviour (Boeve *et al.*, 1999). The classical syndrome of CBD usually presents in the sixth decade with a varied combination of symptoms, including stiffness, clumsiness, jerking and sensory impairment affecting the upper limbs. Alien hand phenomenon develops in approximately 50% of patients (Kompoliti *et al.*, 1998). Typical features of frontal lobe dementia at presentation or early in the clinical course include progressive loss of judgement with disinhibition (including distractibility, impulsiveness, compulsiveness or perserveration), social misconduct, or loss of initiative out of proportion to the degree of anterograde amnesia; affective symptoms, including depression, anxiety, emotional unconcern or abulia; and early primitive reflexes and incontinence. This disorder is characterized further by striking asymmetrical gait and speech apraxia, rigidity, myoclonus and cortical sensory loss, while dementia is usually a late manifestation.

Neuropathology

Recent advances have been made in the pathologic characterization of CBD coupled with recognition of overlapping features with other neurodegenerative diseases, specifically progressive supranuclear palsy (PSP) and Pick's disease. On gross examination, frontoparietal cortical atrophy is usually present but may be mild (Bergeron *et al.*, 1998; Feany *et al.*, 1996). The cortical atrophy is usually moderate in severity and not as sharply delineated as in Pick's disease, and can be correlated with the clinical presentation. Thus, atrophy of the frontal lobes would give rise to dementia, atrophy of the superior parietal regions and the precentral gyri to the CBD syndrome, and involvement of the frontal operculum and anterior superior temporal gyrus to PPA (Ikeda *et al.*, 1996; Black, 1996). The substantia nigra and locus coeruleus are always affected, and may be accompanied by atrophy of the globus pallidus and the dorsomedial thalamus (Black, 1996).

Pathologically, there is pre- and post-central cortical neuronal degeneration with achromatic intracytoplasmic neuronal inclusions (Halliday *et al.*, 1995; Black, 1996; Mori *et al.*, 1994). These inclusions are also found in the thalamus, subthalamic nucleus, red nucleus and substantia nigra. On haematoxylin and eosin-stained sections, the achromatic inclusions are very similar to Pick bodies, and immunohistochemical studies reveal that these inclusions share neurofilament/ubiquitin/crystallin and tau immunoreactivity (Sergeant *et al.*, 1999; Jackson and Lowe, 1996; Komori, 1999; Spillantini and Goedert, 1998).

The pathological findings in CBD also include the presence of cortical ballooned neurons, cortical glial plaques, and white matter oligodendroglial tau inclusions (Ikeda *et al.*, 1994; Takeda *et al.*, 1997). Neuronal loss and gliosis is most prominent in superficial cortical layers, and in the most severely involved areas status spongiosus may be observed. These changes are most evident in grossly atrophic areas, but they can be seen to a milder degree in less overtly atrophic cortex. Subcortical white matter gliosis commonly parallels the degree of cortical degeneration. Neuronal loss, gliosis and corticobasal inclusions are invariably found in the substantia nigra. Other deep grey structures may be involved, including the lateral thalamic nuclei subthalamic nucleus, locus coeruleus, globus pallidus, red nucleus, claustrum, striatum, amygdala, deep cerebellar nuclei and other brainstem nuclei.

Ballooned neurons demonstrate perikaryal swelling, dispersion of Nissl substance, an eccentrically located nucleus, loss of typical cytoplasmic staining (achromasia), and occasional cytoplasmic vacuolation. They are immunoreactive with antibodies to phosphorylated neurofilament epitopes and B-crystallin. Although ballooned neurons were once thought to be unique to Pick's disease (so-called 'Pick cells') and CBD, they have been increasingly recognized in some cases of PSP and, less commonly, in Alzheimer's disease, motor neuron disease and Creutzfeldt–Jakob disease (CJD) (Halliday *et al.*, 1995; Armstrong *et al.*, 1999). In CBD, ballooned neurons are typically plentiful in the cortex, with fewer present in the basal ganglia. CBD, Pick's disease and PSP have numerous

tau-immunoreactive neuronal and glial inclusions in cortical and subcortical regions. Cortical tau-immunoreactive neuronal inclusions in CBD are found in pyramidal and nonpyramidal neurons, and may have a distinctive perinuclear, coiled filamentous appearance, but in general they vary greatly in appearance. The clustering and distribution of ballooned neurons and tau-immunoreactive neurons in a pattern similar to lesions of Alzheimer's disease, Lewy body dementia and Pick's disease is consistent with the degeneration of cortico-cortical projections in CBD.

Corticobasal inclusions are weakly basophilic nigral neuronal inclusions initially thought to be specific for CBD. However, examination of additional cases has revealed considerable morphologic heterogeneity, thus these inclusions are not reliably distinguishable from tau-immunoreactive neurofibrillary tangles commonly associated with PSP. In CBD, cortical tau-immunoreactive neuronal inclusions are considerably more common than in PSP; however, both disorders have a similar density of these structures in deep grey matter and brainstem nuclei. Pick bodies are ubiquitin and tau-immunoreactive spherical cortical intraneuronal inclusions seen predominantly in the hippocampal dentate gyrus and frontotemporal cortex.

In PSP, CBD and Pick's disease, abnormal tau accumulates preferentially in specific subcellular regions of astrocytes (Komori, 1999). In CBD, astrocytic inclusions are more common in the cortex than in deep grey matter or the brainstem. Astrocytic plaques may be specific for CBD; they consist of distinctive annular clusters of thick, short, tau-immunoreactive deposits within distal processes of cortical astrocytes.

Frontotemporal Dementia

Clinical Findings

The mean age of onset for FTD is usually between the fifth and seventh decades, but it can be hard to determine when FTD begins because the illness is so insidious. The first symptoms are often subtle alterations in drive, personality, social behaviour, executive functions and sometimes language (Neary, 1999). These findings emerge because FTD patients lose these functions associated with select regions in the prefrontal and anterior temporal cortex. In contrast, Alzheimer's disease patients show early deficits in memory because the disease begins in the brain's memory regions. Benson and Stuss (1986) suggested a logical system for thinking about frontal lobe functions that helps to understand many of the findings of FTD patients with selective frontal involvement. However, different symptoms emerge in patients with selective temporal lobe involvement and sparing of frontal lobes (Edwards-Lee *et al.*, 1997). Furthermore, the relative involvement of the right versus left frontotemporal region is an important factor in the type of symptoms that a patient will manifest.

Table XV-9.2 lists the types of deficits that occur when certain brain regions degenerate.

The disinhibition and antisocial behaviours due to orbital-basal involvement occur in approximately one-half of the patients with FTD, and can be presenting findings (Bozeat *et al.*, 2000). Theft, hit-and-run accidents and indecent exposure commonly occur in FTD. In contrast, violent assaults upon others are rare. The behavioural disturbances of FTD place a special burden on families caring for such patients and is a major factor leading to nursing home placement. Patients with asymmetric right-sided disease are more likely to exhibit socially unacceptable behaviours than those with asymmetric left-sided involvement (Edwards-Lee *et al.*, 1997), while left-sided patients show more language disability (Mesulam, 2001).

The occurrence of FTD in families has been a long-standing observation, and the presence of a similar disorder in the family of a proband is supportive of the diagnosis of FTD (*Journal of Neurology, Neurosurgery, and Psychiatry*, 1994). Recently, progress has been made in linking FTD to particular chromosomes, and in identifying mutations in some familial and rare sporadic cases. Linkage of a substantial number of families to chromosome 17, and of a few families to chromosome 3, has been achieved (Foster *et al.*, 1997). Over ten mutations in the tau gene of chromosome 17 have been identified in FTD families (Heutink, 2000). The most common mutation occurs in exon 10, resulting in the conversion of proline to leucine (P301L) (Kodama *et al.*, 2000; Houlden *et al.*, 1999; Poorkaj *et al.*, 2001). These mutations appear to reduce the ability of tau to bind microtubules and promote microtubule assembly, resulting in tau inclusions in affected neurons, astrocytes and oligodendrocytes (Nasreddine *et al.*, 1999). Mutations have been identified in approximately 15% of familial FTD patients investigated and 0–4% of sporadic FTD cases investigated (Nasreddine *et al.*, 1999). There is substantial clinical and pathological heterogeneity among cases linked to chromosome 17. Patients have had classical FTD syndromes or disorders identified clinically as CBD, PSP, motor neuron disease, PPA and spastic paraplegia with dementia (Nasreddine *et al.*, 1999). Frontotemporal dementia with parkinsonism (FTDP-17) is an autosomal dominant disorder that presents clinically with dementia, extrapyramidal signs and behavioural disturbances in mid-life and progresses to death within 5–10 years. Pathologically, the disorder is characterized by variable neuronal loss and gliosis in the frontal and temporal lobes, limbic structures and midbrain (Hulette *et al.*, 1999).

In a related mutation (P301S), Bugiani *et al.* (1999) described an individual who presented with frontotemporal dementia, and his son, who had CBD demonstrating that the same primary gene defect in tau can lead to two distinct clinical phenotypes. Neuropathologically, the father presented with an extensive filamentous pathology made of hyperphosphorylated tau protein (Bugiani *et al.*, 1999).

Table XV-9.2 Frontal brain regions with lesional disturbances

Brain region	Hypothesized function	Deficit seen with dysfunction
Orbitobasal	Inhibition, social control	Disinhibited, impulsive, confabulatory, antisocial
Dorsolateral	Attention, working memory (area 46), alternating programmes, generate words, designs, ideas, self-awareness, generation of affect? (right > left)	Poor attention and focus, working memory deficits, poor organization, poor planning, poor word/design generation, poor insight, abnormal expression of affect
Medial frontal	Energy, motivation, drive	Apathy, abulia, depression

Neuropathology

Clinical pathology can be subdivided into three main neuropathological subtypes: frontal lobe dementia, Pick's disease and FTD with motor neuron disease, all characterized by distinct histological features (Jackson and Lowe, 1996).

Grossly, there is slight symmetrical frontal and anterior temporal atrophy with frontal ventricular enlargement (Jackson and Lowe, 1996). The atrophy is usually not as severe as in classic Pick's disease. No striatal, amygdala or hippocampal atrophy is seen. The cellular neuropathology is variable. In some instances, these conditions appear to be focal variants of Pick's disease, Alzheimer's disease or Lewy body disease (Jackson and Lowe, 1996). In others, the histopathology is not congruent with these disorders, raising the

possibility that some of the non-Alzheimer's dementias represent unique new diseases.

Pathologically, patients with FTD linked to chromosome 17 have variable involvement of basal ganglia and substantia nigra, may or may not have Pick bodies and ballooned neurons, and may or may not exhibit involvement of the spinal motor neurons (Foster *et al.*, 1997). Thus, no specific clinical syndrome or set of pathological changes identifies cases linked to chromosome 17 versus those without such linkage. Conversely, specific types of histopathological changes are not indicative of a corresponding unique genetic aetiology. The clinical and pathological heterogeneity associated with specific mutations suggests that additional genetic or environmental factors substantially modify the expression of the underlying genetic change.

Autopsied individuals from some kindreds with FTD display abundant neurofibrillary change, while others, including a single affected individual from Duke Family 1684, lack distinctive histological features and exhibit only mild neuronal loss and gliosis in limbic structures and subcortical nuclei when examined by routine silver stain (Hulette *et al.*, 1999).

Microscopically, there is microvacuolation and gliosis predominantly of the outer three laminae of cerebral cortex. Neurons are lost in laminae 2 and 3, but lamina 4 is relatively spared (Jackson and Lowe, 1996). No classic Pick bodies, ballooned neurons or Lewy bodies are seen. No neuronal inclusions immunoreactive to tau or ubiquitin are seen. The microscopic alterations are most marked in the frontal cortex. In some cases, there is mild to moderate loss of pigmented neurons of the substantia nigra (Jackson and Lowe, 1996). Patients exhibiting frontal lobe dementia plus these neuropathological findings are now classified has having FTD of the frontal lobe degeneration type.

Some patients have the clinical features of frontal lobe dementia but have much more pronounced frontal lobe atrophy; also, in addition to microvacuolation, gliosis and neuronal loss, they have ballooned or inflated neurons and Pick bodies. The gliosis may extend throughout the cortex and into the white matter. Such patients are classified as having FTD of the Pick type. This semantic manoeuvre leaves open the possibility that these patients have Pick's disease whilst maintaining sufficient flexibility to encompass atypical cases.

Finally, there is FTD of the motor neuron type, in which the previously described clinical and neuropathological findings are coupled with spinal motor neuron degeneration. The motor neuron loss is usually most severe in the cervical and thoracic segments. In Japan and, more recently, in Western countries, sporadic cases of ALS plus dementia have been described and designated amyotrophy dementia complex, which appears to be identical to FTD of the motor neuron type (Tsuchiya *et al.*, 2001; Mitsuyama, 2000).

Until recently, the presence of ubiquitin-positive intraneuronal inclusions in the dentate gyrus and the temporal and frontal cortex was usually associated with the motor neuron disease type. Such inclusions were also observed in a few sporadic cases of FTD with or without parkinsonism in the absence of motor neuron disease. We present here clinical, neuropathological and immunohistochemical data about a Swiss FTD family with FTD with parkinsonism-like features but without motor neuron disease. Spongiosis and mild gliosis were observed in the grey matter. No neurofibrillary tangles, Pick bodies, Lewy bodies, senile plaques or prion-positive signals were present. However, ubiquitin-positive intracytoplasmic inclusions were detected in various structures, but predominantly in the dentate gyrus. These observations support the existence of a familial form of FTD with parkinsonism with ubiquitin-positive intracytoplasmic inclusions (Kovari *et al.*, 2000).

The authors report a presenilin 1 (PSEN1) mutation (L113P) in a family with six cases of dementia. The patients had personality changes and behavioural disorders, whereas spatial orientation and praxis were preserved late in the course of the illness. Neuroimaging features were consistent with the diagnosis of FTD. The authors conclude that PSEN1 mutations can be associated with clinical features of frontotemporal dementia (Raux *et al.*, 2000).

Progressive Supranuclear Palsy

Clinical Findings

PSP, or Steele–Richardson–Olszewski syndrome, is characterized by supranuclear ophthalmoparesis (down gaze in particular), rigidity and gait disturbance. Furthermore, about 70% of PSP patients may exhibit dementia with signs of frontal dysfunction. Conversely, there are familial cases of FTD with tau mutations, such as pallidoponto-nigral degeneration, in which patients develop a progressive PSP-like syndrome followed by FTD (Reed *et al.*, 1998). Also, there are striking parallels between the neuropathology of PSP and FTD, and abnormalities in tau appear to lie at the pathological (Dickson *et al.*, 1996) and genetic (Baker *et al.*, 1997) basis for PSP and FTD.

Neuropathological Findings

Macroscopic examination of the brain shows cortical, subcortical and ponto-mesencephalic atrophy (Bigio *et al.*, 1999; Lantos, 1994). There is widespread diencephalic, mesencephalic, brainstem and cerebellar nuclear neuronal loss. Globose neurofibrillary tangles and variable neuron loss with gliosis occur in the globus pallidus, subthalamic nucleus, periaqueductal grey matter and substantia nigra. PSP is characterized neuropathologically by neuronal degeneration of the basal ganglia, brainstem and cerebellum. In addition, cortical neuronal degeneration associated with neurofibrillary tangles formation has been identified over wide areas of the brain in patients with PSP.

Hauw *et al.* (1994) published neuropathological criteria for PSP as proposed at a workshop held at the National Institutes of Health in 1993. The criteria distinguish typical, atypical and combined PSP. A high density of neurofibrillary tangles and neuropil threads in the basal ganglia and brainstem is crucial for the diagnosis of typical PSP. Tau-positive astrocytes or their processes in areas of involvement help to confirm the diagnosis. Atypical cases of PSP are variants in which the severity or distribution of abnormalities deviates from the typical pattern. Criteria excluding the diagnosis of typical and atypical PSP are large or numerous infarcts, marked diffuse or focal atrophy, Lewy bodies, changes diagnostic of Alzheimer's disease, oligodendroglial argyrophilic inclusions, Pick bodies, diffuse spongiosis, and prion protein-positive amyloid plaques. The diagnosis of combined PSP is proposed when other neurological disorders exist concomitantly with PSP (Hauw *et al.*, 1994).

Patients with PSP often develop dementia, and cortical pathology has been documented in PSP. Bigio *et al.* (1999) found striking cortical tau pathology in demented PSP patients, suggesting that neurofibrillary pathology is central to the cause of dementia in PSP. Cordato *et al.* (2000) found that PSP could be differentiated from other dementias by the marked atrophy of the internal globus pallidus.

The presence of tau-positive glial inclusions has been found to be a consistent feature in the brains of patients with PSP, CBD and Pick's disease. These inclusions are classified based on cellular origin as tau-positive astrocytes, presumably either fibrillary or protoplasmic, coiled bodies and glial threads. Immunohistochemically, their major structural component is abnormal tau proteins, similar to those found in Alzheimer's disease. Nevertheless, their morphology, including ultrastructural profile, has been suggested to be distinctive for each disease. The profile and extent of particular glial inclusions correlate well with disease phenotype. Highly

characteristic correlations include tufts of abnormal fibres in PSP, astrocytic plaques and dense glial threads in CBD, and ramified astrocytes and small Pick-body-like inclusions in Pick's disease (Komori, 1999).

Severe neurodegeneration within the dopaminergic pars compacta was seen in all cases of PSP. There was a striking 70% reduction in the number of pars reticulata neurons in PSP, with no cell loss observed in Parkinson's disease compared with controls (Hardman *et al.*, 1997).

Mackenzie and Hudson (1995) found significant numbers of achromatic neurons in the cerebral cortex in five out of seven cases that had been diagnosed pathologically as PSP.

We studied the distribution of alpha B-crystallin-positive degenerating neurons in cases with PSP and compared them with those in Pick's disease, Alzheimer's disease, senile dementia of Alzheimer type, and normal aged individuals. A large number of alpha B-crystallin-positive neurons was found in the cerebral cortices of four out of nine patients with PSP. In particular, alpha B-crystallin-positive ballooned neurons were observed frequently in deep cortical pyramidal cell layers of the limbic and paralimbic systems in these diseases. The involvement of the limbic and paralimbic systems may thus contribute to personality changes as well as to memory and cognitive impairment in some patients with PSP (Higuchi *et al.*, 1995).

Multiple System Atrophy

Clinical Findings

This group of disorders is characterized by extreme clinical variability and nosological uncertainty. Multiple system atrophies (MSAs) can be familial and are then transmitted either dominantly or recessively, but sporadic cases are very common. Olivopontocerebellar atrophy (OPCA) is one of the commonest and most variable of these conditions. Ataxia, rigidity, spasticity and oculomotor movement disturbances are present to variable degrees in combination. OPCA is currently classified as one of the MSAs, which also include striatonigral atrophy and the Shy–Drager syndrome.

Neuropathology

There is considerable clinical and pathological overlap among these syndromes. Glial cytoplasmic inclusions have been recognized as apparently unique cellular pathological markers of MSA. These are intracytoplasmic predominantly oligodendroglial argyrophilic inclusions that exhibit strong tau and ubiquitin immunoreactivity. Oligodendroglial cytoplasmic inclusion-rich structures occur in the suprasegmental motor systems, in the supraspinal autonomic systems and in their targets. In contrast, the visual and auditory pathways, olfactory structures, somatosensory systems, association and limbic cortical areas and subcortical limbic structures contain no or only a few glial cytoplasmic inclusions (Papp and Lantos, 1994). Alpha-synuclein has been implicated as a major component of the abnormal filaments that form glial cytoplasmic inclusions in MSA (Duda *et al.*, 2000).

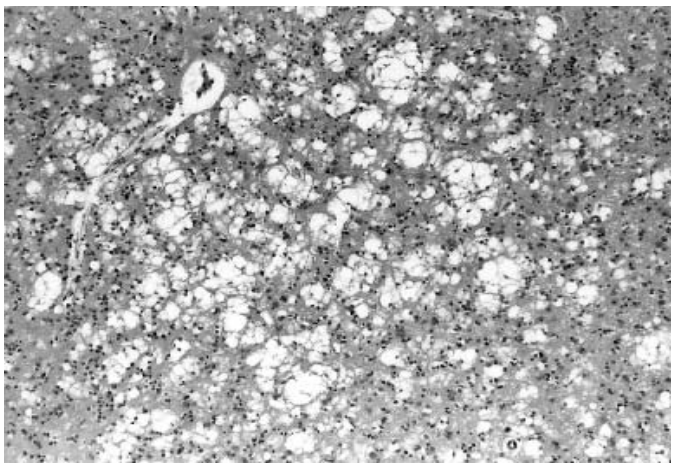
Transmissible Spongiform Encephalopathies

Clinical Characteristics

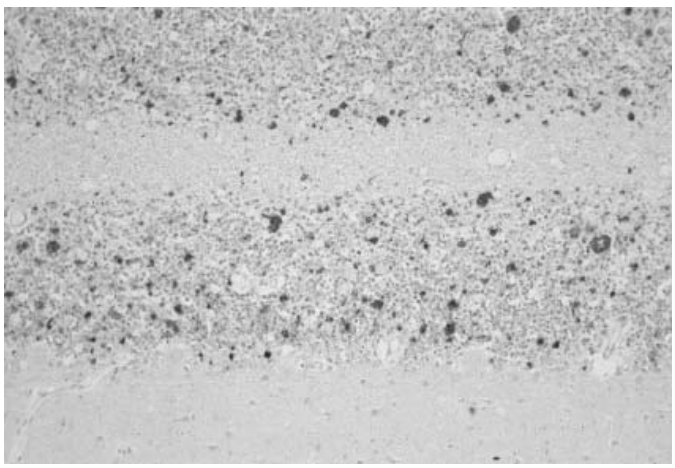
CJD is a rare spongiform encephalopathy that affects about one per one million inhabitants in most countries (Will, 1993). CJD is a transmissible spongiform encephalopathy characterized by a rapidly developing dementia associated with cerebellar, pyramidal and extrapyramidal signs, myoclonus and typical electroencephalographic (EEG) changes. The disease can be transmitted by direct intracerebral inoculation of infectious brain tissue, dura and cornea transplants, and injections of contaminated human growth and gonadotropic hormones. Prion diseases or transmissible spongiform encephalopathies affect both humans and animals. They include CJD, Kuru, Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia, and some rare atypical dementias. A further classification of CJD can be made according to the mode of transmission: sporadic, iatrogenic, familial and the recently described variant CJD (vCJD), which has been linked to bovine spongiform encephalopathy (BSE) (Gambetti, 1999).

Neuropathology

Human spongiform encephalopathies display a large array of histopathological phenotypes. Three basic histological lesions can be observed in prion diseases: spongiosis, astrogliosis and neuronal loss (Figure XV-9.8). Prion protein deposits and rarely neurofibrillary tangles can be demonstrated with appropriate pretreatment and monoclonal antibodies. The relative amounts of these individual basic lesions and their rostrocaudal distribution within the brain result in the three basic pathological phenotypes that are commonly



(a)



(b)

Figure XV-9.8 Neuronal loss, spongiosis and reactive gliosis in the cortex of a patient with CJD. (a) Haematoxylin eosin stain (magnification 145×); (b) prion protein immunostain (magnification 320×)

distinguished in prion diseases — CJD, fatal familial insomnia (FFI) and GSS — as well as in the variants within these major phenotypes. A minority of cases, generally associated with insertion mutation, cannot be accommodated in any of these phenotypes and form a heterogeneous group (Gambetti, 1999).

Inherited Prion Diseases

At present, there are 23 known mutations and three polymorphisms in the prion protein gene (PRNP). These mutations are of three types: (1) point mutations, which include 14 mutations distributed predominantly in the carboxyl two-thirds of the PRNP coding regions; (2) insertional mutations, made of 24 additional base-pair repeats located between codons 51 and 91; and (3) stop codon mutations, one of which is known to result in the premature termination of synthesis and to yield a truncated prion protein. Moreover, the 129 M/V polymorphism may modify the expression of the mutation, resulting in a different phenotype according to the codon 129 present in the mutant allele. Therefore, it is more appropriate to identify each PRNP genotype associated with inherited prion diseases not only by the mutation but also by the codon 129 polymorphism. Currently, 28 disease-associated PRNP haplotypes are known that result in CJD, fatal familial insomnia, GSS, mixed CJD and GSS, and undefined phenotypes. The latter two phenotypes are seen in the insertion mutations (Gambetti, 1999).

Neuropathology of the Inherited Creutzfeldt-Jakob Disease Phenotype

The histopathology of inherited CJD is very similar to that of sporadic CJD and is characterized by spongiosis, gliosis and neuronal loss of the cerebral cortex, basal ganglia and, to a lesser extent, diencephalon and cerebellum. Astrogliosis and neuronal loss appear to be a function of the disease duration. The histopathological pattern is remarkable for the intense cerebral cortical gliosis often with large gemistocytic astrocytes and the loss of neurons, while many of those remaining are ballooned resulting in the disorganization of the cerebral cortical layers. Spongiosis is invariably present throughout the cerebral cortex; it is more severe at the level of the basal ganglia, while it is mild in the thalamus and brainstem and absent in the cerebellum (Gambetti, 1999).

Fatal Familial Insomnia Phenotype

The mean age at onset for 45 proven patients with fatal familial insomnia (30 homozygotes, 15 heterozygotes) is 50 ± 10 years (range 25–72 years) in homozygotes and 45 ± 11 years (range 20–56 years) in heterozygotes. The mean clinical course is 12 ± 4 months (range 6–21 years) in homozygotes and 21 ± 15 months (range 7–33 years) in heterozygotes (Gambetti *et al.*, 1995). Insomnia is an early symptom that progresses in some months to a total or almost total inability to sleep. The insomnia is not the trivial difficulty in initiating or maintaining sleep, as in other neurodegenerative diseases, but rather a severe, persistent and complete disorganization of sleep cycles. The inability to sleep is a more prominent early symptom that deteriorated rapidly in 129 homozygous patients. Indifference to surroundings, inability to express feelings or emotions, and prolonged episodes of stupor associated with automatic behaviour, such as enacting dreams, are other prominent clinical features. Coma precedes death by days or weeks. Difficulties in micturition, impotence, lacrimation, salivation and sweating, increased body temperature, high heart rate, and high blood pressure usually appear in the earlier stages of the disease. Tachypnoea, paradoxical breathing, loud snoring and obstructive apnoeas are later signs. Dysarthria and ataxic gait are the first motor disturbances and usually appear some months after insomnia and dysautonomia. Speech becomes progressively unintelligible; ataxic gait can evolve to astasia-abasia. Segmental and diffuse, spontaneous and evoked myoclonus can appear transiently. Motor disorders were more severe in 129 heterozygous patients. Diplopia and saccadic ocular movements, sporadic generalized tonic-clonic seizures, and dystonic attacks are transient and less consistent late signs. A progressive impairment of vigilance, attention and visuomotor performances associated with selective memory disturbances and behavioural features of a confusional state in the absence of a global decline in intellectual function are common findings. EEG background activity becomes progressively slow and less reactive. Visual, brainstem and somatosensory evoked potentials are normal. Sympathetic skin response is reduced or abolished. The duration of sleep EEG activity shortens and results in a complete or nearly complete inability to generate sleep patterns. Sleep spindle activities disappear. Only brief episodes of REM sleep or, more seldom, slow-wave sleep arise abruptly and randomly from wakefulness. Total sleep time decreases progressively. Body temperature, breathing and heart rates, and systemic blood pressure are persistently elevated, with reduced circadian oscillations. Cardiovascular balance is lost, with higher background and stimulated sympathetic activity. Adrenaline and noradrenaline plasma concentrations are persistently and markedly elevated. The hypothalamic-pituitary function is normal. Plasma cortisol concentration is abnormally high; corticotropin is constantly low. In addition, sleep-coupled (growth hormone, prolactin) and uncoupled (cortisol, corticotropin) hormones lack circadian oscillations. The nocturnal increase in melatonin secretion subsides progressively.

CT and MRI are normal. Positron emission tomography (PET) scan in seven patients showed an early severe hypometabolism of the thalamus associated with a milder impairment of the cingulate gyrus. The cerebral metabolic deficit is more widespread in heterozygotes (M/V) at codon 129, who have a longer clinical course.

The hallmark is loss of neurons and astrogliosis in the thalamus, which is present in all subjects, independently of the disease duration (Gambetti, 1999). The mediodorsal and anterior ventral thalamic nuclei are invariably and severely affected, while the involvement of other thalamic nuclei varies. The inferior olives also show neuronal loss and gliosis in most cases. In contrast, the pathology of the cerebral cortex varies in proportion to the disease duration and is more severe in the limbic lobe than in the neocortex. The entorhinal cortex and, to a lesser extent, the piriform and paraolfactory cortices show spongiosis and astrogliosis in most subjects. The neocortex is spared in subjects with a disease duration of less than 1 year, is focally affected by spongiosis and gliosis in those with a course between 12 and 20 months, and is diffusely involved only in subjects with a disease of longer duration (more than 20 months). In addition, the frontal, temporal and parietal lobes are affected more severely than the occipital lobe. The other structures are virtually normal or show only mild focal pathology. Overall, the thalamus is involved more severely and consistently than any other brain region. Therefore, on the basis of the pathology, fatal familial insomnia can be defined as a preferential thalamic degeneration (Gambetti, 1999).

Gerstmann–Sträussler–Scheinker Syndrome Phenotype

The GSS phenotype has only been observed linked to PRNP mutations. Currently, six point mutations in PRNP have been found to be associated with GSS. All variants share a relatively chronic course (longer than 2 years in most cases) and the presence of Congo red positive amyloid plaques composed of prion protein fragments. Despite this, they show striking phenotypic differences. The onset can be characterized by ataxia (102 and 117 mutations), spastic

paraparesis (105 mutation), extrapyramidal signs (217 mutation) or dementia (145, and 117 mutations). Pathologically, the amyloid plaques can coexist with significant spongiform degeneration (102 mutation), neurofibrillary degeneration (145, 198 and 217 mutations) or amyloid angiopathy (145 mutation).

Codon 129 has been shown to influence age at onset and duration of the disease in the phenotype associated with the 198 mutation. More recently, codons 129 and 219 on the mutant alleles have been shown to affect basic features of the phenotype of the P102L mutation as it was originally shown to occur in the D178N mutation associated with FFI and CJD (Gambetti, 1999).

Familial Creutzfeldt–Jakob Disease with Insertions

The PRNP gene has an unstable region of five variant octapeptide coding repeats between codons 51 and 91. Insertions ranging from one to nine extra repeats in this region have been found in 12 families and nine affected individuals from uninformative families (Gambetti, 1999). The phenotype associated with the insertion mutations shows a high variability, particularly in the duration of the disease, which ranges between 3 months and 15 years. The clinicopathological features range from the typical CJD phenotype, i.e. rapidly progressive dementia of less than 1 year duration, to a chronic disease of several years, lacking specific histopathological changes and, therefore, difficult to include in the CJD, GSS and fatal familial insomnia phenotypes. In this group, the common presenting signs include frontal release, dysphasia, apraxia, visual disturbances or personality disorders associated with memory loss. Cerebellar, extrapyramidal and pyramidal signs are also common. Chorea and generalized seizures are rare. EEG is either normal or characterized by a slow-wave activity. Eight cases out of the 34 reported had bilateral synchronous repetitive triphasic discharges, usually at a late stage of the disease. Among the 18 autopsied cases of this group, only six showed classic CJD pathology, five showed a mild and focal spongiosis, and in the others there were no distinctive pathological features (Gambetti, 1999).

The molecular basis underlying this variable phenotype is largely unknown. Patients carrying the shortest insertions (from one to four repeats) show the typical clinical CJD phenotype more often than those carrying an insertion longer than four repeats (67% versus 8%). In contrast, subjects with long insertions are more likely to display a long duration with atypical features. In addition, patients that carry the shortest insertion usually have a negative family history (100%), whereas those with an insertion longer than four repeats are more likely to be familial (82%). Thus, both the penetrance and the phenotypic expression appear to be influenced by the length of the extra repeat insertion.

The correlation between length of the extra repeat insertion and the disease phenotype also extends to the neuropathological features of the disease (Gambetti, 1999). While widespread spongiform degeneration is the typical finding in those patients carrying insertions of one to four extra repeats, in those with an insertion longer than four repeats the neuropathological changes are extremely variable, ranging from severe spongiform encephalopathy to cases lacking any obvious pathology. Preliminary analysis indicates that the number of the inserted octapeptide repeats and, again, the genotype at codon 129 of PRNP may play a role as modulator of the phenotypic expression of the disease.

Diffuse Axonal Injury

Some head-injured patients are severely impaired in the absence of gross lacerations or haematomas. These patients are generally comatose from the instant of injury and subsequently have only limited recovery. They have sustained widespread microscopic axonal injury evidenced by the presence of ruptured axons that retract to form spheroids. This syndrome is called diffuse axonal injury (DAI), and is believed to result from shearing forces that damage axons during acceleration or deceleration. There is strong evidence that this axonal alteration matures over a period of hours to days, and may therefore be amenable to therapeutic intervention. Initially, the axons appear normal, but soon there is focal accumulation of axoplasmic cytoskeletal components and organelles, indicating a malfunction of axonal transport. After several hours of increasing focal swelling, the axon splits and the severed ends retract. In grade 1 DAI, there is histological evidence of axonal injury in the white matter of the cerebral hemispheres, corpus callosum, brainstem and, less commonly, cerebellum. In grade 2 DAI, there is also a focal lesion in the corpus callosum. In grade 3 DAI, there is, in addition, a focal lesion in the dorsolateral quadrant or quadrants of the rostral brainstem. The focal lesions can often only be identified microscopically. DAI was identified in 122 patients of a series of 434 fatal non-missile head injuries (10 grade 1, 29 grade 2, 83 grade 3) (Adams *et al.*, 1989). In a series of 53 children with fatal head trauma, severe hypoxic brain damage was present in 77% of cases. While vascular axonal damage was found in 21 out of 53 cases, DAI was present in only three. Eleven additional cases, all infants, showed evidence of localized axonal injury to the craniocervical junction or the cervical cord (Geddes *et al.*, 2001). Study of DAI is providing insight into the brain's acute stress response, which may in turn give us clues about the cellular stress response in other neurological disorders. DAI, for example, results in a rapid upregulation of APP expression and formation of diffuse neuritic plaques.

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XV-10

Brain Imaging of Cognitive Disorders

P.H. Robert, J. Darcourt and M. Benoit

Imaging techniques are major tools for investigating brain–behaviour relationships. Brain imaging is one of the main instruments used in cognitive neuroscience, allowing the visualization and measurement of processes in the living brain. Measuring regional brain activity with general markers of brain function, such as blood flow and metabolic activity, is a highly successful way of elucidating the neuroanatomic substrates of cognitive processes. Using a subtraction method and dynamic positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), it becomes possible to study the functional neuroanatomy of the healthy human brain during cognitive tasks. The main challenge is to design two tasks that differ in only one meaningful component. After subtraction, the resulting image shows a pattern of activation that is assumed to represent local blood flow changes associated with this task component.

In clinical practice, brain imaging is used as a complementary and powerful diagnostic tool in dementia and other neuropsychiatric diseases.

Brain imaging includes structural techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), and functional techniques, such as PET, single positron emission computed tomography (SPECT), fMRI and magnetic resonance spectroscopy (MRS).

This chapter reviews structural and functional brain imaging findings in the main clinical syndromes chiefly characterized by cognitive disturbances.

ALZHEIMER'S DISEASE

Alzheimer's disease is characterized clinically by cognitive manifestations, which, in combination with behavioural disturbances, interfere significantly with activities of daily living. Memory impairment is an early and prominent cognitive symptom. Progression of the disease over time affects several brain regions, starting from the hippocampal, parahippocampal structures and entorhinal cortex, and extending to the cortical region, especially the posterotemporal and inferotemporal lobes (Braak and Braak, 1991; Geddes *et al.*, 1997).

Structural Imaging

CT is the oldest imaging technique used for scanning the brain. Today, in dementia CT is used only for differential diagnosis in order to exclude subdural haematoma, normal-pressure hydrocephalus, and other brain diseases such as tumours.

MRI provides the same information as CT but is more powerful in separating grey from white-matter signals and is not hampered by bone artefacts. It is useful for showing morphological modifications, such as cortical atrophy, ventricular dilation and enlargement of the subarachnoid spaces. Several studies (George *et al.*, 1987; Kido *et al.*, 1987) have shown temporal atrophy in early Alzheimer's disease. As a result, the internal temporal region has been a major focus of attention.

Linear and volumetric measurements of the hippocampus (Kesslak *et al.*, 1991), the parahippocampic gyrus (Ikeda *et al.*, 1994), the amygdala (Lehericy *et al.*, 1994), the subiculum and the perihippocampal clefts have been made (De Leon *et al.*, 1993). In Alzheimer's disease patients, these studies generally show marked atrophy (30–40%) relative to healthy controls. In the De Leon *et al.*, study of 80 subjects free of dementia at baseline, of the 25 patients who developed Alzheimer's disease 4 years later, 23 showed an increase of perihippocampal cerebrospinal fluid on baseline CT (91% sensitivity and 89% specificity). In the same way, the loss of brain volume, measured by MRI (Fox *et al.*, 1999), correlated with the global cognitive severity of dementia.

By studying histological sections of brains from patients with autopsy-confirmed Alzheimer's disease ($n = 16$) and from normal controls ($n = 4$), Bobinski *et al.* (1999) validated a method for measuring entorhinal cortex volume that relies on gyral and sulcal landmarks visible on magnetic resonance images. In another study (Bobinski *et al.*, 2000), the calculated number of neurons in the hippocampus and hippocampus/subiculum correlated strongly with the MRI-measured volume of these structures. Overall, the best MRI predictor of Alzheimer's disease is medial temporal lobe atrophy combined with medial occipito-temporal gyrus atrophy (Convit *et al.*, 2000). However, large studies of the specificity of these measurements are still needed.

Functional Imaging

The neurons affected in Alzheimer's disease are predominantly projection neurons. This neuronal alteration is reflected by a dysfunction of association cortical areas. This is supported, by PET data on regional cerebral glucose metabolism (regional cerebral metabolic rate of glucose, rCMRGL) reported consistently by many authors (Haxby *et al.*, 1986; McGeer *et al.*, 1986). These results indicate a typical pattern of hypometabolism that is most prominent in the temporoparietal region but is also present in frontal cortical association areas. Primary visual and sensory motor cortices are typically spared. SPECT studies (Bartenstein *et al.*, 1997; Jobst *et al.*, 1994; Hirsch *et al.*, 1997) have shown a reduction of regional cerebral blood flow (rCBF) in the same regions. Using hexamethyl propylene amineoxime (HMPAO) SPECT, Holman *et al.* (1992) found that patients with bilateral temporoparietal hypoperfusion had the highest probability of having Alzheimer's disease.

A software package for PET and SPECT (Frackowiak *et al.*, 1997), known as statistical parametric mapping (SPM), allows spatial normalization of images to a standardized stereotactic

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space and statistical group image analysis. Using this technique, Kogure *et al.* (2000) demonstrated that rCBF was significantly bilaterally decreased in the posterior cingulate and precunei of patients with early mild Alzheimer's disease compared with healthy volunteers.

Studies correlating brain imaging data with clinical features and disease progression are also of great interest. Several authors (Robert *et al.*, 1992; Imran *et al.*, 1999; Ashford *et al.*, 2000) have shown that rCBF in the temporoparietal areas correlated negatively with the global severity of the disease. More specifically, PET studies of resting brain glucose utilization were conducted to determine the neural basis of the memory impairment in Alzheimer's disease. Verbal episodic memory was related to changes in a network including the mesial temporal cortex, thalamus, cingulate gyrus (left predominance) and parietotemporal and frontal association cortices of the right hemisphere (Desgranges *et al.*, 1998), whereas semantic memory performance correlated with activity in the left inferior temporal gyrus (Hirono *et al.*, 2001). Other studies showed that functional brain imaging can predict the rate of cognitive decline in Alzheimer's disease (Jagust *et al.*, 1996; Claus *et al.*, 1999).

Functional brain imaging is also useful for understanding the origin of behavioural disturbances observed frequently in Alzheimer's disease. Recently, SPECT was used to compare the rCBF pattern in two groups of ten demented subjects with and without aggression and agitation. Subjects with aggression had significant hypoperfusion of the left anterior temporal cortex and the right and left dorsolateral frontal cortex (Hirono *et al.*, 2000).

Apathy is another frequent behavioural manifestation of Alzheimer's disease and other organic brain disorders in the elderly. It is defined as a lack of motivation not attributable to a diminished level of consciousness, cognitive impairment or emotional distress (Marin *et al.*, 1991; Marin, 1995). SPECT studies of Alzheimer's disease subjects have shown a correlation between apathy and frontal and cingulate hypoperfusion (Craig *et al.*, 1996; Benoit *et al.*, 1999). In another study (Migneco *et al.*, 2001), 41 elderly subjects with Alzheimer's disease or organic personality disorders with dysexecutive syndrome were subdivided according to the presence or absence of apathy. SPECT images were compared using SPM. The major difference between subjects without and with apathy is the presence of an anterior cingulate hypoperfusion in the latter group (Figure XV-10.1). Using the same method, Alzheimer's disease patients with and without apathy were compared with healthy elderly controls (Figure XV-10.2). The apathy-free group had significantly lower perfusion of the inferior temporal and occipital regions. The apathy group had significantly decreased perfusion of the left anterior cingulate, the right inferior and medial gyrus frontalis, the left orbitofrontal gyrus, and the right gyrus lingualis. The differences in the brain areas with reduced perfusion between the apathy-free subjects (mainly the posterior regions) and the apathetic subjects (mainly the anterior regions) could indicate that behavioural disorders such as apathy participate in the heterogeneity of brain perfusion in Alzheimer's disease.

In the future, techniques such as SPM will play an important role in, for example, monitoring responses to cholinergic treatment of Alzheimer's disease.

Finally, brain imaging has also been used to map neurochemical processes in Alzheimer's disease. As a cholinergic deficit is most consistently found in the disease, major efforts have been made to develop PET or SPECT tracers of cholinergic systems. The density of muscarinic and nicotinic receptors (Nordberg *et al.*, 1995) appears to be diminished in the cortex of patients with Alzheimer's disease. However, methodologic limitations — due mainly to the influence of local changes in brain perfusion on cortical abnormalities in radioligand uptake — make it difficult to determine the pathophysiological significance of these results.

Recently, more specific tracers of cortical cholinergic function have been developed and modelled, such as markers of acetylcholinesterase, the acetylcholine-degrading enzyme, and ligands of vesicular acetylcholine transporters, which are potential markers of the integrity of cholinergic nerve endings. Other neurochemical anomalies have been identified *in vivo* in Alzheimer's disease. A marked cortical reduction in serotonin 5-HT2 receptors was found by Blin *et al.* (1993), affecting parietal and frontal cortices and, to a lesser extent, the occipital cortex. A cortical reduction in benzodiazepine receptors has also been described (Soricelli *et al.*, 1996).

Proton MRS (^{1}H-MRS) and phosphorus MRS (^{31}P-MRS) are other opportunities for studying the biochemical composition of brain tissue *in vivo*. The most consistent results come from ^{1}H-MRS concerning the amino acid *N*-acetylaspartate (NAA) localized exclusively in the neurons and the sugar-alcohol myo-inositol, which could be a marker of glial cell number. Several studies found a 15% decrease of NAA in the temporoparietal regions and the occipital and frontal lobes, and a 20% increase of myo-inositol in white and grey matter in Alzheimer's disease patients. The NAA/myo-inositol ratio as been proposed for discriminating

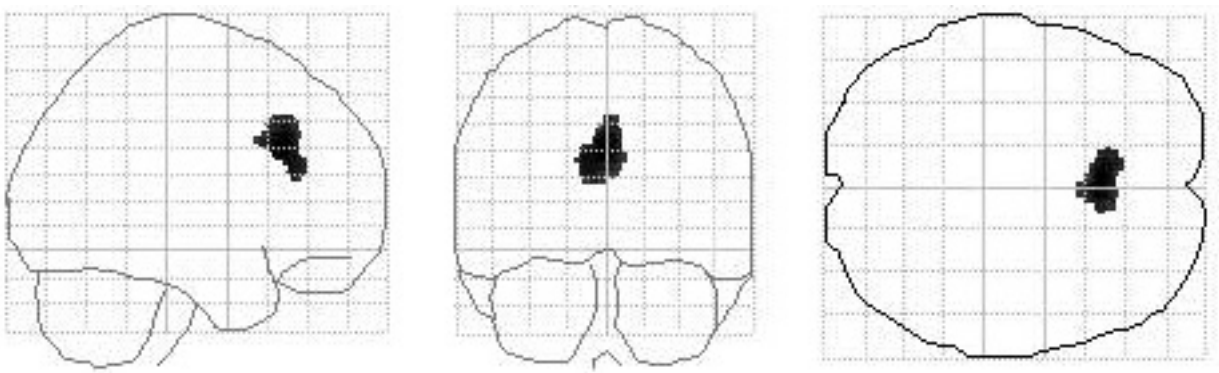

Figure XV-10.1 Anatomical correlate of apathy. Forty-one subjects with Alzheimer's disease or organic personality disorders according to ICD-10 diagnostic criteria were subdivided into two group of subjects: 21 subjects were apathetic and 20 were not. The groups were comparable in terms of age, sex, mini-mental state examination and diagnostic repartition. Brain perfusion was measured by ethylene-cysteine-dimer bicisate (ECD) brain SPECT, and the images were compared using SPM software. The Z map showed significant decreased ECD uptake for the apathetic patients in the anterior cingulate

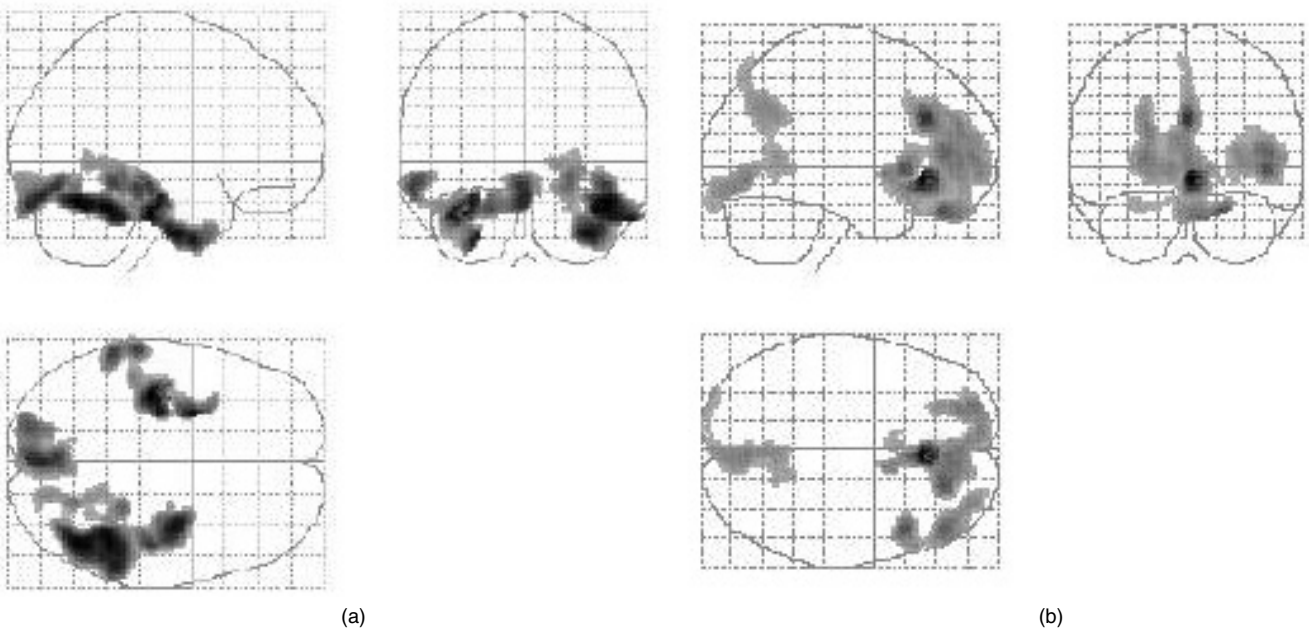

Figure XV-10.2 Fifteen Alzheimer's disease patients without apathy (mean age 76.6 years, SD 5.1) and 15 Alzheimer's disease patients with apathy (mean age 77.6 years, SD 6.1) were studied. The Neuropsychiatric Inventory (NPI) was used to evaluate apathy. Brain perfusion was measured by ^{99m}Tc-labelled ethylene-cysteine-dimer bicisate (ECD) SPECT. The images of the two groups (AD-NA, Alzheimer's disease without apathy; AD-A, Alzheimer's disease with apathy) were compared by SPM with corresponding images of 11 healthy elderly control subjects (obtained from the Society of Nuclear Medicine database). (a) AD-NA versus control; (b) AD-A versus control

Alzheimer's disease patients from aged-matched, controls and has been shown to correlate with mini-mental status examination (MMSE) scores.

MILD COGNITIVE IMPAIRMENT

Another important challenge is to predict the development of Alzheimer's disease in patients with mild cognitive impairment (MCI) in order to treat them early (Scheltens and Korf, 2000). Subjects with MCI have a memory impairment beyond that expected on the basis of age and education, yet they are not demented (Petersen *et al.*, 1999). The diagnosis of MCI is made if the patient meets the following criteria: (1) memory complaints, (2) normal activities of daily living, (3) normal general cognitive function, and (4) abnormal memory for age. As indicated earlier, the entorhinal cortex and hippocampus may be involved before the neocortex in Alzheimer's disease, and massive loss of entorhinal neurons occurs even in very mild cognitive impairment. In an MRI study, Jack *et al.* (2000) identified 129 subjects who met established criteria for normal control subjects, MCI and probable Alzheimer's disease, both at inclusion and at clinical follow-up 3 ± 1 years later. The rates of hippocampal atrophy matched both baseline cognitive status and the change in cognitive status over time in these elderly subjects who lay along the cognitive continuum from normal over MCI to Alzheimer's disease. In the very early stages of Alzheimer's disease, several studies suggest that variants of the apolipoprotein E gene account for the majority of cases of late-onset Alzheimer's disease (Corder *et al.*, 1993; Corder *et al.*, 1994; Roses *et al.*, 1994): people carrying two copies of the E4 allele have a higher risk of Alzheimer's disease. In an rCMRGL PET study, Reiman *et al.* (1996) compared cognitively normal elderly subjects who reported a family history of probable Alzheimer's disease, on the basis of the E4 allele. Only E4 homozygotes had significantly reduced glucose metabolism in the same posterior cingulate, parietal, temporal and prefrontal regions as in previously studied patients with probable Alzheimer's disease. This underlines the role of brain imaging in the early stages of Alzheimer's disease.

VASCULAR DEMENTIA

Vascular factors are recognized increasingly to be important causes of cognitive impairment in the elderly. Even in subjects with MCI without signs of cerebrovascular disease, CT reveals more frequently extensive white-matter abnormalities (Wolf *et al.*, 2000). Two types of vascular disease potentially causing cognitive dysfunction are usually considered. The first is due to subcortical white-matter vascular damage related to microangiopathy; the second is due to multiple small cortical infarcts (multi-infarct dementia) and is related to macroangiopathy. Both can be associated with atrophy.

These white-matter lesions, also called leukoaraiosis, can be patchy or diffuse; they are associated with thickening of deep arterioles and they are nonspecific. Garde *et al.* (2000) carried out a standardized neuropsychological assessment of a cohort of almost 700 subjects born in 1914, at ages 50, 60, 70 and 80 years. Of the 129 subjects tested fully at age 80 years, 68 also underwent MRI scanning. The degree of white-matter changes was related significantly to the decline in performance intelligent quotient on the Wechsler Intelligence Scale (WAIS). However, large studies of elderly populations with serial MRI and neuropsychological testing are scarce.

In multi-infarct dementia, randomly distributed multiple transcortical defects are detected easily by perfusion SPECT or fluorodeoxyglucose (FDG) PET. However, white-matter changes are difficult to assess by these techniques. These limitations do not apply to morphological imaging: lacunar infarcts and deep white-matter lesions are detected readily by CT and MRI, but they are not seen on SPECT or PET. However, these lesions are only epiphenomenona, morphologically characterizing cerebral

microangiopathy. Related neuropsychological deficits are essentially reflected by cortical dysfunction revealed by PET and SPECT (Mori *et al.*, 1999; Sabri *et al.*, 1998) in the absence of cerebral atrophy. If present, atrophy becomes the main determinant of neuropsychological impairment (Sabri *et al.*, 1995). Recently, a relationship between subcortical hyperintensities on MRI, cortical perfusion on SPECT, and cognitive function were studied in 26 patients with vascular dementia (Cohen *et al.*, 2001). The results suggest a complex relationship: subcortical hyperintensities on MRI correlated with frontal lobe perfusion but not with global brain perfusion measured by SPECT. However, frontal lobe perfusion did not correlate reliably with performance on executive function tests.

Using ^{1}H-MRS, cortical metabolic abnormalities through reduction of NAA concentrations secondary to subcortical vascular neuronal loss were also found. Interestingly, in this study decrease of hippocampal NAA was observed specifically in Alzheimer's disease and not in subcortical ischaemic vascular dementia.

FRONTOTEMPORAL LOBAR DEGENERATION

Circumscribed atrophy of the frontal and temporal lobes has been grouped into three prototypical clinical syndromes designated as frontotemporal lobar degeneration (FTLD). FTLD accounts for about one-fifth of primary degenerative dementia cases occurring before age 65 years. A consensus on the diagnostic criteria of FTLD includes functional brain imaging characteristics (Neary *et al.*, 1998).

The most common form of FTLD is frontotemporal dementia, characterized by personality changes, profoundly altered social behaviour, and bilateral atrophy of the frontal and anterior temporal lobes. SPECT, PET, CT and MRI have shown abnormalities, unilaterally or bilaterally, most marked in the anterior (frontal, temporal) part of the brain (Miller *et al.*, 1991; Starkstein *et al.*, 1994). However, on the basis of case studies, Gregory *et al.* (1999) indicated that neuropsychological tests as well as brain imaging may not be sensitive to the very early changes that occur in the ventromedial frontal cortex and that are expressed only though behavioural disorders.

The second syndrome, progressive non-fluent aphasia, is characterized by difficulties with verbal expression, anomia and phonemic errors, despite relative preservation of comprehension; it is associated with atrophy predominating in the left hemisphere. SPECT usually demonstrates a striking reduction in tracer uptake in the left frontal, temporal and parietal regions (Snowden *et al.*, 1996).

Finally, patients with semantic dementia have fluent speech with semantic errors and severely impaired comprehension and naming, together with visual associative agnosia resulting from bilateral atrophy of the inferior and middle temporal gyri. Functional brain imaging shows reduced tracer uptake in the temporal region, bilaterally or markedly asymmetric with a major defect on the left side (Snowden *et al.*, 1996).

DEMENTIA WITH LEWY BODIES

Lewy bodies are found in the subcortical nuclei, limbic cortex and neocortex, and are associated with significant neuronal loss, including extensive disruption of ascending cholinergic and dopaminergic projections. Fluctuating cognitive impairment, visual hallucinations and parkinsonism are core features of dementia with Lewy bodies (DLB) (McKeith *et al.*, 1996). In a recent study (Barber *et al.*, 2001), medial and lateral temporal lobe atrophy on MRI was compared in DLB patients ($n = 26$), Alzheimer's disease patients ($n = 22$) and normal controls ($n = 26$). All patients with dementia had a loss of hippocampal asymmetry compared with the normal controls. Furthermore, hippocampal and parahippocampal volumes were significantly larger in subjects with DLB than in those with Alzheimer's disease. This may help explain differences in the pattern of cognitive deficits between these disorders.

PET studies (Albin *et al.*, 1996; Ishii *et al.*, 1998) showed, in comparison to Alzheimer's disease, a greater reduction in glucose metabolism in the occipital lobe of DLB patients, including the primary visual areas and visual association cortices. In addition, patients with visual hallucinations had hypoperfusion of the occipital cortex and a relatively spared temporoparietal cortex compared with patients without hallucinations (Imamura *et al.*, 2001). SPECT studies have also shown posterior rCBF abnormalities in DLB (Ishii *et al.*, 1999; Defebvre *et al.*, 1999). Specific dopaminergic degeneration was demonstrated in DLB when compared with Alzheimer's disease patients using a SPECT presynaptic ligand iodine-123-carbomethoxy-3 beta (4-iodophenyl) tropane (F-CIT) (Walker *et al.*, 1999). This appears to be a promising tool for this differential diagnosis.

OTHER ORGANIC BRAIN DISEASES

Parkinson's Disease

Morphological changes on MRI are inconsistent, difficult to quantify and common to parkinsonian patients, both with and without dementia (Huber *et al.*, 1989). Several functional imaging studies have shown that dementia in parkinsonism is accompanied by a bilateral reduction in cortical metabolism (Shapiro *et al.*, 1990; Peppard *et al.*, 1990) or perfusion in posterior parietotemporal areas (Spampinato *et al.*, 1991). This metabolic pattern is close to that observed in Alzheimer's disease, from which Parkinson's disease may differ by some involvement of the sensorimotor and visual cortices (Herholz, 1995).

In addition to the dopaminergic system, the cholinergic system could also be involved in the development of the cognitive disorders encountered in Parkinson's disease. Khul *et al.* (1996) showed abnormal cortical cholinergic innervation in parkinsonian patients. The decline was moderate (20%) and localized at the parietal and occipital cortices of non-demented patients, but was more diffuse and severe in parkinsonian patients with dementia.

Steele–Richardson Disease

This is characterized by an atypical parkinsonian syndrome, dementia, and lesions generally localized at the basal ganglia and brainstem. Functional imaging has shown a marked reduction in cortical (predominantly frontal) metabolism and cortico-subcortical circuits involved in motor function, executive function and attention (Salmon *et al.*, 1997).

Involvement of pre and postsynaptic striatal dopaminergic function characterizes this disease. A marked decrease in ^{18}F-Dopa accumulation has been found in the caudate nucleus and putamen (Brooks *et al.*, 1990). Contrary to Parkinson's disease, the number of dopamine D2 receptors is also diminished (Baron *et al.*, 1986). These changes point to a loss of intrinsic striatal neurons and may account for the lack of response to dopa therapy. A study by Pappata *et al.* (1997) suggests that this striatal involvement affects mainly cholinergic neurons, as striatal uptake of ^{11}C-physostigmine is reduced significantly in these patients.

AIDS and Creutzfeldt–Jakob Disease

Brain damage caused by human immunodeficiency virus (HIV) can lead to dementia. Morphological imaging inconsistently shows atrophy or white-matter involvement, which can be present in

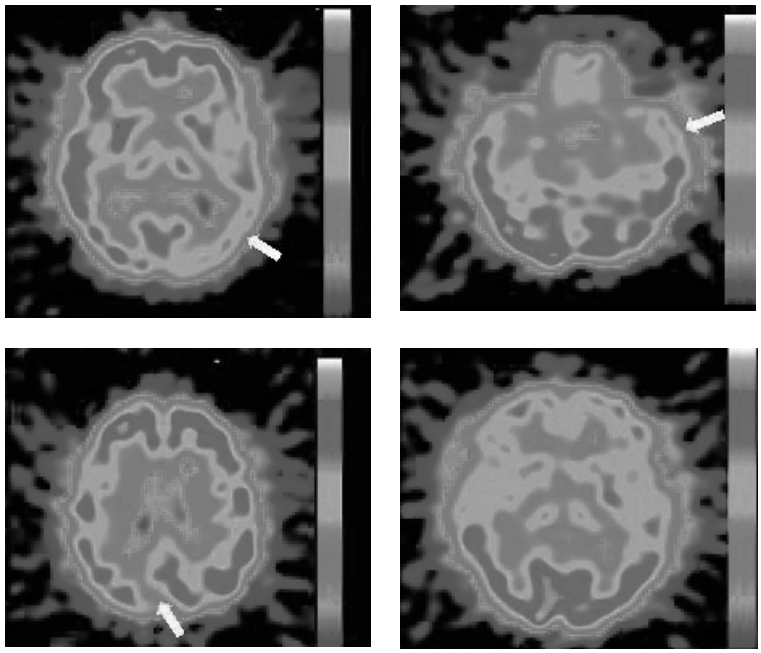

Figure XV-10.3 Perfusion brain SPECTs of four subjects with different pathologies: On the upper right: *Alzheimer disease*: 70 years old woman; MMSE = 19; performance deficit at the following tests; Boston naming test, Token test, verbal fluency and memory recall; Perfusion SPECT: Middle transaxial cut showing left temporo Parietal hypoperfusion. On the upper left: *Mild Cognitive Impairment*: 70 years old woman; MMSE = 28; performance deficit at the following tests; Grober Buschke memory recall; Perfusion SPECT: lower transaxial cut showing left temporal pole hypoperfusion. On the lower right: *Multi-infarct dementia*: 65 years old man; MMSE = 21; performance deficit at the following tests; Benton orientation/praxis/memory recall; Perfusion SPECT: Upper transaxial cut showing a right occipito parietal transcortical hypoperfusion. On the lower left: *Frontotemporal dementia*: 61 years old woman with apathy Irritability & disinhibition; MMSE = 30; performance deficit at the following tasks; attention dual test/capture error sequencing; Perfusion SPECT: Middle transaxial cut showing a diffuse frontotemporal hypoperfusion (See Colour Plate XV-10.3)

combination or alone. The atrophy is mainly subcortical. The signal anomalies initially affect the corpus callosum, the fornix and, later, the white matter. Functional imaging has shown either asymmetric subcortical anomalies or multiple or isolated cortical defects with a variable distribution in patients with a subacute encephalopathy (Kramer and Sanger, 1990). Serial examinations show that the increase in perfusion deficits runs parallel to the clinical deterioration.

Creutzfeldt–Jakob disease is a transmissible spongiform encephalopathy caused by an infectious prion protein. MRI scan may initially be normal or subnormal but subsequently shows severe and rapidly progressive cortical atrophy.

BRAIN IMAGING AND DIFFERENTIAL DIAGNOSIS OF DEMENTIA

A particularly important use of brain imaging is differential diagnosis of dementia (Figure XV-10.3). Using MRI, Barber *et al.* (1999) created a visual rating scale in order to evaluate medial temporal lobe atrophy in 26 patients with DLB, 28 patients with Alzheimer's disease, 24 patients with vascular dementia and 26 control subjects. The results suggest that the absence of medial temporal lobe atrophy had a specificity of 100% (sensitivity 38%) for separating DLB from Alzheimer's disease, and a specificity of 88% for separating vascular dementia from Alzheimer's disease. In another study with SPECT, Talbot *et al.* (1998) prospectively studied 364 patients with dementia (132 Alzheimer's disease, 78 vascular dementia, 24 DLB, 58 frontotemporal dementia, 22 progressive aphasia) for a median of 3 years (range 1–6 years). To evaluate the diagnostic gain of individual brain imaging results (rCBF abnormalities), likelihood ratios were calculated pairwise for disease group comparisons (e.g. disease A *v.* disease B). (Likelihood ratios provide an indication of the degree to which a particular result modifies the pretest odds of one subject having a particular disease to provide a new post-test odds ratio.) A bilateral posterior rCBF abnormality significantly increased the odds of having Alzheimer's disease as opposed to vascular dementia or frontotemporal dementia. 'Patchy' rCBF abnormalities significantly increased the odds of having vascular dementia as opposed to Alzheimer's disease. The technique was, however, less useful for differentiating Alzheimer's disease from DLB.

CONCLUSION

The main message of this chapter is that brain imaging is useful for both positive and differential diagnosis of dementia. Future improvements could result from better standardization of both structural and functional brain imaging methodologies. Brain imaging might also help clinicians to predict the onset of cognitive behavioural disturbances and, possibly, the effectiveness of therapeutic intervention. This approach provides the basis for what has been called cognitive neuropsychiatry (Halligan and David, 2001), which attempts to establish the functional organization of psychiatric disorders within the framework of cognitive neuropsychology.

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XV-11

Neurogenetics of Dementia

Bart Dermaut and Christine Van Broeckhoven

INTRODUCTION

Whereas intensive genetic study has so far failed to convincingly identify genes for psychiatric disorders like schizophrenia or bipolar disorder, the molecular defect of a number of dementia syndromes has been identified unambiguously. Indeed, for rare familial forms of dementia, such as Alzheimer's disease, frontotemporal dementia (FTD), Creutzfeldt–Jakob disease (CJD), and other rare atypical dementias, we are able to pinpoint highly penetrant mutations in genes as the sole cause of the disease. This genetic approach has been successful because of two specific properties of dementia mostly absent in other psychiatric disorders. First, detailed neuropathological study of brains of demented individuals has made possible the classification of dementia syndromes into clinicopathological and biochemical entities amenable for a molecular genetic approach. Second, some of these cognitive disorders or subtypes behave as Mendelian traits, making them suitable for classical positional cloning of disease genes. Although this has led to the identification of the molecular cause of a number of monogenic dementias, genetic study of these diseases remains considerably complicated. First, there is the problem of variable expression, meaning that the same or similar genetic defect can result in different phenotypes. This is particularly well illustrated by the enormous phenotypic heterogeneity observed in inherited tau and prion diseases. Second, the same dementia can be caused by different mutations in different genes. These phenomena are referred to as allelic and genetic heterogeneity and are evident from genetic studies in familial early-onset Alzheimer's disease (EOAD), which can be caused by different mutations in at least three genes. Third, the significant clinical overlap between diseases with totally different neuropathological and molecular diagnoses adds to their clinical diagnostic complexity.

From an academic point of view, the complementary roles of molecular genetics and neuropathology cannot be overestimated. In many cases, important clues to the identification of a disease gene have come from the careful neuropathological and biochemical study of proteins that are present in brain lesions of demented individuals. Historical examples of this approach include the identification of mutations in the amyloid precursor protein gene (*APP*) and prion protein gene (*PRNP*) causing Alzheimer's disease and CJD, respectively. On the other hand, as illustrated by the mutated presenilins in familial EOAD, 'blind' scanning of the whole genome in large families has resulted in the identification of unexpected genetic defects, thereby often providing completely novel insights into the biochemical mechanisms of a disease.

Unfortunately, primary degenerative dementias are presently untreatable and patients therefore benefit little from receiving a correct molecular diagnosis. It is hoped, however, that novel pathogenetic insights obtained from molecular genetic studies of these diseases will finally result in efficient therapeutic strategies.

More recently, for the more frequent occurring late-onset Alzheimer's disease (LOAD) and some other neurodegenerative dementias, common gene polymorphisms and chromosomal loci have been identified that contribute to disease susceptibility. As these gene polymorphisms are common DNA variations, their contribution to the phenotype within the general population is expected to be high. Nevertheless, progress in the field of genetic epidemiology has been slow, and the biochemical significance of the findings is often unclear. This is not surprising as it is likely that these genetic variations will interact with both the environment and other genes. With the enormous amount of frequent genetic variations becoming available through the human genome project and the development of new statistical methods, the area of complex genetics will become increasingly important.

Presently, genetic research is still focused on both the rare monogenic and common complex types of dementia. New chromosomal loci and genes are still being identified for monogenic presenile dementia, and an enormous number of genes are under scrutiny for harbouring variations affecting susceptibility to the common forms of dementia. In this chapter, we try to highlight the major topics in both areas. For reasons of clarity, we have also added the Online Mendelian Inheritance in Man (MIM: http://www.ncbi.nlm.nih.gov/Omim/) numbers of the described genes and phenotypes.

ALZHEIMER'S DISEASE

Alzheimer's disease (MIM 104300) is the most common type of dementia in the elderly. It is characterized by slowly progressive impairment in intellectual function resulting in a state of complete dependency and death after 5–10 years. The definite diagnosis is made at autopsy by the demonstration in brain of extracellular amyloid deposits called neuritic plaques and intracellular neurofibrillary tangles (Mirra *et al.*, 1991). Whereas neuritic plaques are composed mainly of extracellular deposited amyloid peptide (Aβ) fibrils derived from the amyloid precursor protein (APP), hyperphosphorylated tau protein is the main constituent of neurofibrillary tangles. In research settings, an arbitrary cut-off of 65 years is often used to distinguish between the rare EOAD, or presenile type of Alzheimer's disease, and the frequent LOAD, or senile Alzheimer's disease, which are clinically and pathologically highly similar, if not identical. The first genetic evidence suggesting genetic causes of AD came from large, multigenerational families that included many individuals with EOAD (Kennedy *et al.*, 1994; Mirra *et al.*, 1991). Later epidemiological studies of risk in first-degree relatives also revealed a strong familial clustering of the disease. It is estimated that first-degree relatives of AD patients have a 3.5-fold increased risk for developing AD (van Duijn *et al.*, 1991). This relative risk decreases with increasing age at onset of the proband, but

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even at the age of 80 years the relative risk is 2.6-fold increased for first-degree relatives. The lifetime cumulative risk for first-degree relatives of AD patients was found to be 39%, compared with about 12% in first-degree relatives of controls (Lautenschlager *et al.*, 1996; van Duijn *et al.*, 1993). Although there is substantial evidence for genetic factors in the aetiology of both EOAD and LOAD, the most important genetic breakthroughs have been established by investigation of EOAD families segregating the disease in an autosomal dominant manner.

Molecular Genetics

Chromosomes 21, 14 and 1: APP, PSEN1 and PSEN2

Initial genetic linkage studies targetted mainly chromosome 21 because the brains of almost all middle-aged Down's syndrome patients, who have a trisomy of chromosome 21, contain the neuropathological hallmarks of AD (Mann, 1988; Wisniewski *et al.*, 1985). The underlying hypothesis was that in Down's syndrome patients, AD is caused by overrepresentation of a gene on chromosome 21 as a result of the trisomy 21, while in AD, a mutation in that gene might result in the production of an abnormal protein or the overproduction of a normal protein. In some EOAD families, linkage was found to 21q11.2-21 (St George-Hyslop *et al.*, 1987); around the same time, *APP* (MIM 104760) was characterized and localized to the same chromosomal region (Tanzi *et al.*, 1987) (Figure XV-11.1). Since APP is cleaved endoproteolytically to produce the Aβ peptide, the major component of neuritic plaques in AD brains, *APP* was a strong candidate gene for AD. Moreover, a Glu693Gln mutation in *APP* was found in families suffering from hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D), a rare autosomal dominant disorder found in a few families in the Netherlands (Bakker *et al.*, 1991; Levy *et al.*, 1990). HCHWA-D is characterized by recurrent cerebral haemorrhages caused by excessive deposition of Aβ in the blood vessel walls in the brain (Haan *et al.*, 1991). The discovery of this mutation demonstrated that mutations in *APP* can lead to Aβ deposition and encouraged mutation analysis of *APP* in EOAD families. Sequence analyses of *APP* revealed a Val717Ile mutation in a chromosome-21-linked EOAD family (Goate *et al.*, 1991). To date, 12 different Alzheimer's disease-related *APP* mutations are known (AD mutation database: http://molgen-www.uia.ac.be/ADmutations) (Table XV-11.1). The *APP* Val717Ile mutation was found in multiple EOAD families of different ethnicity, which underlined the causative character of the mutation. *APP* is a housekeeping gene comprising 18 exons encoding a single membrane-spanning protein with a large ectodomain and a small cytoplasmic tail (Tanzi *et al.*, 1987; Konig *et al.*, 1992; Ponte *et al.*, 1988; Sandbrink *et al.*, 1994). All mutations are located in exons 16 and 17 at or near proteolytic cleavage sites involved in the generation of Aβ.

Since *APP* mutations explain the development of EOAD in only a small number of cases, a genome-wide search in families segregating EOAD was started in many laboratories. This led to the discovery of strong linkage evidence of EOAD with markers at chromosome 14q24.3 (Mullan *et al.*, 1992; Schellenberg *et al.*, 1992; St George-Hyslop *et al.*, 1992; Van Broeckhoven *et al.*, 1992). The gene encoding presenilin 1 (*PSEN1*; MIM 104311) was isolated from this region, and missense mutations were identified that cosegregated with EOAD (Figure XV-11.1). To date, 82 different AD mutations have been identified (Table XV-11.1). *PSEN1* contains 13 exons encoding a 467-amino-acid integral membrane protein with six to eight transmembrane domains (Doan *et al.*, 1996; Lehmann *et al.*, 1997; Li and Greenwald, 1998). In contrast to *APP* mutations, the *PSEN1* mutations are scattered over the entire coding region of the gene, with some clustering in exons 5 and 8 encoding the regions that correspond to

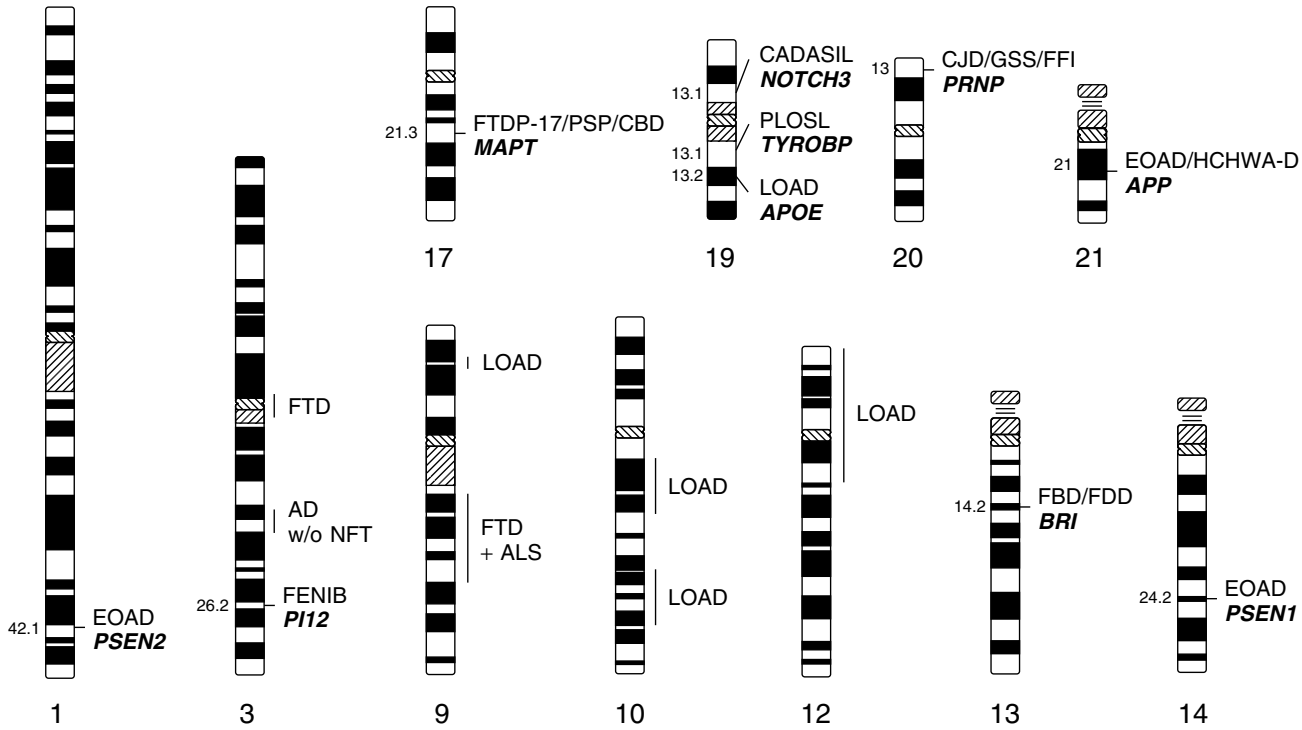

Figure XV-11.1 Human chromosomes with genetic loci that influence different types of dementia syndromes. When known, the responsible gene is highlighted in bold. (AD, Alzheimer's disease)

Table XV-11.1 Monogenic dementias with known molecular defect

Dementia subtype	Main clinical features	Neuropathological features	Mode of inheritance	Mutated gene	Type of mutations	Number of mutations reported
Familial EOAD	Memory impairment, cognitive disturbances, various accompanying features in later stages	Neuritic amyloid plaques (Aβ), neurofibrillary tangles (tau), amyloid angiopathy (Aβ)	A.D.	*APP*	Clustered missense mutations	12
				PSEN1	Mainly missense mutations	82
				PSEN2	Missense mutations	6
FTDP-17	Behavioural, cognitive and motor disturbances (highly variable)	Gliosis, spongiosis, neuronal loss, neurofibrillary tangles (tau), rare Pick bodies (tau)	A.D.	*MAPT*	Clustered missense, silent, intronic mutations	21
Familial prion disease	Cognitive disturbances, ataxia, myoclonus (highly variable)	Gliosis, severe spongiosis, neuronal loss, prion deposits	A.D.	*PRNP*	Missense mutations Octapeptide insertions	22 19
CADASIL	Strokes, migraine, cognitive deterioration	Arteriopathy, osmiophilic deposits near vascular smooth muscle cells	A.D.	*NOTCH3*	Stereotyped missense mutations	25
FBD and FDD	*FBD*: dementia, spastic paraparesis, ataxia *FDD*: dementia, cataracts, deafness, ataxia	Alzheimer's disease-like amyloid plaques (ABri, ADan), neurofibrillary tangles (tau), amyloid angiopathy (ABri, ADan)	A.D.	*BRI*	Stop codon mutation (FBD) and ten nucleotide insertion (FDD) leading to longer precursor protein	2
FENIB	Cognitive decline, epilepsy	Collins bodies (neuroserpin)	A.D.	*PI12*	Missense mutations	2
PLOSL	Bone cysts, psychosis, frontal dementia	Loss of myelin, gliosis, calcifications, obliterated arterioles and capillaries	A.R.	*TYROBP*	Deletion mutations	2

A.D., autosomal dominant; A.R., autosomal recessive.

the second transmembrane domain and the N-terminal part of the sixth hydrophilic loop (Cruts and Van Broeckhoven, 1998). *PSEN2* (MIM 600759) was found based on its homology with *PSEN1* (Levy *et al.*, 1995a). Interestingly, *PSEN2* was located at 1q42.3 (Figure XV-11.1), a region that was genetically linked to AD in the Volga-German group of families who first emigrated from Germany to Russia and later to the USA (Levy *et al.*, 1995b). In these families, the Asn141Ile mutation was identified in *PSEN2*. Now, five additional missense mutations are known (Table XV-11.2).

The majority of *PSEN* mutations are missense mutations giving rise to the substitution of a single amino acid. However, two splicing defects and two genomic deletions have been reported (De Jonghe *et al.*, 1999; Kwok *et al.*, 1997; Perez-Tur *et al.*, 1995; Prihar *et al.*, 1999; Smith *et al.*, 2001). It has been shown in functional studies that all *PSEN* mutations distort APP processing by promoting the secretion of the longer form of the Aβ peptide, suggesting a gain of function mechanism for *PSEN* mutations (Citron *et al.*, 1997).

Table XV-11.2 Genes and chromosomal loci that influence susceptibility to dementia

Dementia subtype	Chromosome	Gene	Risk polymorphism
LOAD	19	*APOE*	*APOE*-ε4 allele
	12	Unknown	Unknown
	10	Unknown	Unknown
	9	Unknown	Unknown
CJD (sporadic, iatrogenic, variant)	20	*PRNP*	*PRNP* -Met129Val
PSP and CBD	17	*MAPT*	*MAPT* spanning haplotype

Chromosome 19: APOE

In LOAD families, no mutations are found in *APP*, *PSEN1* or *PSEN2*. Therefore, a genomic screen was undertaken in a set of LOAD families using the 'affected pedigree members' method in which the segregation patterns of genetic markers are considered only in affected individuals (Pericak-Vance *et al.*, 1991). These analyses supported linkage to 19q13.2. In 1991, the apolipoprotein E (apoE) encoded by *APOE* (MIM 107741) was localized to the lesions in AD brain (Namba *et al.*, 1991). Also, in cerebrospinal fluid (CSF), apoE binds with high avidity to immobilized Aβ (Strittmatter *et al.*, 1993a), and later *APOE* was localized to chromosome 19q13.2 near the candidate region defined by genetic analysis (Figure XV-11.1). Two polymorphisms in the coding region of *APOE* result in three major isoforms of the protein, differing in the presence of a Cys or Arg residue at amino acid positions 112 and 158, respectively. *APOE*-ε3 ($Cys_{112}Arg_{158}$) is the most common isoform, with an allele frequency of 75% in the Caucasian population. The other two common alleles are *APOE*-ε2 ($Cys_{112}Cys_{158}$) and *APOE*-ε4 ($Arg_{112}Arg_{158}$), with frequencies of 10% and 15%, respectively. Genetic association of the *APOE*-ε4 allele was first demonstrated with familial LOAD (Strittmatter *et al.*, 1993a), and later also with sporadic LOAD (Saunders *et al.*, 1993) and EOAD (van Duijn *et al.*, 1994). It was observed that *APOE*-ε4 was associated with a dose-dependent increased risk of developing AD that was confirmed consistently in all studies (Table XV-11.2). Also, the *APOE*-ε2 allele was found to have a protective effect against AD (Corder *et al.*, 1994), but this observation was not confirmed in all studies (van Duijn *et al.*, 1995). In a multicentric meta-analysis, almost a three times increased risk was observed for carriers of one *APOE*-ε4, and a 15 times increased risk was found for homozygote carriers of *APOE*-ε4 (Farrer *et al.*, 1997). There is evidence that the risk associated with *APOE*-ε4 is dose-dependent, as reflected in the age at onset of disease: individuals with two *APOE*-ε4 alleles have an earlier onset than those without an *APOE*-ε4 allele (Corder *et al.*,

1993). Despite the highly consistent association between *APOE*-ε4 and AD in Caucasian populations, this association seems to be absent in populations of African origin, where the *APOE*-ε4 frequency is relatively high (Kalaria *et al.*, 1997). The reasons for these discrepant findings are still unclear, although a difference in linkage disequilibrium patterns between African populations and evolutionarily more recent Caucasian populations might offer a clue (de Knijff and van Duijn, 1998). More recently, several polymorphisms in the promoter region of *APOE* were reported to be associated with AD independently of *APOE* isoforms, suggesting that apoE expression levels might be important in the development of AD (Artiga *et al.*, 1998a; Artiga *et al.*, 1998b; Bullido *et al.*, 1998; Lambert *et al.*, 1998a; Lambert *et al.*, 1998b). However, this was not confirmed in all studies (Roks *et al.*, 1999). On the biochemical level, it was demonstrated that the apoE-ε4 isoform binds Aβ much faster than the apoE-ε3 isoform (Strittmatter *et al.*, 1993b). Nevertheless, the exact mechanism by which apoE isoforms influence the development of AD remains unknown.

Chromosomes 9, 10 and 12

In a complete genomic screen using different linkage methods in families affected with LOAD, possible or suggestive linkage was detected on chromosomes 4, 6, 12 and 20, with the strongest evidence for markers in the pericentric region of chromosome 12 (Pericak-Vance *et al.*, 1997) (Figure XV-11.1). Using parametric linkage analysis, the presence of an AD susceptibility locus on chromosome 12 was confirmed in an independent set of families with LOAD (Rogaeva *et al.*, 1998). Although the precise location of the gene could not be determined, it included the previously reported region. In the first stage of a full genome scan in 292 affected sibling pairs (ASPs) with LOAD, only moderate evidence for a somewhat overlapping region on 12p was detected (Wu *et al.*, 1998), while the highest peaks were on chromosomes 1, 9, 10 and 19 (Kehoe *et al.*, 1999). In the second stage using 429 ASPs, a multipoint lod score of 3.83 was found near D10S1225 (Myers *et al.*, 2000) (Figure XV-11.1). Interestingly, in an independent set of LOAD families, Aβ42 plasma levels again showed strongest evidence for linkage at the same marker (Ertekin-Taner *et al.*, 2000), while another study of 435 multiplex AD families, partially overlapping with the ASP sample, found linkage with AD more downstream at D10S583 (Bertram *et al.*, 2000) (Figure XV-11.1). In another study using 730 ASPs, strongest evidence was found for a marker on chromosome 9p22.1 (Pericak-Vance *et al.*, 2000) (Figure XV-11.1). The chromosome 9, 10 and 12 AD genes remain to be identified.

Other Candidate Genes

Numerous genes have been reported to affect genetic susceptibility to AD. Although there is substantial functional evidence for an involvement in AD for most of these genes, initial positive genetic associations are often not replicated in independent populations (Prince *et al.*, 2001; Emahazion *et al.*, 2001). One gene that has recently attracted the attention of many researchers is the gene encoding α2-macroglobulin (α2m), *A2M*, which is located within the putative chromosome 12 AD locus. α2m is an abundant panprotease inhibitor (Borth, 1992) and, like apoE, a major ligand for the lipoprotein-related receptor protein (Strickland *et al.*, 1990). Moreover, α2m has been implicated in the binding (Hughes *et al.*, 1998), degradation (Qiu *et al.*, 1996) and clearance (Narita *et al.*, 1997) of the Aβ protein. Two polymorphic loci in *A2M* have been reported to increase the risk for AD (Blacker *et al.*, 1998; Liao *et al.*, 1998), but subsequent replication studies on both polymorphisms were inconsistent. A meta-analysis of all published case–control studies, including our own results in the Dutch population, showed no demonstrable contribution of *A2M* to AD (Koster *et al.*, 2000).

PSEN1, which causes autosomal dominant EOAD when mutated, has also been investigated as an AD susceptibility gene. A genetic association between LOAD and a polymorphism in intron 8 of *PSEN1* was reported, with a two-fold increased risk for the AA genotype (Wragg *et al.*, 1996). This finding was replicated in some other LOAD populations, while others showed no association. This can be explained if the *PSEN1* intron 8 polymorphism is not functionally relevant but is genetically close to another, biologically functional polymorphism. Recently, we performed an association study of *PSEN1* in a population-based series of EOAD patients, suggesting that a functional polymorphism in the promoter of *PSEN1* might contribute to the genetic risk of EOAD (van Duijn *et al.*, 1999; Theuns *et al.*, 2000). Interestingly, our results were replicated in an independent AD sample, where the effect was strongest in EOAD cases (Lambert *et al.*, 2001). However, in a Dutch population-based sample of LOAD, we were unable to demonstrate association with the *PSEN1* promoter (Dermaut *et al.*, 2001a). It is important to realize that since the identification of *APOE*, genetic association studies in AD have generally yielded no conclusive results. As such, *APOE* remains the only established genetic risk factor in AD. Without denying the complex nature of a multifactorial disease, empirical experience with genetic association studies in AD over the past 10 years raises questions on the utility of this strategy in unravelling the genetic components of AD (Emahazion *et al.*, 2001).

Genotype–Phenotype Correlations in Familial Early-Onset Alzheimer's Disease

A recent study compared age at onset and age at death in 90 subjects with dominantly inherited EOAD caused by *PSEN1*, *PSEN2* and *APP* mutations (Lippa *et al.*, 2000). Although highly variable in each group, it was found that both age at onset and death were significantly earlier in *PSEN1* and *APP* mutation carriers than in the *PSEN2* group. Mean ages at onset were 44 ± 8 years in the *PSEN1* group, 59 ± 7 years in the *PSEN2* group, and 49 ± 7 years in *APP* mutation carriers, with a similar mean disease duration of about 10 years in the three groups. There is evidence that *APOE* genotype modulates age at onset in some but not all *APP* families. In one well-studied family, the age at onset of individuals with a mutation who are *APOE*-ε3ε3 homozygous is 55 years, whereas *APOE*-ε4ε4 homozygotes have an onset at 45 years and *APOE*-ε2ε3 heterozygotes have an onset of 60 years (Alzheimer's Disease Collaborative Group, 1993). In *PSEN1* families, the *APOE* genotype does not seem to modulate age at onset (Van Broeckhoven *et al.*, 1994).

A number of neurological symptoms can accompany *APP*- and *PSEN*-related dementia syndromes in later stages of the disease course. These include myoclonus, epileptic seizures, pyramidal signs, extrapyramidal signs, cerebellar signs and ataxia (Fox *et al.*, 1997; Haltia *et al.*, 1994; Kennedy *et al.*, 1995; Lampe *et al.*, 1994; Martin *et al.*, 1991). However, these features are not unique to familial EOAD, as they are also observed in other inherited dementias and even typical LOAD cases (McKhann *et al.*, 1984). Therefore, accompanying neurological features generally do not allow a reliable prediction on which causal EOAD gene is mutated. Recently, however, it has been suggested that a subset of *PSEN1* mutations leads to 'variant' AD, presenting with spastic paraparesis and so called 'cotton-wool plaques' in the brain (Houlden *et al.*, 2000). The first report concerned a Finnish pedigree in which a 4.6-kb genomic deletion containing exon 9 of *PSEN1* leads to this EOAD subtype (Crook *et al.*, 1998; Prihar *et al.*, 1999). It was suggested that such cases with variant AD, also seen with other *PSEN1* mutations, represent an aggressive subtype of EOAD,

since mutations causing this condition lead to exceptionally high amyloid concentrations in *in vitro* experiments (Houlden *et al.*, 2000).

At the neuropathological level, amyloid deposition in the cerebral blood vessel walls, called cerebral amyloid angiopathy (CAA), has received much attention. CAA is found in 90% of autopsied AD brains, but its severity and quantity are highly variable (Glenner *et al.*, 1981; Yamada, 2000). CAA is classically considered an important cause of cerebral haemorrhage, and is a prevailing feature in carriers of mutations affecting codons 692, 693 and 694 of *APP*, which are located at the α-secretase site within the coding region of the Aβ peptide. While patients carrying Dutch APP (Glu693Gln) suffer from recurrent cerebral haemorrhages (Van Broeckhoven *et al.*, 1990), Flemish APP (Ala692Gly) is associated with both AD and cerebral haemorrhages (Hendriks *et al.*, 1992; Roks *et al.*, 2000). Iowa APP (Asp694Asn) mainly presents as AD without obvious stroke-related events, although small haemorrhagic lesions are demonstrable by magnetic resonance imaging (MRI) and at neuropathological examination (Grabowski *et al.*, 2001).

Interestingly, some *PSEN* mutations also present with a comparably severe CAA but mostly in the absence of haemorrhages or infarcts. It is noteworthy that a recent systematic neuropathological study of *PSEN1*-related EOAD showed a strong correlation between CAA and *PSEN1* mutation codon position (Mann *et al.*, 2001). It was demonstrated that a severe degree of CAA was much more frequent among cases with a *PSEN1* mutation occurring after codon 200. Consistent with these findings, we have described a Belgian EOAD pedigree with unusual severe CAA segregating a novel *PSEN1* mutation at codon 282 (Leu282Val) (Dermaut *et al.*, 2001b). Immunohistochemical study of the brain of a *PSEN1* Leu282Val carrier revealed that the CAA lesions were capable of instigating a strong local dystrophic and inflammatory reaction, similar to the neuritic plaques that are classically considered the pathogenic amyloid lesion in AD brains.

Population Attribution

Several studies have estimated the genetic contribution of *PSEN* and *APP* mutations to EOAD. The estimated frequencies of *APP* and *PSEN* mutations to autosomal dominant EOAD depend largely on the ascertainment strategy of families. In families with a very early onset of disease that are documented thoroughly for use in genetic linkage studies or genetic counselling, mutation frequencies are clearly higher. In a French study, it was estimated that about 71% of all autosomal dominant EOAD families were explained by mutations in *APP*, *PSEN1* or *PSEN2* (Campion *et al.*, 1999). However, in a more systematic population-based sample of autosomal dominant EOAD in the Netherlands, we showed that only 20% could be explained by mutations in *APP*, *PSEN1* or *PSEN2* (Cruts *et al.*, 1998). In the same Dutch EOAD population, including both sporadic and familial cases, mutation frequencies were 0.5% for *APP*, 6% for *PSEN1* and 1% for *PSEN2*. Since the fraction of EOAD in the total AD population is very small (<1%) (Ott *et al.*, 1995), the contribution of the three dominant genes together to AD in general is probably less than 0.1%. Moreover, other dominant EOAD genes are expected, since in an important percentage of familial EOAD cases the genetic cause is unknown, including large, well-documented autosomal dominant pedigrees with neuropathological confirmation.

At the level of the population, *APOE* seems to be a more important determinant of AD. Although the relative risk associated with carriership of one *APOE*-ε4 allele is increased only moderately, the proportion of patients with dementia that is attributable to the *APOE*-ε4 allele was estimated to be 20% (Slooter *et al.*, 1998). This substantial contribution to the occurrence of AD in the general population is explained by the large amount of heterozygote carriers of the *APOE*-ε4 allele ($\sim$25% in the Caucasian population). Because of the rarity of the homozygous *APOE*-ε4ε4 genotype (<2%), the contribution of this genotype to AD in the population is limited, despite the high relative risk associated with this genotype.

FRONTOTEMPORAL DEMENTIA

Between 1892 and 1906, Arnold Pick described several patients with frontal and temporal atrophy presenting with language and/or behavioural disturbances. The term 'Pick's disease' (MIM 172700) has since been used to classify such cases. However, the clinical and pathologic heterogeneity of these focal non-Alzheimer's dementias has led historically to a proliferation of names. In 1994, the Lund and Manchester Groups published a consensus statement and introduced the term 'frontotemporal dementia' (FTD), along with its clinical and neuropathological criteria. Pick's disease is now considered to be a subtype of FTD that is defined neuropathologically by the presence of intraneuronal inclusion bodies (Pick bodies) and ballooned neurons (Pick cells), the microscopic lesions originally described by Aloïs Alzheimer in 1911 (Alzheimer, 1911). The most common type of FTD neuropathology is characterized by neuronal loss and spongiform change, together with mild or moderately severe gliosis in the outer cortical layers (Lund and Manchester Groups, 1994). In another type, spinal motor neuron degeneration is an additional feature. In a recent Dutch population-based study, the approximate prevalence of FTD varied between 1.2 of 10^6 in the age group 30–40 years and 28.0 of 10^6 in the age group 60–70 years (Stevens *et al.*, 1998). It is estimated that FTD comprises 3–12% of all demented patients (Brun, 1987; Giannakopoulos *et al.*, 1995; Knopman *et al.*, 1990) and may account for up to one-quarter of patients presenting before the age of 65 with dementia due to primary cerebral atrophy (Brun, 1987; Neary *et al.*, 1988). A positive family history for dementia is found in 38–50% of FTD patients (Knopman *et al.*, 1990; Neary *et al.*, 1988; Stevens *et al.*, 1998), and in the majority of these familial cases the disease is transmitted as an autosomal dominant trait.

Molecular Genetics

Chromosome 17: MAPT

In 1939, autosomal dominant presenile dementia was described in a Dutch family with prominent behavioural disturbances, language problems and mild memory impairment (Sanders *et al.*, 1939). This was the first description of the familial form of FTD. Between 1994 and 1996, several families with disinhibition-dementia-parkinsonism-amyotrophy complex, familial progressive subcortical gliosis and pallidopontonigral degeneration were linked to a locus on chromosome 17q21-22 (Petersen *et al.*, 1995; Wijker *et al.*, 1996; Wilhelmsen *et al.*, 1994) (Figure XV-11.1). In 1997, the original Dutch family (now known as Dutch family 2) was also assigned to this locus (Heutink *et al.*, 1997). By then, definite or probable linkage was established for 13 families with early-onset FTD, and in a consensus conference the clinical and morphological phenotypes of these families were compared (Foster *et al.*, 1997). Although the clinical phenotype of these families is highly variable, the term 'frontotemporal dementia and parkinsonism linked to chromosome 17' (FTDP-17) (MIM 600274) was adopted to emphasize the predominant symptoms of these families. Macroscopic examination of these FTDP-17 brains reveals severe frontotemporal atrophy as a consistent pathological feature, while involvement of the medial temporal lobe, amygdala, basal ganglia and substantia nigra is variable. However, in nearly all FTDP-17 affected brains, there is a pathological accumulation of the microtubule-associated protein tau, hence FTDP-17 is

often classified as a 'tauopathy'. Tau is involved in stabilizing microtubules, which are essential for cell shape, support and intracellular transport. As the gene encoding tau (*MAPT*; MIM 157140) maps within the FTDP-17 locus (Neve *et al.*, 1986), *MAPT* became the obvious candidate gene to screen for pathological mutations; and in 1998, the first *MAPT* mutations were reported (Hutton *et al.*, 1998; Poorkaj *et al.*, 1998; Spillantini *et al.*, 1998).

MAPT contains a total of 15 exons, with the major tau protein isoforms being encoded by 11 of these (Andreadis *et al.*, 1992). The gene undergoes complex alternative splicing, resulting in six different tau isoforms (Goedert *et al.*, 1989). Exons 9 to 13 encode four microtubule-binding motifs, and alternative splicing of exon 10 generates tau protein with either three or four microtubule-binding repeats. Presently, around 20 different mutations in *MAPT* have been identified in at least 50 known pedigrees worldwide (Reed *et al.*, 2001) (Table XV-11.1). All mutations are located in the carboxy-terminus of the protein, and include missense, silent and intronic variations as well as a codon deletion. They are all clustered in or near the microtubule-binding domains, and they affect microtubule-binding properties of tau in some cases by altering the three to four microtubule-binding repeats ratio resulting from distorted alternative splicing of exon 10.

MAPT: A Susceptibility Gene for Progressive Supranuclear Palsy and Corticobasal Degeneration

Progressive supranuclear palsy (PSP; MIM 601104) and corticobasal degeneration (CBD) are sporadic atypical parkinsonian syndromes that are often clinically confused with each other (Litvan *et al.*, 1999). These disorders share a rapid disease progression and parkinsonism with poor response to dopaminergic therapy, and display associated signs, such as ocular abnormalities (supranuclear gaze palsy), pyramidal signs and cognitive involvement often resembling FTD. Neuropathologically, both disorders are associated with tau inclusions in neurons and glia and are thus, like FTDP-17, classified as primary tauopathies (Dickson, 1999). Both PSP and CBD have similar biochemical alterations in the tau protein, with the abnormal tau containing predominantly four repeat tau. However, microscopically, the tau lesions appear different for the two disorders: while PSP is characterized by the presence of globose shaped neurofibrillary tangles in affected neurons, in CBD mainly fine filamentous tau inclusions are seen.

A genetic association between an intronic dinucleotide polymorphism in *MAPT* and PSP was reported (Conrad *et al.*, 1997). Later, it was shown in several studies that homozygosity of a common haplotype spanning the whole *MAPT* gene was significantly overrepresented in PSP patients (Baker *et al.*, 1999; Higgins *et al.*, 2000), and recently these results have been extended to CBD (Houlden *et al.*, 2001) (Table XV-11.2). The causal variation explaining this association is still unknown. These results suggest that PSP and CBD share a similar genetic cause. It is of interest that familial atypical PSP has been reported to be caused by homozygosity of an *MAPT* deletion at codon 296 located in the second tubulin-binding domain of the tau protein (Pastor *et al.*, 2001). No association between the extended *MAPT* haplotype was observed in a sample of patients with Pick's disease, a primary tauopathy in which predominantly three repeat tau accumulates in Pick bodies (Russ *et al.*, 2001).

Other Familial Frontotemporal Dementia Loci

A Danish FTD family lacking distinctive neuropathological features was linked to the centromeric region of chromosome 3 (MIM 600795) (Brown *et al.*, 1995) (Figure XV-11.1). Since this pedigree shows anticipation, an unstable trinucleotide expansion has been suggested as the genetic cause of FTD in this family (Ashworth *et al.*, 1999). Another family with clinical AD and neuritic plaques but no tangles (MIM 604154) was also mapped to a locus on chromosome 3 that was about 40 centimorgans (cM) more downstream from the marker, giving the highest lod score in the Danish FTD pedigree (MIM 604154) (Poduslo *et al.*, 1999) (Figure XV-11.1). The families are probably phenotypically and genetically distinct.

The combination of amyotrophic lateral sclerosis (ALS) and FTD is well known (Hudson, 1981; Neary *et al.*, 1990). Moreover, the presence of amyotrophy is a supportive diagnostic feature in the Lund and Manchester criteria for FTD (Lund and Manchester Groups, 1994). In a set of families in which people develop both ALS and FTD, linkage was found with markers on 9q21-22 (MIM 105550) (Hosler *et al.*, 2000) (Figure XV-11.1). This locus is distinct from the juvenile ALS locus on 9q34 (MIM 602433).

Genotype–Phenotype Correlations in FTDP-17

The principal clinical features in FTDP-17 families consist of behavioural, cognitive and motor disturbances, albeit with substantial variability within and between families (Foster *et al.*, 1997). In typical FTD, the disease is characterized by behavioural and personality changes involving disinhibition, stereotypy, antisocial acts and language disorders, leading to apathy, mutism and late neurological (frontal release or extrapyramidal) signs (Neary and Snowden, 1996). However, the spectrum of disease extends wider and comprises cases where severe parkinsonism or amyotrophy is the major disabling feature (Lynch *et al.*, 1994; Wszolek *et al.*, 1992; Spillantini *et al.*, 1997). The duration of disease in FTDP-17 families is highly variable; it usually lasts for 10 years, but it can be as short as 3 years and as long as 35 years (Foster *et al.*, 1997). In terms of duration and progression, the parkinsonism seen in FTDP-17 is generally more aggressive than that observed in Parkinson's disease but is similar to that in other extrapyramidal tauopathies, such as PSP and CBD, diagnoses that are also found within FTDP-17 families. This type of parkinsonism is also poorly responsive to dopaminergic therapy (Reed *et al.*, 2001).

Population Attribution

Several investigators have studied the contribution of *MAPT* mutations to the occurrence of FTD, and estimates vary widely among studies (Houlden *et al.*, 1999; Poorkaj *et al.*, 2001; Rizzu *et al.*, 1999). However, the percentage of *MAPT* mutations is consistently higher among FTD cases with a positive family history (10.5–43.0%) and tau-related neuropathological features (13.6–33.0%) compared with total FTD (5.9–17.8%), including both sporadic and familial patients with or without tau neuropathology. So far, no *MAPT* mutations have been detected in sporadic FTD patients, and in one study it was estimated that the occurrence of *MAPT* mutations in dementia in general is extremely rare (<0.2%) (Houlden *et al.*, 1999). In all studies, *MAPT* Pro301Leu was the most frequent mutation. It is of interest that for some FTDP-17 families, including one family with conclusive linkage to 17q21-22, no *MAPT* mutations have been found (Bird *et al.*, 1996; Froelich *et al.*, 1997; Heutink *et al.*, 1997; Lendon *et al.*, 1998), suggesting either the presence of another FTD gene on 17q21-22 or the existence of other unidentified *MAPT* mutations. Moreover, the relatively low *MAPT* mutation frequency in familial FTD, and the fact that 64% of FTD patients do not have tau pathology as shown by immunohistochemistry (Mann *et al.*, 2000), suggest that only a minority of FTD cases can be classified as tauopathies. Therefore, other FTD genes, as already suggested by the FTD loci on chromosomes 3 and 9, are to be expected.

PRION DISEASE

Transmissible spongiform encephalopathies (TSE) are subacute or chronic neurodegenerative disorders of humans and animals that are characterized neuropathologically by the classical triad of spongiosis, neuronal loss and reactive gliosis. In 1982, it was found that a proteinaceous infectious particle devoid of nucleic acid (prion) was the disease-causing agent of scrapie, a TSE of sheep and goats (Prusiner, 1982). In humans, around 85% of TSEs or prion diseases are sporadic and described clinically as CJD (MIM 123400). Familial prion disease, iatrogenic CJD and the bovine spongiform encephalopathy (BSE)-related variant CJD account for the remainder. Human prion disease has an estimated yearly incidence of 1/1 000 000/year (Haywood, 1997). The core clinical syndrome of classical sporadic CJD is a rapidly progressive (disease course of <6 months), generalized early-onset dementia, usually accompanied by myoclonus and periodic synchronized discharges in the electroencephalogram (EEG). More recently, it was shown that the presence of the 14-3-3 protein in the CSF significantly increases diagnostic accuracy (Zerr *et al.*, 2000).

Molecular Genetics

Chromosome 20: PRNP

Prion diseases are inherited in about 10% of cases and have been described under different names, including CJD, Gerstmann–Sträussler–Scheinker syndrome (GSS; MIM 137440) and fatal familial insomnia (FFI; MIM 600072). In 1989, the first disease-causing mutations in the gene encoding the prion protein (*PRNP*; MIM 176640) on chromosome 20 were described (Figure XV-11.1). The first report involved an insertion in *PRNP* (Owen *et al.*, 1989), shown later to comprise 144 bp encoding six extra octapeptide repeats in addition to the five present in normal prion protein (Owen *et al.*, 1990). The report of this insertion was followed quickly by several missense mutations (Hsiao *et al.*, 1989; Doh-ura *et al.*, 1989). Presently, more than 20 pathogenic *PRNP* mutations have been reported that can be subdivided into two groups: (1) point mutations within coding sequence, resulting in amino acid substitutions in the prion protein or, in one case, production of a stop codon resulting in truncated prion protein; (2) insertions encoding additional copies of an octapepetide repeat present in tandem array of five copies in the normal protein (Collinge, 1997) (Table XV-11.1). All inherited prion diseases are caused by *PRNP* mutations, suggesting no locus heterogeneity for this condition.

Genetic Susceptibility to Sporadic and Acquired Prion Diseases

In sporadic and acquired CJD, no *PRNP* mutations have been observed. However, homozygosity for a common *PRNP* polymorphism at codon 129, where either Met or Val can be encoded, is a key determinant in the genetic susceptibility in sporadic (Palmer *et al.*, 1991) and iatrogenic CJD (Collinge *et al.*, 1991) (Table XV-11.2). Also, all cases with the BSE-related variant CJD have been homozygous for the Met allele (Collinge *et al.*, 1996). In a recent multicentric study including 749 sporadic CJD patients, it was shown that the Val homozygous genotype is significantly more frequent in patients younger than 49 years compared with those aged 50 years and over (Alperovitch *et al.*, 1999). These Val homozygous patients are generally younger, have an atypical clinicopathological phenotype, and lack the typical EEG findings (Parchi *et al.*, 1996).

Genotype–Phenotype Correlations in Inherited Prion Disease

Generally, phenotypic expression of inherited prion disease is highly variable. However, symptoms, disease course and neuropathological findings usually fall within a broad spectrum defined by classical CJD (with dementia, a rapid course, and marked spongiform changes) and GSS (with cerebellar ataxia, a slow course, and multicentric amyloid plaques) at the extremes. Familial prion diseases tend to have an earlier age at onset and a more protracted course than sporadic CJD. Also, the typical EEG findings are often missing and the 14-3-3 protein in CSF is detected in only about one-half of the cases (Zerr *et al.*, 2000). It is important to note that even within the same family, the disease can present as, or mimick conditions like, EOAD, FTD, multiple sclerosis, CBD, GSS or CJD (Mallucci *et al.*, 1999). With the exception of FFI, most *PRNP* missense mutations are associated with CJD. The *PRNP* Glu200Lys is the most frequent cause of hereditary CJD, accounting for >70% of the families worldwide (Lee *et al.*, 1999). Unlike most inherited prion diseases, these cases present clinically with a rapidly progressive dementia (<12 months) with myoclonus and typical periodic sharp wave complexes on EEG reminiscent of sporadic CJD (Brown *et al.*, 1992). Prevalence of this mutation and hence CJD is particularly high in Slovakia, Chile and Italy, and among populations of Libyan and Tunisian Jews. Recent haplotype analysis suggests that at least four independent mutational events are responsible for the current geographic distribution of Glu200Lys related CJD (Lee *et al.*, 1999). The classical GSS presentation, on the other hand, is a chronic cerebellar ataxia accompanied by pyramidal features, with dementia occurring later. Neuropathologically, the disease is characterized by the presence of multicentric amyloid plaques. The first *PRNP* missense mutation (Pro102Leu) reported was described in two GSS families (Hsiao *et al.*, 1989). Other missense mutations also led to a GSS phenotype (Prusiner, 1993). Another *PRNP* mutation (Asp178Asn) is of particular interest because its phenotypic expression is modulated by the codon 129 polymorphism. When inherited together with a Met allele on the same haplotype, Asp178Asn results in FFI, a rapidly progressive disease characterized clinically by untreatable insomnia, dysautonomia, motor signs and prominent atrophy of the thalamic nuclei. However, when coupled with Val at codon 129, Asp178Asn results in familial CJD (Goldfarb *et al.*, 1992). The stop codon 145 mutation results in a vascular variant of prion disease with severe CAA (Ghetti *et al.*, 1996). Clinically, the patient with this mutation was diagnosed as having AD.

PRNP insertions of extra copies of an octapeptide coding repeat (normal = 5) are the second major type of *PRNP* mutations. These tend to give rise to a form of CJD with an earlier onset and a relatively slow progression (>1 year) (Goldfarb *et al.*, 1991). Interestingly, there seems to be a relationship between the number of inserted repeats and the disease presentation. Although exceptions can be found, the majority of patients with long insertions of more than four extra repeats display a long disease duration ranging between 2 and 18 years, whereas CJD associated with shorter insertions usually mimics the classical sporadic CJD course (Capellari *et al.*, 1997). Also, elongated prion protein deposits in the molecular layer of the cerebellum are a typical feature in *PRNP* insertion carriers. It was demonstrated that these elongated prion protein deposits are associated with four to seven extra repeats, whereas the larger plaques are present only in patients carrying eight or nine octapeptide repeats (Vital *et al.*, 1998).

OTHER MENDELIAN EARLY-ONSET DEMENTIAS

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL; MIM 125310) is an inherited stroke syndrome that leads to dementia. The key features of the disorder are recurrent subcortical ischaemic events (85%), dementia

(40%), migraine with aura (20–30%), mood disturbances (20%) and diffuse white-matter abnormalities on neuroimaging (Bousser and Tournier-Lasserve 2001). CADASIL is characterized by an arteriopathy that affects mainly the small cerebral arteries and by prominent alterations of the vascular smooth muscle cells that ultimately disappear. Ultrastructural examination shows granular osmiophilic deposits located close to the membrane of the vascular smooth muscle cells in both cerebral and peripheral arteries, including skin arterioles (Ruchoux *et al.*, 1995). The disease was mapped to chromosome 19 in two families (Tournier-Lasserve *et al.*, 1993), and subsequent linkage analysis of 13 additional pedigrees revealed genetic homogeneity of the condition and allowed refinement of the locus to a 2 cM interval on 19p13.1 (Ducros *et al.*, 1996) (Figure XV-11.1). In 1996, it was found that CADASIL is caused by highly stereotyped missense mutations in the gene encoding the Notch3 receptor (*NOTCH3*; MIM 600276), predicted to result in either the gain or loss of a cysteine residue within one of the 34 epidermal growth factor repeats of the extracellular domain of the Notch3 receptor (Joutel *et al.*, 1996). It has been shown that in CADASIL brains, there is a dramatic and selective accumulation of the extracellular domain of the Notch3 receptor at the cytoplasmic membrane of the smooth muscle cells but not within the granular osmiophilic material (Joutel *et al.*, 2000a). Worldwide, more than 400 families have been reported since 1993, and a sporadic case with a *NOTCH3* mutation has been described suggesting that CADASIL might be more frequent than anticipated (Joutel *et al.*, 2000b) (Table XV-11.1).

Familial British Dementia and Familial Danish Dementia

Familial British dementia (FBD; MIM 176500), also called Worster-Drought syndrome, was first described in 1933 as a familial presenile dementia with spastic paraparesis and cerebellar ataxia (Worster-Drought *et al.*, 1933). The disease is transmitted as an autosomal dominant trait in a large family, and is characterized neuropathologically by the presence of prominent CAA and Alzheimer's disease-like neuritic and non-neuritic plaques and neurofibrillary tangles. By immunohistochemistry, however, the amyloid deposited is negative for antibodies directed to the classical β-amyloid of AD (Plant *et al.*, 1990). On MRI, the disease is manifest as deep white-matter lesions and lacunar infarcts (Mead *et al.*, 2000).

In 1999, a point mutation at the stop codon of a novel gene, *BRI* (MIM 603904), located on chromosome 13 was reported to cause FBD (Vidal *et al.*, 1999) (Figure XV-11.1). This mutation generates a longer open reading frame, resulting in a larger precursor protein of which the release of the 34 carboxy-terminal amino acids generates the British amyloid fibril ABri that is deposited in FBD brains. Another *BRI* mutation causes familial Danish dementia (FDD; MIM 117300), also known as heredopathia ophthalmo-oto-encephalica, characterized by cataracts, deafness, progressive ataxia, dementia and Alzheimer's disease-like neuropathology similar to FBD (Vidal *et al.*, 2000a). The FDD mutation is a ten-nucleotide duplication producing a frame shift in the *BRI* sequence, again generating a larger-than-normal precursor protein, of which the amyloid subunit (designated Danish amyloid or ADan) comprises the last 34 carboxy-terminal amino acids. By comparing FBD and FDD with AD, it is clear that different types of amyloid peptides can trigger similar neuropathological changes, leading to neuronal loss and dementia, suggesting that amyloid peptides are the primary event in the neurodegenerative process (Vidal *et al.*, 2000b).

Familial Encephalopathy with Neuroserpin Inclusion Bodies

In 1999, two Caucasian families living in the USA with autosomal dominant early-onset dementia were described (Davis *et al.*, 1999). In one family, the disease presented in the fifth decade with cognitive decline, including deficits in attention and concentration and impaired visuospatial skills. Memory was also impaired, albeit to a lesser extent than in AD. In the other family with an earlier onset around the second and third decades of life, the disease presented with both epilepsy and cognitive decline (Yerby *et al.*, 1986). The principal neuropathological finding in both families was the presence of eosinophilic neuronal inclusions, termed Collins bodies, in the cerebral cortex and many subcortical nuclei (Davis *et al.*, 1999). These inclusions were different from other intraneuronal inclusions, such as Pick bodies, Lewy bodies and Lafora bodies, and were composed primarily of a single protein, identified as neuroserpin. The disease was therefore called familial encephalopathy with neuroserpin inclusion bodies (FENIB; MIM 604218); in each family, a causative missense mutation in the gene encoding neuroserpin (*PI12*; MIM 602445) at 3q26 was identified (Figure XV-11.1; Table XV-11.1). However, in a French study, no mutations were found in a series of familial early-onset dementia, suggesting that *PI12* mutations are a rare cause of inherited dementia (French Alzheimer's Disease and Fronto-Temporal Dementia Genetics Study Groups, 2000).

Presenile Dementia with Bone Cysts

Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; MIM 221770), also called Nasu–Hakola disease or presenile dementia with bone cysts, is a rare recessive disorder characterized by bone cysts restricted to ankles and wrists, followed by a combination of psychotic symptoms and progressing rapidly to presenile dementia of the FTD type. There is a long period between the early skeletal and the neuropsychiatric symptoms, which can be as long as 20 years. Most patients have been diagnosed in Finland and Japan, with an estimated population prevalence in Finland of $2/10^6$. Neuropathologically, the disease is characterized by frontally accentuated loss of myelin, astrocytic gliosis, calcifications and atrophy of the basal ganglia, atrophy of the corpus callosum, and thick-walled or obliterated small arterioles and capillaries. In 1998, the disease was mapped to 19q13 (Pekkarinen *et al.*, 1998), and recently two different loss-of-function mutations in the gene encoding TYRO protein tyrosine kinase binding protein (*TYROBP*; MIM 604142) were identified (Paloneva *et al.*, 2000) (Figure XV-11.1; Table XV-11.1). However, the molecular and cellular pathogenesis remains unknown.

GENETIC COUNSELLING AND TESTING

As described above, dementia in both its late-onset and early-onset forms is a disorder with a high genetic contribution. Therefore, asymptomatic relatives of demented patients often seek genetic advice about their own and their children's risks. Alternatively, molecular genetic testing is sometimes requested by clinicians as a differential diagnostic tool in patients presenting with cognitive symptoms. Regardless of the purpose (prediction or diagnosis), molecular genetic testing should not be performed without a thorough pedigree analysis, including careful determination of the age at onset of dementia in the affected family members.

Autosomal Dominant Dementia

When the dementia has an early onset and is clearly familial, pedigree analysis might reveal an inheritance pattern that is consistent with autosomal dominant transmission. However, in the absence of molecular genetic confirmation, counselling is often uncomfortable because of problems of insufficient family information, diagnostic uncertainty, and the possible confounding by phenocopies.

Therefore, molecular genetic screening might be requested in order to alter an individual's a priori risk of 50% to almost zero (mutation absent) or 100% (mutation present). Depending on the clinical or pathological diagnosis, genetic screening in an affected individual of the most frequently mutated familial dementia genes can be performed. At present, these include *APP*, *PSEN1*, *PSEN2*, *MAPT* and *PRNP*. If AD is the suspected diagnosis, then *APP*, *PSEN1* and *PSEN2* are the main candidates for screening. However, it is important to realize that a considerable proportion of autosomal dominant EOAD is not explained by any of these AD genes (see above). In such cases, but also justified by the high amount of genetic and phenotypic heterogeneity in familial dementia, screening of *MAPT* or *PRNP* should be considered. It is of interest in this context that in a recent study, *PRNP* mutations were found in three out of 16 familial early-onset dementia patients (19%), while *APP*, *PSEN1* and *PSEN2* together accounted for 37% of the cases (Finckh *et al.*, 2000). In this study, none of the *PRNP* mutation carriers were expected clinically to have inherited prion disease. Unfortunately, systematic studies on the relative contribution of *APP*, *PSEN1*, *PSEN2*, *MAPT* and *PRNP* mutations in familial early-onset dementia are presently unavailable. In case a causative mutation is found, presymptomatic predictive testing can be offered to at-risk family members. Presymptomatic testing for Huntington's disease is considered the paradigm for predictive testing in genetic disorders of adulthood and has shown the psychological importance of pretest and post-test genetic counselling (Meiser and Dunn, 2000; Wiggins *et al.*, 1992). Given the complex nature of such counselling, this should preferentially be carried out in a medical genetics centre by a multidisciplinary team.

A major problem contributing to the complexity of genetic counselling of familial dementia is the interpretation of a mutation as being causative or not causative. Clues to the causative nature of a mutation come from different angles, including location in a functionally important region of the protein, evidence of cosegregation of the mutation in the family, absence of the mutation in a large series of controls, and *in vitro* functional evidence that the mutation is pathogenic. An illustrative example of misinterpreting the pathogenic character of a mutation is the *PSEN1* Glu318Gly missense substitution. This mutation was reported by several investigators and interpreted as a causative mutation (Cruts *et al.*, 1998; Forsell *et al.*, 1998; Sandbrink *et al.*, 1996). However, we demonstrated later that the Glu318Gly carrier frequencies were similar in elderly nondemented controls (9/256; 4.1%) and demented individuals (13/390; 3.3%) and that this substitution had no *in vitro* effect on APP processing (Dermaut *et al.*, 1999). This study therefore demonstrated that *PSEN1* Glu318Gly is a rare benign polymorphism rather than a causative mutation, and emphasizes the need to be careful when assigning a pathological nature to *PSEN1* mutations, especially when they are reported in isolated cases or in the absence of conclusive evidence for cosegregation with the disease. In a presymptomatic testing programme, the burden of an incorrect molecular diagnosis should be prevented by all means.

Some authors have advocated genetic testing as a diagnostic tool in familial forms of dementia and emphasized its use in excluding the presence of treatable forms of dementia (Finckh *et al.*, 2000). However, given the low prevalence rates of autosomal dementias, the sometimes complex interpretation of genetic variations, and the untreatable character of these diseases, genetic testing is presently of limited use in clinical practice (Croes *et al.*, 2000).

Common Forms of Dementia

The identification of causative mutations for autosomal dominant dementia has probably overinflated the public's perception of the role of genes in causing the far more common forms of dementia like LOAD. Presently, only *APOE* is known to affect susceptibility to LOAD. However, as many people with *APOE*-ε4 alleles will never get AD and, conversely, many AD patients do not have *APOE*-ε4 alleles, the predictive value of *APOE* genotyping in asymptomatic individuals is of limited value (McConnell *et al.*, 1998). Therefore, genetic counselling can be performed only in the broadest terms. In general, it is probably safe to say that the risk for first-degree relatives of typical LOAD patients is three- to fourfold increased, resulting in an absolute risk of between one in five and one in six (Liddell *et al.*, 2001).

APOE genotyping has also been advocated by some as a diagnostic test. Although it was shown that *APOE* genotyping might increase diagnostic certainty when applied to people already diagnosed clinically with probable AD (Mayeux *et al.*, 1998), *APOE* genotyping alone can never establish a diagnosis of AD. As currently a patient's prognosis and treatment would not change based on the increased likelihood that the dementia is caused by AD or another cause of dementia, the use of *APOE* genotyping as a diagnostic tool should not be encouraged (McConnell *et al.*, 1998).

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XV-12

Gene–Environment Interactions in Cognitive Disorders

Kala M. Mehta and Kristine Yaffe

Gene–environment interactions are critical to understanding complex chronic disease processes, such as those involved in cognitive disorders. Genetic factors may increase the likelihood of a cognitive disorder developing. Environmental factors may also increase the risk of developing a cognitive disorder. However, neither genes nor environmental factors alone are sufficient to explain the development of these disorders. More likely, genes and environment interact to confer risk, e.g., an environmental factor may affect genetic predisposition by altering the time of onset, progression or the severity of disease. By studying gene–environment interactions, further advances can be made regarding the aetiology and possible treatment of cognitive disorders.

PREVALENCE OF COGNITIVE DISORDERS AND DEMENTIA

Cognitive disorders in the elderly range from mild cognitive deficits to severe dementia (Petersen, 2000). Worldwide incidence of dementia increases steeply with age: for people aged 60–64 years, the incidence is 0.8–4.0 per 1000 person-years, but in people over 95 years, it is 50–136 per 1000 person-years (Fratiglioni *et al.*, 1999). The most common forms of dementia are Alzheimer's disease and vascular dementia, accounting for 50–70% and 20–30% of all dementia patients, respectively (Plassman and Breitner, 1996).

Most of the research concerning the aetiology, genetics and environmental risk factors in cognitive disorders in the elderly has focused on Alzheimer's disease (Mayeux, 1998). Alzheimer's disease has two patterns of inheritance: familial and sporadic. The familial form tends to be inherited via autosomal dominance, can have a more severe course, and typically has an onset before age 65 years. This form of Alzheimer's disease accounts for less than 5% of all cases. The sporadic form of Alzheimer's disease tends to have a more diverse genetic aetiology, with a more gradual course, and often has an onset after age 65 years (National Institute on Aging National Institutes of Health, 1999). The past decade has seen tremendous advances in the genetics of both forms of Alzheimer's disease.

REVIEW OF ALZHEIMER'S DISEASE MOLECULAR BIOLOGY AND GENETICS

Senile plaques and neurofibrillary tangles are the two pathological brain lesions found in Alzheimer's disease. Neurofibrillary tangles are comprised of microtubule-associated proteins, tau. Hyperphosphorylation of tau by kinases leads to disassociation of microtubules and subsequent aggregation into filaments. The senile plaques consist of extracellular deposits of amyloid β protein (Aβ). Aβ comes in two forms, Aβ_{40} and Aβ_{42}, the latter of which is more hydrophobic and prone to aggregation. A higher ratio of Aβ_{42}/Aβ_{40} is thought to lead to greater plaque deposition. Aβ is derived from the amyloid precursor protein (APP). APP is a single transmembrane polypeptide, common to neurons and other cells such as astrocytes, microglia, endothelial and smooth-muscle cells. Aβ is formed when APP is cleaved sequentially by secretases. When APP is cleaved by a β-secretase, a 99-residue fragment is formed. The 99-residue fragment can be cleaved by a γ-secretase resulting in either Aβ_{40} or Aβ_{42} (Selkoe, 2001). At least three known autosomal dominant gene mutations are involved in Alzheimer's disease: APP on chromosome 21, Presenilin-1 (PSEN1) on chromosome 14, and Presenilin 2 (PSEN2) on chromosome 1 (Table XV-12.1). APP, PSEN1 and PSEN2 all alter APP processing, producing more Aβ_{42} than Aβ_{40} (Hardy, 1997).

Apolipoprotein E allele ε4 (APOE-ε4) on chromosome 19 is a genetic risk factor for the sporadic form of Alzheimer's disease. The APOE locus has three common alleles, ε2, ε3 and ε4. The ε4 allele has consistently been found to predispose individuals to Alzheimer's disease, with increasing allele-dose risk per ε4 allele (Corder *et al.*, 1993; Saunders *et al.*, 1993; Strittmatter *et al.*, 1993). It is different from the three familial gene mutations in that it increases the likelihood that a person will develop Alzheimer's disease in people aged over 65 years, but it is not conveyed by a dominant pattern of inheritance. APOE contributes approximately 50–70% to sporadic Alzheimer's disease risk; other susceptibility genes or environmental factors may account for the remainder (Pericak-Vance *et al.*, 2000). APOE may also increase the risk of developing other cognitive disorders, such as preclinical cognitive impairment (Hyman *et al.*, 1996; Yaffe *et al.*, 1997), vascular dementia (Skoog *et al.*, 1998), and dementia associated with neuromuscular diseases (Bedlack *et al.*, 2000). There are two main hypotheses for APOE ε4's effects on the increased risk of developing Alzheimer's disease: either ε4 increases the aggregation of Aβ into fibrils found in plaques, or APOE-ε4 helps with

Table XV-12.1 Genetic factors involved in Alzheimer's disease

Factor	Chromosome
Autosomal dominant genes	
β-Amyloid precursor protein	21
Presenilin-1	14
Presenilin-2	1
Genetic risk factors	
Apolipoprotein E allele ε4	19
Alpha-2-macroglobulin	12
Low-density lipoprotein-related protein 1	12

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deposition or clearance of Aβ (probably Aβ_{40}). Both of these pathways could result in increased numbers of senile plaques.

Other recent genetic factors that may be involved in sporadic Alzheimer's disease include alpha-2 macroglobulin (A2M), a potent pan-protease inhibitor, and low-density lipoprotein-related protein 1 (LRP-1), a multifunctional receptor. A2M may also be associated with the accumulation of Aβ in senile plaques. As LRP-1 is a multifunctional receptor and binds three important Alzheimer's disease-related proteins (APOE, APP and A2M), it may increase the risk by a common neurogenetic pathway affecting senile plaque deposition or other molecular processes involved in Alzheimer's disease.

ENVIRONMENTAL FACTORS ASSOCIATED WITH THE RISK OF ALZHEIMER'S DISEASE AND EXAMPLES OF GENE–ENVIRONMENT INTERACTIONS

Identifying environmental risk factors and how they are involved in the risk of developing Alzheimer's disease is important because this could lead to interventions and possibly a reduction in disease prevalence. Most of the environmental factors that are associated with the risk of developing Alzheimer's disease can be categorized into three subgroups: medications or supplements, atherosclerotic risk factors or lifestyle factors (Table XV-12.2). A hypothetical scenario of how genes and environmental factors may act together to confer earlier cognitive impairment is depicted in Figure XV-12.1. Since the sporadic form of Alzheimer's disease affects far more people than the familial form, and the APOE-ε4 allele is a genetic risk factor for the sporadic form of Alzheimer's disease, much of the research has focused on APOE-ε4–environment interaction.

Medications or Supplements

Based on observational studies and biological plausibility, several medications and supplements have been investigated as having an effect on the risk of developing Alzheimer's disease. The three most studied medications are oestrogen, anti-inflammatory agents, and antioxidants.

Oestrogen supplementation may be an important contributor to cognitive health. Several potential mechanisms of action have been suggested (Yaffe *et al.*, 1998). Oestrogen may affect Aβ deposition through neurotransmitter modulation, specifically acetylcholine, serotonin and monoamine oxidase. Alternatively, oestrogen may have a direct effect on neurons through either synaptic regulation or dendritic sprouting, or oestrogen may have a positive effect on cognition via a positive effect on lipids. Some studies on oestrogen in nondemented elderly women support the idea that oestrogen improves cognition (Jacobs *et al.*, 1998), but other studies have not shown this effect (Barrett-Connor and Kritz-Silverstein, 1993). A recent multicentre trial in community-dwelling women suggests that using a selective oestrogen receptor modulator, raloxifene, has no positive or negative effect on cognitive function over 3 years (Yaffe *et al.*, 2001b). Oestrogen has also been studied as a potential treatment for Alzheimer's disease, with some trials, mostly uncontrolled, showing a beneficial effect (Honjo *et al.*, 1989; Asthana *et al.*, 1999; Ohkura *et al.*, 1994; Ohkura *et al.*, 1995), but more recent controlled trials not showing a positive effect of oestrogen on Alzheimer's disease (Wang *et al.*, 2000; Mulnard *et al.*, 2000; Henderson *et al.*, 2000). Observational studies of oestrogen as a potential preventive agent for Alzheimer's disease have mostly reported that oestrogen does decrease the risk of developing Alzheimer's disease (Kawas *et al.*, 1997; Tang *et al.*, 1996a; Paganini-Hill and Henderson, 1994), but there are some exceptions (Brenner *et al.*, 1994; Graves *et al.*, 1990b). Results from four ongoing primary prevention trials will extend the understanding of whether oestrogen may be used for the prevention of Alzheimer's disease.

A recent study of over 2700 women found that the effect of current oestrogen use on cognitive decline varies by APOE-ε4 allele status (Yaffe *et al.*, 2000). Women who did not have an ε4 allele had

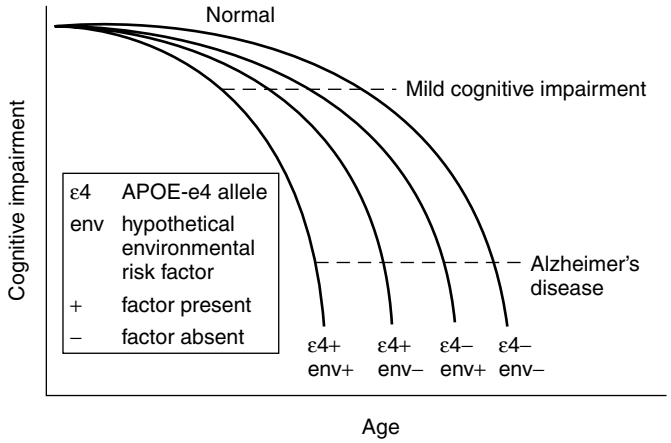

Figure XV-12.1 Trajectory of cognitive impairment by APOE-ε4 allele status, a hypothetical environmental risk factor and age

Table XV-12.2 Environmental risk factors associated with Alzheimer's disease

	Strength of evidence[†]	Gene Interaction
Medication and supplements		
Oestrogen replacement	Decrease risk (+)	(+)
Anti-inflammatory agents (e.g., NSAIDs)	Decrease risk (++)	
Antioxidants (e.g., vitamin E)	Decrease risk (+)	
Atherosclerotic risk factors		
Hypertension	Increase risk (+)	(+)
Lipids	Increase risk (+/−)	(+/−)
Diabetes	Increase risk (+/−)	(+/−)
Homocysteine	Increase frequency	
Lifestyle factors		
Smoking	Increase risk (+)	(+/−)
Moderate alcohol consumption	Decrease risk (+)	(+/−)
Physical activity	Decrease risk (+)	(+)
< 8 years of education	Increase risk (+++)	
Intellectual activity	Decrease risk (+/−)	
Moderate or severe head injury	Increase risk (++)	(+)

NSAID, non-steroidal anti-inflammatory drug.

[†] *Convincing* (+++): epidemiological studies show consistent associations, with little or no evidence to the contrary.
Probable (++): epidemiological studies showing associations are either not so consistent, with a number and/or proportion of studies not supporting the association, or else the number or type of studies is not extensive enough to make a more definite judgement; mechanistic and laboratory evidence are usually supportive or strongly supportive.
Possible (+): epidemiological studies are generally supportive, but are limited in quantity, quality or consistency. There may or may not be supportive mechanistic or laboratory evidence. Alternatively, there are few or no epidemiological data, but strongly supportive evidence from other disciplines.
Insufficient (+/−): there are only a few studies, which are generally consistent, but really do no more than hint at a possible relationship; often, more well-designed research is needed.
Source: World Cancer Research Fund and American Institute for Cancer Research, 1997.

a 40% lower risk of developing cognitive impairment associated with oestrogen use, whereas women who were $\varepsilon 4$ positive did not obtain a protective effect from oestrogen (Figure XV-12.2). This study supports the hypothesis that the environmental factor (oestrogen replacement) affects risk of cognitive impairment and that it varies by genetic profile (APOE). Since the $\varepsilon 4$-positive women in this study had greater internal carotid wall thickness than $\varepsilon 4$-negative women, and this varied by oestrogen use as well, a potential mechanism of oestrogen's effect on cognitive impairment could be via carotid atherosclerosis.

Inflammation may be related to Alzheimer's disease pathology either as a primary or secondary process, since Aβ plaques are associated with inflammation with resultant increases in cytokines, such as interleukin 1β, interleukin-6 and tumour necrosis factor. Anti-inflammatory agents potentially could decrease Alzheimer's disease severity by altering the disease's inflammatory response. A meta-analysis of 17 observational studies found that non-steroidal anti-inflammatory drugs (NSAIDs) may decrease the development of Alzheimer's disease by 30–50% (McGeer *et al.*, 1996). Few studies have investigated the NSAIDs–gene interaction on cognitive decline or Alzheimer's disease. One study showed a decreased occurrence of Alzheimer's disease associated with NSAIDs in people with and without APOE-$\varepsilon 4$ (Anthony *et al.*, 2000). Further work on the interaction between the APOE-$\varepsilon 4$ allele and anti-inflammatory agents is needed. A form of NSAID that selectively binds the cyclooxygenase-2 (COX-2) receptor is of particular interest. Because COX-2 inhibitors bind receptors more selectively and cause fewer side effects than other NSAIDs, they may be useful for treating or preventing Alzheimer's disease (McGeer, 2000). An ongoing primary prevention trial will compare an NSAID (ibuprofen), a COX-2-selective agent (celecoxib) and placebo in 2625 older dementia-free subjects with a family history of Alzheimer's disease. The results of this trial will further the understanding of the effect of NSAIDs on the prevention of Alzheimer's disease.

Antioxidants, such as vitamins E and C, have various health benefits and may be beneficial to cognitive health. Vitamin E may prevent or help treat Alzheimer's disease by reducing oxidative stress (Smith *et al.*, 2000). Some observational studies have investigated the association between high doses of vitamin E and the risk of developing Alzheimer's disease, showing a lower incidence of Alzheimer's disease in vitamin E users (Morris *et al.*, 1998). One clinical trial investigated the effects of alpha-tocopherol (vitamin E, 2000 IU per day), a selective monoamine oxidase inhibitor (selegiline, 10 mg per day), and a placebo in patients with moderate to severe Alzheimer's disease (Sano *et al.*, 1997). Both vitamin E and selegiline were associated with fewer adverse outcomes, such as nursing home placement, compared with the placebo, but there were no differences on cognitive test scores. Although preliminary basic science studies have suggested differential oxidative effects by APOE allele type (Ramassamy *et al.*, 2001; Veinbergs *et al.*, 2000), no population-based studies have investigated the effects of vitamin E or other antioxidants according to the APOE allele.

Summary

Oestrogen, NSAIDs and antioxidants potentially decrease the risk of developing Alzheimer's disease. The oestrogen use–Alzheimer's disease relationship varies by APOE-$\varepsilon 4$ presence, suggesting an interaction. Whether an interaction exists between NSAIDs or antioxidants and APOE-$\varepsilon 4$ remains to be investigated.

Atherosclerotic Risk Factors

Atherosclerotic risk factors, such as hypertension and diabetes mellitus, are known to be associated with vascular disease, such as coronary artery disease, stroke and vascular dementia. Vascular pathology often coexists with Alzheimer's disease pathology, so the relationship between atherosclerotic risk factors and Alzheimer's disease is of growing interest. It is clear that atherosclerotic risk factors play a large role in both diseases and therefore are important in preserving cognitive health.

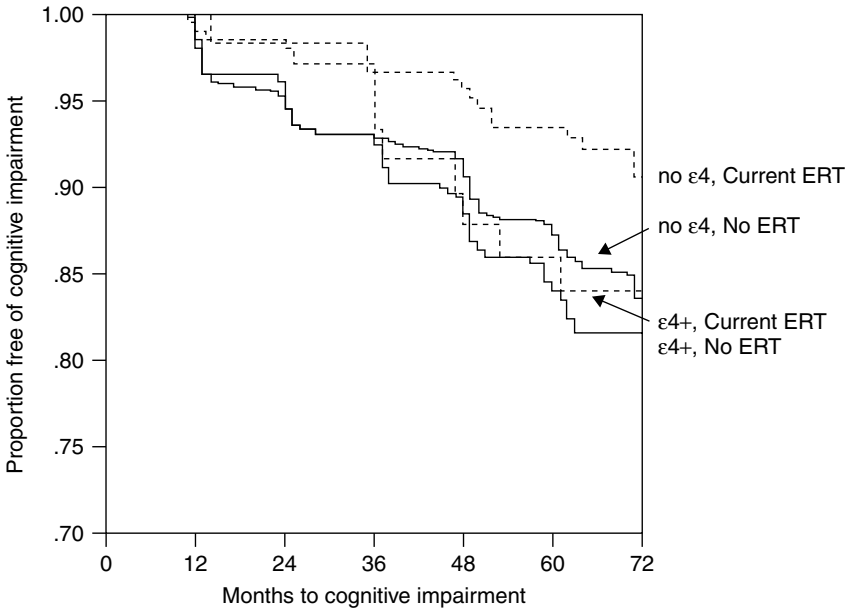

Figure XV-12.2 Time to cognitive impairment (modified mini-mental score <80) according to APOE-$\varepsilon 4$ status and baseline oestrogen use after adjusting for age, education, race and stroke history. Reproduced from Yaffe, K., Haan, M., Byers, A., Tangen, C. and Kuller, L., 2000. Estrogen use, APOE, and cognitive decline: evidence of gene–environment interaction. *Neurology*, 54, 1949–1954 Figure 1 by permission of Lippincott Williams & Wilkins

Elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP) are known risk factors for stroke and are associated with central nervous system vascular changes, such as small-vessel disease and white-matter hyperintensities seen on brain imaging (Schoenberg, 1979; Ketonen, 1998). The hypertension–Alzheimer's disease relationship is less well understood. Several longitudinal studies suggest that SBP and DBP are correlated negatively with cognition after a 12–15-year follow-up (Elias *et al.*, 1993; Skoog *et al.*, 1996) and a 25-year follow-up (Launer *et al.*, 1995). It is possible that a J-shaped relation exists between blood pressure and Alzheimer's disease, similar to coronary artery disease, in which low blood pressure increases the risk of Alzheimer's disease slightly and high blood pressure increases the risk much more, relative to pressures within the normal range. In one study, people with either the APOE-ε4 allele or with low ankle–arm blood pressure declined about three times as much as people with neither risk factor, whereas people with both low ankle–arm blood pressure and the ε4 allele were eight times as likely to decline, suggesting a gene–atherosclerosis risk factor interaction (Haan *et al.*, 1999).

Since APOE-ε4 increases susceptibility for Alzheimer's disease and is related directly to lipid metabolism (increases in serum total cholesterol and low-density lipoproteins), serum lipid levels may be associated with Alzheimer's disease. The few studies on the relationship between total cholesterol levels and Alzheimer's disease are inconclusive. Both high total cholesterol (Evans *et al.*, 2000; Notkola *et al.*, 1998) and high intake of fat (Kalmijn *et al.*, 1997) were associated with Alzheimer's disease in some studies but not in others (Breteler, 2000). APOE-ε4 may modify the relationship between total cholesterol and Alzheimer's disease risk, as suggested in two studies where total cholesterol levels were found to be higher in Caucasian people with the ε4 allele (Jarvik *et al.*, 1995) and Chinese people with the ε4 allele (Liu *et al.*, 1999). In African-American and in Finnish cohorts, increased total cholesterol, independent of ε4 allele, was associated with increased Alzheimer's disease risk, suggesting an alternative mechanism (Evans *et al.*, 2000; Notkola *et al.*, 1998). Further studies in diverse racial groups are needed to increase the understanding of APOE-ε4 and total cholesterol. As the LRP receptor also binds to cholesterol, further investigation of APOE-ε4, cholesterol and LRP receptor is warranted.

Studies are conflicting regarding diabetes and Alzheimer's disease. One case–control and several longitudinal studies have shown a positive association between diabetes mellitus and cognitive impairment, with up to a two-fold increased risk of developing Alzheimer's disease (Leibson *et al.*, 1997; Ott *et al.*, 1999) or cognitive impairment (Gregg *et al.*, 2000). Several other studies have shown an inverse association between diabetes and risk of Alzheimer's disease (Bucht *et al.*, 1983; Launer *et al.*, 1995; Landin *et al.*, 1993). Studies vary regarding the relationship between diabetes, APOE-ε4 and Alzheimer's disease. In one study, diabetics with dementia had low frequency of the ε4 allele as compared with non-diabetics with dementia (Nielson *et al.*, 1996). In another study, participants who had an ε4 allele and diabetes mellitus declined 1.7 times more on cognitive tests than participants with neither risk factor. However, people with ε4 alone or diabetes alone declined three times and two times worse, respectively (Haan *et al.*, 1999). This suggests that diabetes may increase the risk of Alzheimer's disease in people who do not have a strong genetic disposition, or that an increased risk of Alzheimer's disease cannot be detected in people with diabetes and ε4 due to inadequate sensitivity of instruments. Further studies are needed to elucidate whether diabetes is associated with the risk of developing Alzheimer's disease, and, if so, whether it is modified by APOE-ε4 status.

Hyperhomocysteinaemia increases the likelihood of cardiovascular, peripheral vascular and cerebrovascular disease (Refsum *et al.*, 1998). Higher levels of homocysteine have been found more frequently in Alzheimer's disease patients compared with controls (Clarke *et al.*, 1998), and were associated inversely with cognitive function (Kalmijn *et al.*, 1999). More work on homocysteine is needed to determine its association with Alzheimer's disease and its interaction with APOE-ε4 and other genetic markers.

Most of the published gene–environment studies have focused on APOE-ε4. However, other genetic factors, such as presenilins, alpha-2 macroglobulin and the low-density lipoprotein-related protein 1 receptor, may interact with atherosclerotic risk factors and further study is needed.

Summary

Atherosclerotic risk factors such as hypertension, total cholesterol and diabetes increase the risk of cognitive impairment and may also be similarly associated with developing Alzheimer's disease. Hypertension has the strongest evidence for a gene–environment interaction with APOE-ε4. Results differ as to whether a gene–environment interaction exists between either cholesterol or diabetes and APOE-ε4. Further studies on atherosclerotic risk factor–gene interactions are warranted.

Lifestyle Factors

Several early studies suggested that smoking is a protective factor for cognitive impairment (Letenneur *et al.*, 1994) and Alzheimer's disease (van Duijn and Hofman, 1991; van Duijn *et al.*, 1995; Brenner *et al.*, 1993; Lee, 1994; Graves *et al.*, 1991). More recent studies with stronger methodology suggest that smoking either has no effect (Doll *et al.*, 2000) or increases the risk of Alzheimer's disease (Launer *et al.*, 1999). Indeed, several mechanisms might explain an association between smoking and a greater likelihood of cognitive decline (Lee, 1994). Smoking is a well-known risk factor for cerebrovascular disease, and smoking may be directly neurotoxic and cause oxidative damage to neurons (Linert *et al.*, 1999). Finally, smoking may promote the deposition of APP into amyloid (Plaschke *et al.*, 1997). Few studies have examined the effects of smoking on cognitive decline by APOE-ε4 status. One study found up to a 75% reduction in risk in smokers with an ε4 allele, consistent with the early reports of a protective effect (van Duijn *et al.*, 1995). More recent population-based studies have reported the opposite, suggesting that smokers who do not have the ε4 allele are up to four times as likely to get Alzheimer's disease compared with nonsmokers, whereas smokers with the ε4 allele are not at any greater risk than nonsmokers with ε4 (Ott *et al.*, 1998; Merchant *et al.*, 1999). In contrast, smokers with ε4 are at significantly greater risk of coronary heart disease compared with nonsmokers with ε4. (Humphries *et al.*, 2001) The modification of APOE-ε4–Alzheimer's disease risk by smoking may be due to selection bias, such that participants who were smokers and had ε4 were more likely to die. Two alternative explanations are that the increased risk of Alzheimer's disease cannot be detected in smokers with an ε4 allele due to inadequate sensitivity of instruments, or that smoking may act through a vascular mechanism on Alzheimer's disease risk, independent of the APOE-ε4-related vascular mechanisms.

A longitudinal study on moderate alcohol consumption and risk of Alzheimer's disease was conducted in Bordeaux, France (Orgogozo *et al.*, 1997). Moderate wine consumption lowered the risk of Alzheimer's disease by 75% compared with nondrinkers, but other studies have not confirmed this finding (Graves *et al.*, 1991; Elwood *et al.*, 1999). Smoking may have confounded the alcohol–Alzheimer's disease relation in the French study (Hebert *et al.*, 1992; Leibovici *et al.*, 1999), or the result may have been obtained due to the sampling strategy. Alcohol consumption may act through several mechanisms to affect the risk of Alzheimer's disease, such

as reducing cardiovascular risk factors, inhibiting platelet aggregation, altering serum lipid profile, or directly affecting acetylcholine release, in turn facilitating learning and memory. The association between moderate alcohol consumption and Alzheimer's disease may be mediated by APOE allele status, as suggested by one study in which the protective effect of alcohol was stronger in people with the ε4 allele (Carmelli *et al.*, 1999). Another study found the opposite, i.e., that people who drank moderately and had the ε4 allele had a lower risk of Alzheimer's disease compared with moderate drinkers without the ε4 allele (Dufouil *et al.*, 2000). These conflicting results highlight the need for more investigation of alcohol consumption, APOE and Alzheimer's disease.

Physical activity has several positive health benefits, including lowering the risk for coronary artery disease, stroke and diabetes. For Alzheimer's disease, physical activity has shown a positive effect in some studies but not others (Yaffe *et al.*, 2001a; Laurin *et al.*, 2001; Schuit *et al.*, 2001; Broe *et al.*, 1998). The one study that has examined physical activity by APOE-ε4 status suggests that people with both ε4 and who perform less than 1 hour of physical activity per week have an increased risk of cognitive decline as compared with people who have ε4 and who engage in more than 1 hour of physical activity per week, suggesting that physical activity may modify the risk of APOE-ε4 on cognitive decline (Schuit *et al.*, 2001). It remains to be seen whether the positive effects of physical activity on cognitive health are replicated, and whether physical activity interacts with other genetic risk factors.

Low educational level is an established risk factor for Alzheimer's disease (Katzman, 1993). Specifically, less than 8 years of education is associated with at least double the increased risk of Alzheimer's disease in Western societies. Potential explanations for the education–Alzheimer's disease relationship include: (1) education is a proxy marker for better socioeconomic, nutrition or housing status, which in turn are associated with decreased risk of Alzheimer's disease; (2) people with higher education might have more brain capacity, which buffers them against later cognitive decline; (3) education and intellectual stimulation could promote brain development, neuronal connectivity, or increased cerebral blood flow (Deary and Caryl, 1997; Stern *et al.*, 1995; Friedland, 1994). Studies indicate that higher performance on intelligence tests is associated inversely with Alzheimer's disease. In one study, army recruits' intelligence scores were measured on the Army General Classification Test early in life and their scores used to predict cognitive function in late life (Plassman *et al.*, 1995). Similarly, in a study of nuns, linguistic ability early in life was inversely proportional to severity of Alzheimer's disease pathology in the neocortex (Snowdon *et al.*, 2000). Whether the effect of intellect or education on Alzheimer's disease varies by APOE status or other genes warrants further investigation.

Head injury has been studied as one of the environmental risks of developing Alzheimer's disease (Mortimer *et al.*, 1985). Insults to the rodent brain may potentiate beta-amyloid neurotoxicity (Smith *et al.*, 1998). In humans, amyloid deposition subsequent to head trauma may represent a milder version of the diffuse Aβ plaques seen in boxers (Clinton *et al.*, 1991). Epidemiological studies conducted to investigate the head injury–Alzheimer's disease relation have had conflicting results. Some studies report up to a ten-fold increased risk of Alzheimer's disease associated with head injury (Mortimer *et al.*, 1991; Graves *et al.*, 1990a; Molgaard *et al.*, 1990; Rasmusson *et al.*, 1995; Mayeux *et al.*, 1993), but other studies show no association between head injury and Alzheimer's disease (Brayne, 1991; Chandra *et al.*, 1987; Chandra *et al.*, 1989; Salib and Hillier, 1997; van Duijn and Hofman, 1992). Factors that underlie these discrepant results include study design, criteria of both head trauma and Alzheimer's disease, and differential survival in head injured and non-injured groups. People with a history of head trauma had earlier symptom onset of Alzheimer's disease compared with people without head trauma in two studies (Nemetz *et al.*, 1999; Schofield *et al.*, 1997). With respect to the APOE–head injury relationship, some studies indicate an additive relation (Katzman *et al.*, 1996), others a synergistic relation (Tang *et al.*, 1996b), and others no relation at all (Mehta *et al.*, 1999). The differences in the studies on gene–head trauma interaction may be explained by reporting bias or by varying degrees of head trauma severity. In one study using documented military head injuries (Plassman *et al.*, 2000), mild head injury was not associated with Alzheimer's disease, whereas moderate head injury doubled the risk and severe head injury quadrupled the risk when compared with non-head-injured individuals. With respect to APOE-ε4, people with any degree of head injury and who were homozygous for the ε4 allele had four times the risk, ε4 heterozygotes had 1.5 times the risk, and no ε4 alleles had no increased risk, compared with people with neither risk factor. These findings, coupled with prior reports, suggest that moderate or severe head injury, but not mild head injury, increases the risk of developing Alzheimer's disease, especially in people with APOE-ε4.

Summary

Lifestyle factors such as having fewer than 8 years of education or a history of moderate head injury sharply increases the risk of developing Alzheimer's disease. Smoking slightly increases the risk of developing Alzheimer's disease, whereas moderate alcohol consumption and physical activity seem to decrease the risk of developing Alzheimer's disease. Moderate to severe head injury has the strongest evidence of a gene–lifestyle factor interaction, followed by preliminary evidence of a physical activity–gene interaction. Whether a lifestyle factor–gene interaction exists for either moderate alcohol consumption or smoking will require further study, as the evidence thus far differs. Similarly, the association of education or intellectual activity and genes on Alzheimer's disease warrants further investigation.

INCIDENCE AND PREVALENCE OF ALZHEIMER'S DISEASE BY ETHNICITY AND APOE-ε4 ALLELE STATUS

The incidence and prevalence of Alzheimer's disease are not consistent across different ethnic groups. Potential explanations for this variation could be due to study methodology, presence of environmental risk factors, or variance in susceptibility allele frequency. Studies in Caucasians indicate a gene–dose risk of ε4 allele presence on the risk of Alzheimer's disease with approximately a three-fold increased risk for heterozygous ε4 allele and a 15-fold increase for homozygous ε4 alleles (ε4/ε4) (Farrer *et al.*, 1997). In Japanese people, gene–dose risk is more robust, with up to a 43-fold increase for ε4/ε4 (Yoshizawa *et al.*, 1994). However, in Hispanic and African-American people, the gene–dose risk is weaker, despite similar cumulative incidence and familial aggregation across racial groups (Tang *et al.*, 1998; Devi *et al.*, 2000), suggesting either environmental determinants or other genetic determinants of Alzheimer's disease risk in these groups. Two recent cross-cultural studies comparing ethnic Japanese people in Japan, Hawaii and Washington state and ethnic African people in Yoruba and America show that the proportion of Alzheimer's disease to vascular dementia in each ethnic group decreases according to westernization (Shadlen *et al.*, 2000; Hendrie *et al.*, 2001). Although a likely explanation for this shift is the increasing vascular risk factors present in Western lifestyles, unidentified genetic causes could also play a role through genetic drift. Further studies to investigate the complex interaction between genetic predisposition and environmental triggers are necessary to explain the differences found in different ethnic groups.

Monozygotic and dizygotic twin pairs have been used to study gene–environment influences in Alzheimer's disease. Some studies have quantified the genetic contribution to Alzheimer's disease in twins, suggesting that hereditary factors may contribute approximately 60–80% of Alzheimer's disease risk (Bergem *et al.*, 1997; Gatz *et al.*, 1997). Others suggest that genes contribute 37% and common environmental factors contribute 35% of the risk (Meyer and Breitner, 1998). The first two studies suggest that genetics strongly influence the risk of developing Alzheimer's disease, with a simple heritability of about 0.83–0.84. The latter study, which has a simple heritability of 0.28, suggests the opposite, i.e., that genes and environment contribute almost equally to developing Alzheimer's disease. As the first two studies had longer follow-ups than the latter study, further studies in twins with long follow-up times and that examine multiple environmental risk factors are needed.

SUMMARY

The elucidation of genetic risk factors, environmental risk factors, and gene–environment interactions that put people at either substantially higher or lower risk for developing cognitive disorders may lead to efforts that can preserve cognitive health. For example, as individuals with $\varepsilon 4$ are at greater risk for developing Alzheimer's disease, interventions to preserve cognitive health in these patients could include increasing physical activity, smoking cessation or maintaining a low to moderate level of alcohol intake, as well as administration of currently available therapeutic agents.

Future studies may examine potentially protective agents and evaluate their potential harms. For example, if vitamin E is determined to have a protective effect against developing cognitive decline and has low side effects, then providing vitamin E supplements and public education for elderly people or those at risk could lower the prevalence of Alzheimer's disease. Whether this is true for people with the $\varepsilon 4$ allele remains to be seen. As knowledge of protective agents increases, clinical practice guidelines will change to include safe and effective protective strategies.

Gene–environment interactions will also need to be studied in non-Alzheimer's disease cognitive disorders, such as preclinical cognitive impairment and vascular dementia. More specifically, APOE and the other genetic risk factors under investigation for Alzheimer's disease may play a role in both mild cognitive impairment and vascular dementia. Future studies addressing gene–environment interactions in cognitive disorders will need to call on closer collaboration between interdisciplinary researchers. Molecular biologists and geneticists investigating genes will have to work more closely with clinical researchers and epidemiologists studying environmental risk factors. In addition, statisticians will need to be involved in modelling gene–environment interactions in cognitive disorders, to aid in predicting an individual's likelihood of disease. The complex nature of cognitive disorders will require innovative and collaborative research involving gene–environment interactions before cures for these diseases are found.

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XV-13

Review of Gender Issues and Oestrogen Replacement in Common Cognitive Disorders

John Stirling Meyer and Yan-Sheng Li

INTRODUCTION

Differences in brain structure and function between men and women are believed to result from gender differences in exposure to sex hormones during foetal life. Although there are no significant sex differences in global cognitive functioning, some gender differences in specific cognitive abilities have been reported. For example, men excel in spatial orientation and some quantitative abilities, particularly in gross motor strength, whereas women excel in verbal abilities, perceptual speed and accuracy, and fine motor skills. Gender differences also exist for cognitive disorders, particularly for dementia of Alzheimer type (DAT) and vascular dementia (VAD). Generally, women have a higher prevalence and incidence of DAT, while men have a higher prevalence of VAD, with symptoms beginning at a younger age (Jorm *et al.*, 1987; Jorm and Jolley, 1998; Fratiglioni *et al.*, 1997; Fratiglioni *et al.*, 2000; Lobo *et al.*, 2000).

GENDER DIFFERENCES IN ALZHEIMER'S DISEASE

Clinical and Epidemiological Studies

Epidemiological investigations from northern America, Europe and Asia have reported gender differences in the prevalence rates of DAT, especially older women exhibiting higher prevalence rates with female to male ratios between 1.5 : 1 and 3 : 1 (Jorm *et al.*, 1987; Lobo *et al.*, 2000; Skoog *et al.*, 1993; Hofman *et al.*, 1991; Zhang *et al.*, 1990). With regard to age-specific prevalence rates, combining the results of 22 prevalence studies between 1945 and 1985, no age-adjusted gender differences for prevalence of dementia were found, but analysis of the type of dementia showed higher rates of DAT for women (Jorm *et al.*, 1987). The Framingham Study displayed higher age-specific prevalences for both dementia and DAT in women older than 75 years (Bachman *et al.*, 1993).

A longitudinal study of 987 subjects aged 75 years or older in Stockholm indicated that women had higher incidence rates than men for both dementia and DAT (Fratiglioni *et al.*, 1997). A meta-analysis study demonstrated modest gender differences for DAT incidence rates, and the odds ratio for women developing DAT relative to men was 1.56 (Gao *et al.*, 1998). Collaborative studies confirm that in Europe, women have higher prevalence and incidence rates of DAT than men in all age ranges (Lobo *et al.*, 2000; Fratiglioni *et al.*, 2000). Gender differences in prevalence rates are thought to be attributed to different incidence and survival rates between the two sexes, since women survive longer than men with similar severities of dementia (Jorm *et al.*, 1987; Jagger *et al.*, 2000; Morrison and Tweedy, 2000).

Gender differences in DAT have also been noted by neuropsychological studies, such as women with DAT manifesting greater deficits on semantic memory but not on letter fluency or word recognition. These differences present even when the effects of age, education, duration of illness, and level of global impairment are controlled (McPherson *et al.*, 1999). A meta-analytic study showed that the sexual dimorphic character in DAT risk is 1.5 times greater in individuals with apolipoprotein E (*APOE*) genotype E3/E4 than in those with E3/E3 (Farrer *et al.*, 1997).

Effects of Oestrogen Replacement Therapy

Early cross-sectional and case–control studies suggested that oestrogen replacement therapy (ORT) was associated with improved cognitive performance and lower risk for dementia (Paganini-Hill and Henderson, 1994; Mortel and Meyer, 1995; Brenner *et al.*, 1994; Henderson, 1997). One prospective study reported that among 727 women, history of ORT use was associated with higher cognitive function at baseline and less cognitive decline during a mean 2.5 years of follow-up, and the beneficial effect of oestrogen on cognition was independent of age, education, ethnicity and *APOE* genotype (Jacobs *et al.*, 1998). Furthermore, both a community-based study in northern Manhattan and the Baltimore Longitudinal Study of Aging showed that oestrogen users had reduced risk for development of DAT compared with non-users (Kawas *et al.*, 1997; Tang *et al.*, 1996). Another cross-sectional study found that among 2338 non-demented women aged 65 years or older, absence of ORT was associated with lower scores on the modified mini-mental status examination (MMSE) (Steffens *et al.*, 1999).

However, not all recently published results support the conclusion that ORT is beneficial for postmenopausal women. The results of both the Rancho Bernardo Study and the Study of Osteoporotic Fractures showed similar patterns of cognitive declines between women with and without ORT (Barrett-Connor and Kritz-Silverstein, 1999; Mattews *et al.*, 1999), and higher endogenous oestrogen levels were not associated with significantly better performance on any cognitive function test. Results from the Alzheimer Disease Cooperation Study, which followed 120 DAT subjects of mild to moderate severity with or without ORT for 15 months, showed no differences in secondary outcomes, and patients with ORT even showed accelerated changes on the clinical dementia rating scale. Furthermore, the benefit of low-dosage oestrogen on the scores of MMSE after brief exposure (2 months) was not sustained (Mulnard *et al.*, 2000).

Some investigators argue that serum total oestrogen and oestradiol levels may not be the best measurements of their biological activities, whereas bioavailable oestrogens cross the blood–brain barrier more readily and should be correlated better with cognitive

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function. A prospective study showed that at baseline, there were no associations between serum bioavailable hormones and cognitive function, while after 6 years follow-up, women with high concentrations of serum bioavailable oestrogen were less likely to develop cognitive impairment (Yaffe *et al.*, 2000).

Although several observational studies showed that ORT had beneficial effects on paragraph recall and verbal recall tests, some large longitudinal studies and some randomized trials showed inconsistent results. In the Nurses' Health Study, 2138 female nurses aged 70–78 years were evaluated; the results showed that after confound adjustment, neither current nor long-term hormone users demonstrated better performance on general cognitive status or immediate and delayed recall. However, on the test of verbal fluency, current users scored significantly better than never-users, and current users had a 30% decrease in their risk of a low score on the test of verbal fluency (Grodstein *et al.*, 2000). These results suggest that although oestrogen use may provide little benefit on overall cognitive function, there may be certain specific benefits, such as on verbal fluency.

A meta-analysis study collected 27 peer-reviewed articles from 1966 to 1997 and showed that the odds ratio for development of dementia was 0.71 among ORT users (Yaffe *et al.*, 1998). However, different designs and multiple outcome measures used in these studies precluded quantitative summary of the data, since the largest and most methodologically sound observations in non-demented populations showed little or no benefit of ORT for prevention of dementia.

Observational studies of the association of ORT with cognitive performance are susceptible to confounds, such as age, education and depression. Women who choose to take oestrogen generally are younger, are less depressed, have more years of education, and follow a healthier lifestyle than those who do not take oestrogen. Another possible factor in results is whether oestrogen was administrated intermittently or continuously. Based on the weakness of the evidence and potential adverse effects of ORT (breast cancer, endometrial cancer, uterine bleeding, pulmonary and venous thrombosis, gallbladder disease) (Morrison and Tweedy, 2000; Yaffe *et al.*, 1998), ORT has not been recommended universally among women for prevention of DAT.

The influences of oestrogen on cognition have been difficult to establish. It is presently unclear whether the effects of oestrogen on cognition refer to the immediate postmenopausal period, effects on cognition in later life, the ability of oestrogen to prevent cognitive decline and onset of DAT, or the effects on cognitive performance in women with established DAT (Shaywitz and Shaywitz, 2000).

Several important conclusions can be drawn from recently published investigations. First, it is apparent that the questions that now need to be addressed are much more refined than simply asking whether oestrogen improves cognition. Future studies need to investigate effects on particular cognitive functions (e.g. memory, reading, attention) of specific oestrogen types when prescribed under particular conditions (dosage, frequency) to well-defined groups of women. Second, there is no simple translation from laboratory experiments to clinical application. Hypotheses, no matter how compelling the results on which they are based, remain provisional suppositions that must be proven before they can be transformed into clinical practice (Yaffe *et al.*, 2000).

Biological Basis for Gender Differences in Alzheimer's Disease

Oestrogen is present in the brain and plasma at low concentrations, ranging from 10^{-7} to 10^{-12} M, after being synthesized by the liver and ovary. Inside the brain, cytochrome P450 aromatase from glial cells converts testosterone to oestrogen. Among postmenopausal women without oestrogen replacement, the age- and obesity-adjusted levels for oestradiol and oestrone are $12\,\mathrm{pg\,ml^{-1}}$ and $38\,\mathrm{pg\,ml^{-1}}$, respectively, while age-matched non-smoking men have oestradiol and oestrone levels of $39\,\mathrm{pg\,ml^{-1}}$ and $48\,\mathrm{pg\,ml^{-1}}$, respectively. There are two kinds of oestrogen receptors, ERα and ERβ (Kuiper *et al.*, 1996). The receptors are distributed in the CA1 region of the hippocampus, hypothalamus, preoptic area, anterior pituitary, and several other brain regions.

Oestrogen has multiple genomic and non-genomic mediated functions. It can regulate neurotransmitter systems, modulate neurite and synapse reorganization, enhance cerebral blood flow (CBF), upregulate antiapoptotic proteins in the bcl-2 family, act as an antioxidant, and modulate lipid metabolism. In addition to these favourable effects, it also exerts a neuroprotective function among neurons against ischaemic injuries, which are now considered to be more common in DAT than believed previously. Oestrogen and nerve growth factor (NGF) receptors colocalize in neurons in the cerebral cortex and hippocampus. Oestrogen enhances NGF receptor expression; conversely, NGF increases the binding of oestrogen to the same neuron. So, each type of molecule may amplify the other's growth responses. Furthermore, oestrogen has been found to modulate the expression of the *APOE* in rodent tissues and theoretically can reduce the risk of Alzheimer's disease in humans via *APOE* alteration (Dubal *et al.*, 1999).

Modulation of Neurotransmitter Systems

Oestradiol was found to increase choline acetyltransferase activity in the forebrains of oophorectomized rats. Later studies showed that oestrogen also increased potassium-stimulated acetylcholine release in certain brain regions, and prolonged survival of cholinergic neurons (Gibbs *et al.*, 1997). Effects of oestrogen on cholinergic neurons may be in synergy with neurotropic factors (Toren-Allerand *et al.*, 1992). In rats, oestradiol induces serotonin receptors in regions involved in cognition, such as the frontal lobe, cingulate gyrus and nucleus accumbens. Oestrogen affects the dopamine transport system by modulating presynaptic dopamine uptake, turnover and release and D_2 receptor affinity.

Direct Effects on Neurons

Electrophysiological and biochemical studies indicate that oestrogen has rapid effects on membrane excitability of neurons and rapid modulation of presynaptic neuronal events. Moreover, some effects are related not to neuronal communication but rather to morphology, membrane permeability and cell–cell interactions. Oestrogen regulates synaptic plasticity by stimulating axonal sprouting and dendritic spine formation in the hippocampus (McEwen and Woolley, 1994). Oestrogen modulates growth proteins specifically associated with axonal elongation, enhances the outgrowth of nerve processes in cultured neurons, and enhances long-term potentiation (Warren *et al.*, 1995). The effects of oestrogen on morphological plasticity and physiological and cognitive processes in the brain are believed to occur, at least in part, via post-transcriptional regulation of *N*-methyl-D-aspartate (NMDA) receptors, which are important for learning, memory and pattern recognition (Gazzaley *et al.*, 1996). Oestrogen replacement induces a 30% rise in both NMDA receptors and spines in the hippocampus of ovariectomized female rats.

Oestrogen as a Neuroprotectant

Oestrogen protects rodent neurons against oxidative stress, excitotoxins and β-amyloid-induced toxicity. Oestrogen increases cultured hippocampal neurons expression of Bcl-x_L and decreases both the caspase-mediated proteolysis and cell death induced by β-amyloid (Pike, 1999). *In vitro*, oestradiol promotes the breakdown of the amyloid precursor protein to fragments less likely to

accumulate as the β-amyloid peptide; it also attenuates oxidative impairment of synaptic Na^+/K^+ ATPase activity, glucose transport, and glutamate transport induced by amyloid β-peptide and iron (Jaffe *et al.*, 1994; Keller *et al.*, 1997).

In DAT brains, hippocampal subfields such as CA3 exhibiting oestrogen receptor and Bcl-x colocalization are relatively resistant to neurodegeneration. As inflammatory mechanisms are implicated in neuritic plaque formation, and anti-inflammatory agents may protect against DAT, oestrogen has the potential to modify some aspects of the inflammatory response (Bauer *et al.*, 1992).

GENDER DIFFERENCES IN VASCULAR DEMENTIA

Clinical and Epidemiological Studies

The prevalence and incidence of VAD have not been studied as comprehensively as DAT because of large variations in subtypes in the heterogeneous population samples investigated as well as problems with clinical criteria. However, it is known that VAD is more common in men than in women (Jorm and Jolley, 1998; Nyenhuis and Gorelick, 1998). In Europe, under age 85 years, the prevalence of VAD has been reported to be higher among men compared with women; after age 85 years, prevalence in women becomes higher, despite increasing incidence rates with age, but without gender differences (Lobo *et al.*, 2000; Fratiglioni *et al.*, 2000).

Most vascular risk factors for VAD correlate with heightened risk from stroke. Gender differences have long been recognized for stroke. Overall incidence of strokes is uniformly higher in men than in women in all countries, and incidence increases with age in both sexes despite decreases in the male to female ratio. Strokes of embolic origin are the only type to occur more commonly in women than in men. Women demonstrate strikingly less cerebral atherosclerosis relative to men until age 65 years. Women who use oral contraceptives have increased risk for ischaemic stroke, particularly when associated with additional risk factors, such as hypertension, smoking and migraine. Women with stroke have lower mortality compared with men (Holroyd-Leduc *et al.*, 2000).

The value of exogenous hormone replacement in improving risk and outcome is unclear. Although several reports suggest protection from fatal strokes by ORT, some clinical studies did not report significant protection against stroke by ORT. A case–control study compared ORT on the risk of stroke among 1422 stroke patients and 3171 controls aged 45–64 years. The results showed no differences between ORT users' and non-users' brains in non-fatal haemorrhagic and ischaemic stroke. Moreover, a significantly increased incidence of transient ischaemic attack was found in users of oestrogen and progestin (Pedersen *et al.*, 1997). The prospective Cardiovascular Health Study compared the relationship between history of ORT use and magnetic resonance imaging (MRI)-demonstrated cerebral infarcts, white-matter lesions and atrophy among 2133 women aged 65 years or older. The study showed no difference in prevalence of MRI-demonstrated infarcts between current or past ORT users and non-users (Luoto *et al.*, 2000).

The Heart and Oestrogen/Progestin Replacement Study was the first randomized, double-blind, placebo-controlled study to evaluate the outcome of hormone therapy on subsequent cardiac events in 2763 postmenopausal women with established coronary heart disease. The results were surprising as there were no significant differences between the treatment and placebo groups, despite a net 11% reduction on low-density-lipoprotein (LDL) cholesterol and a net 10% increase in high-density-lipoprotein (HDL) cholesterol after 1 year follow-up (Wells and Herrington, 1999). So, the use of oestrogen for secondary prevention of heart disease is more complex than was believed initially.

Possible Mechanisms for Gender Differences in Vascular Dementia

Endogenous oestrogen exerts profoundly protective actions against cerebral ischaemia by several genomic and non-genomic mechanisms, including central nervous system vasodilation with improved cerebral perfusion, reduction of injury responses of arterial smooth muscle, inhibition of platelet aggregation and lipid peroxidation, and modulation of expression of Bcl-2.

Effects on Blood Vessels

The effect of oestrogen on blood vessels is multiple, but the net effect is a promotion of vasodilation. Improved cerebral perfusion and increased cerebral vasodilatory responses by oestrogen have been shown by xenon inhalation contrast computerized tomography (CT), single positron emission computerized tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Kastrup *et al.*, 1999). Oestrogen increases the expression of prostacyclin synthesis and nitric oxide synthase (NOS). In physiological concentrations, oestrogen stimulates the opening of calcium-activated potassium channels through a nitric oxide- and cyclic guanosine monophosphate (cGMP)-dependent pathway, thus relaxing smooth muscle. Blocking calcium mobilization, modulating the cyclic adenosine monophosphate (cAMP) signalling cascade, and reducing L-type and T-type calcium channel peak currents are non-genomic actions of oestrogen on vascular smooth muscle. Oestrogen reduces the sensitivity of blood vessels to endothelin, noradrenaline and thromboxane. It reduces arterial cholesterol ester influx and lipoprotein-induced smooth muscle proliferation, and prevents neointimal thickening after vascular injuries and atherogenesis over time. Oestrogen also elaborates growth factors, decreases collagen and elastin production, and inhibits foam cell formation (Wild, 1996).

Effect on Endothelium

Oestrogen has considerable beneficial functions on vascular endothelium. Oestrogen binding to membrane-sited ERα can activate NOS and cause a rapid release of nitric oxide through the tyrosine kinase pathway or the mitogen-activated protein kinase signalling pathway (Caulin-Glaser *et al.*, 1997). It can inhibit endothelial expression of adhesion molecules, thus inhibiting platelet aggregation. The rapid re-endothelialization induced by oestrogen after vascular injury may be due, in part, to increased local expression of vascular endothelial growth factor.

Effects on Metabolism

Oestrogen increases pancreatic insulin secretion and improves insulin sensitivity. It also inhibits aberrant lipid effects contributing to atherosclerosis by acting at the liver and arterial walls. The net effects of oestrogen on lipoprotein lipid metabolism are increased HDL cholesterol and triglyceride concentrations and reduced LDL cholesterol and lipoprotein A (Lp(a)) levels (Mendelsohn and Karas, 1999).

Effects on Coagulation and Fibrinolysis

Oestrogen regulates the hepatic expression of the genes for several proteins involved in coagulation and fibrinolysis of the blood. It decreases plasma concentrations of fibrinogen, antithrombin III, plasminogen-activator inhibitor type I, factor VII and protein S. Long-term use of oestrogen is associated with decreased plasma concentrations of renin, angiotensin-converting enzyme and endothelin-1 (Mendelsohn and Karas, 1999).

Effects on Experimental Stroke

Oestrogen blunts cerebral ischaemic excitotoxic cascades by reducing NMDA-induced calcium influx and promoting γ-aminobutyric acid A ($GABA_a$) receptor-mediated activity. Oestrogen can alter GABA conduction, induce synaptosomal and hippocampal GABA release, upregulate $GABA_a$ receptor numbers, increase GABA agonist binding, and alter glutamic acid decarboxylase mRNA (Hurn and Macrae, 2000).

GENDER ISSUES IN OTHER COGNITIVE DISORDERS

Age-Related Cognitive Decline

It is believed that age-related degenerative processes affect only certain domains of cognitive functioning, such as encoding and retrieval of new information. In a study concerned with predictors of cognitive change in adults with memory loss, older women were found to have more rapidly declining visual-spatial abilities than older men (Small *et al.*, 1995). In the Italian Longitudinal Study on Aging, women had higher prevalence rates in all age groups for both cognitive impairment but no dementia and age-related cognitive decline (Di Carlo *et al.*, 2000).

Pseudodementia of Depression

It is estimated that in all age ranges, the prevalence rates ratio for depression in women versus men is about 1.9 : 1 to 5 : 1, with an incidence rate ratio of 2 : 1. Lifetime prevalence of depressive disorders is 21% in women and 13% in men. A recent study reported that depressive symptoms in older women were associated with impaired cognitive function with greater cognitive declines at baseline, as well as greater risk of clinically evident cognitive deterioration (Sherwin, 1988). Depression in the elderly is associated with cognitive problems, and about 30% of patients with DAT or VAD have depressive symptoms (Li *et al.*, 2001). Whether depressive disorders are associated with accentuated cognitive decline or the development of dementia or mild cognitive impairment is controversial.

GENDER ISSUES IN DEMENTIA DUE TO OTHER GENERAL MEDICAL CONDITIONS

Dementia due to Head Trauma

In the USA, there is an incidence of over 2 000 000 head injuries per year that are sufficiently serious to require hospitalization, with 30 000–50 000 per year suffering serious intellectual dysfunction. Incidence of post-traumatic dementia is difficult to estimate, but among those with a history of post-traumatic amnesia exceeding 3 weeks, some degree of permanent measurable cognitive deficit is the rule. In some cases, substantial post-traumatic amnesia and residual intellectual deficit may follow head injury without a history of loss of consciousness. Traumatic brain injury occurs twice as often in men as in women. Between the ages of 12 to 24 years, men are involved four times as often as women in cases suffering injury from a motor vehicle accident. Traumatic head injury, particularly from boxing (dementia pugilistica), is a risk factor for DAT.

Dementia due to HIV Infection

Cognitive, motor and behavioural functional changes are the most frequent neurological complications of human immunodeficiency virus (HIV) infection. The principle risk factors for HIV infection include homosexual activities and intravenous administration of illicit drugs with contaminated needles. A total of 72% of patients are homosexual or bisexual men. Direct invasion of the brain has been shown to be causative in the majority of HIV dementia cases.

Dementia due to other Neurodegenerative Diseases

About 20–30% of patients with a clinical diagnosis of DAT were found to meet neuropathological criteria for a diagnosis of Lewy body dementia (LBD), making LBD the second most common cause of neurodegenerative dementia after DAT. Although gender distribution in LBD has not been addressed in the literature, more men than women seem to be affected, with a male to female ratio of 3 : 2, similar to the distribution for Parkinson's disease (Cercy and Bylsma, 1997). Of patients with Parkinson's disease, 10–80% have mild problems in language, visual-spatial function and executive functions, with an incidence rate of about 2.27% at 65 years and 13.6% at 80 years. There is no comprehensive study investigating gender differences in Parkinson's disease-related cognitive disorders, although men with Parkinson's disease have a higher risk for mortality than do women.

Alcohol-Associated Dementia

Over 75% of men and 50% of women in western countries drink alcoholic beverages more than occasionally. Alcohol abuse is usually associated with deterioration of memory, abstract thinking, judgement and personality. Dementia occurs in 10% of all alcoholics and 45% of alcoholics over age 65 years. The role of alcohol in alcohol-associated dementia has been considered secondary to other conditions such as Wernicke–Korsakoff syndrome (WKS), alcoholic pellagra, alcoholic neurotoxicity, Marchiafava–Bignami disease and hepatic encephalopathy. Although the incidence ratio of WKS in men versus women is 1.7 : 1, dementia appears earlier and with less consumption of alcohol among women than men.

Vascular Headache Related to Cognitive Changes

Epidemiological studies demonstrate that women have two to three times the prevalence for migraine than men. Migraine is often associated with temporary cognitive impairments, especially during the time of headache attack with or without aura. Most common cognitive impairments include deficits of attention, concentration, and verbal and visual-spatial memory. A longitudinal study demonstrated that there are temporary or transient global cognitive declines at the time of the headache measured by MMSE and cognitive capacity screening examinations. Female gender and younger age were associated with maximal cognitive decline (Meyer *et al.*, 2000).

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XV-14

Pharmacological Treatment of Dementia, with the Emphasis on Dementia of the Alzheimer Type

P.P. De Deyn

INTRODUCTION

In this chapter, we will focus on the pharmacological treatment possibilities of dementia. Major advances were achieved during the 1990s in the field of Alzheimer's disease, which will therefore be the main topic of this chapter. Nevertheless, whenever applicable, we will also refer to other dementias, such as vascular dementia, mixed dementia, and diffuse Lewy body disease (DLBD). We will not give a review of all treatment strategies or substances investigated, or present guidelines as formulated by some authors (e.g. Doody *et al.*, 2001b), but we will focus mainly on agents with proven efficacy that are currently available for the treatment of cognitive and non-cognitive symptomatology of dementia.

Dementia of the Alzheimer type (DAT) is the most frequent type of dementia, accounting for 60% of cases. The prevalence of DAT has been estimated to be about 10% among people aged 65 years or more, and 40% among people aged 85 years or more (Evans *et al.*, 1989). DAT is, by its prevalence and nature, an important burden on the life of older people, their immediate family and the community.

The clinical-diagnostic criteria for DAT are defined in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association, 1994). The symptoms are presented in Table XV-14.1. There are two groups of symptoms: the impairment of cognitive functioning of which memory impairment is the hallmark in DAT, and the behavioural and psychological symptoms associated with dementia (BPSD) (Finkel *et al.*, 1996). A complete medical treatment of DAT should therefore consist of treatment of both groups of symptoms whenever needed.

Table XV-14.1 Symptoms of DAT

(a) Symptoms of cognitive impairment
Memory impairment
One or more of the following cognitive disturbances:
– aphasia
– apraxia
– agnosia
– disturbance in executive functioning
Cognitive deficits cause significant impairment in social or occupational functioning
Consciousness is preserved
(b) Behavioural and psychological symptoms
Aggression and violence
Agitation
Hallucinations and delusions
Sleeplessness
Pacing and wandering
Depression
Anxiety
Inappropriate sexual behaviour

Figure XV-14.1 shows the hypothetical cascade of events leading to the formation of senile plaques and the development of DAT. The brains of patients with DAT contain a number of structural alterations, including senile plaques, neurofibrillary tangles and cell death. Among a number of deficits of neurotransmission, the most important are cholinergic and noradrenergic (Davis *et al.*, 1982; Rossor and Iversen, 1986). Deficient cerebral glucose consumption and pyruvate dehydrogenase activity are some of the metabolic derangements observed in DAT.

OVERVIEW OF TREATMENT STRATEGIES

Treatment Strategies Based on Knowledge of Aetiology and Pathogenic Mechanisms of Dementia of the Alzheimer Type

These treatment strategies should consist of preventing abnormal β-amyloid production and/or deposition, antagonizing β-amyloid neurotoxicity, and antagonizing inflammatory mechanisms. The β-amyloid protein is deposited as insoluble amyloid fibrils in the brains of patients with DAT. This deposition plays an important role in the pathogenesis of DAT. β-Amyloid protein is produced from its precursor, β-amyloid precursor protein, through enzymatic cleavage by secretase enzymes. γ-Secretase is of particular interest since it is responsible for production of the β-amyloid protein carboxyl terminus, whose length influences the pathogenicity of the protein (Cordell *et al.*, 1998). Currently, research is ongoing into a novel therapy using γ-secretase inhibitors to reduce amyloid deposition. Their effects were confirmed in an *in vivo* situation using transgenic animal models (Felsenstein, 1998). This class of drugs may become the next generation of agents for treatment of DAT.

Interaction with neuronal cell death has been the subject of earlier research, with studies being carried out on the efficacy of neuroprotectants, mainly drugs acting against oxidative stress, which occurs as a result of action by oxygenized free radicals. However, no clear-cut benefits have resulted so far from experimental data with selegiline and/or α-tocopherol (Freedman *et al.*, 1998; Sano *et al.*, 1997). The selective irreversible inhibitor of monoamine

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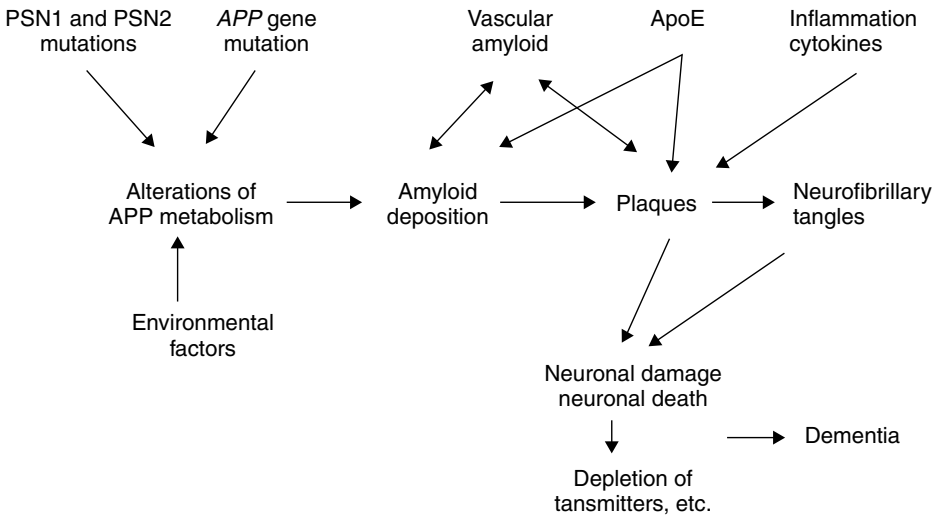

Figure XV-14.1 Hypothetical model of amyloid-related cascade of events leading to the formation of Alzheimer's disease plaques and the development of dementia. (APP, amyloid precursor protein; PSN1, presenilin 1; PSN2, presenilin 2)

oxidase B (MAO-B), selegiline, has been investigated for its presumed beneficial effects on the cognitive symptoms in DAT. While preliminary studies with short-term treatment had suggested promising results, long-term treatment could not confirm this (Schneider *et al.*, 1991; Koivisto *et al.*, 1995). α-Tocopherol interacts with the cellular membrane and is a free-radical scavenger. Sano *et al.* (1997) showed that α-tocopherol alone and in combination with selegiline could slow down the functional deterioration of patients with DAT, but that it had no effect on the outcome of cognitive parameters.

Ginkgo biloba, an extract of the ginkgo biloba tree, has been used widely for the treatment of memory disorders for many years, despite proof of efficacy. The flavonoids, terpenoids and organic acids probably have an antioxidative effect. In a 52-week, double-blind, randomized, placebo-controlled trial with ginkgo biloba in patients with mild to severe DAT and vascular dementia, the active treatment group had an Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog) score slightly better than the placebo group (1.4). However, no difference was seen in the clinical global impression of change evaluation (CGIC) (Le Bars *et al.*, 1997).

Symptomatic Treatment of Core Symptomatology

While not healing the disease and probably not slowing the pathogenic process, symptomatic treatment may contribute during a limited time period to slowing down the evolution of the symptoms. This mode of action is currently the most used method and consists mainly of neurotransmitter replacement therapy. In theory, this can be accomplished by increasing the synthesis and/or release of neurotransmitter, inhibiting the neurotransmitter breakdown, or directly administering receptor agonists. Cholineacetyltransferase (ChAT) is the neurotransmitter marker whose decline best parallels the severity of neuronal loss, density of senile plaques, and impairment on cognitive tests in DAT (Davis *et al.*, 1982). Drugs originally aiming to improve cognitive functioning in dementia were based on acetylcholine replacement treatment strategies by inhibition of acetylcholinesterase. Later, some of these substances were also shown to improve activities of daily living and finally BPSD. A series of psychotropic agents has been tested for control of BPSD. Recently, major progress has been made in the development of efficacious and safe novel antipsychotic agents in this indication.

Outcome Parameters for the Evaluation of Novel Antidementia Agents

Cognitive Symptomatology

To establish the effectiveness of new drugs, we have to evaluate the effect of the drug on the symptoms. This is done in double-blind, placebo-controlled studies. Evaluation is performed by applying a number of instruments developed for this research. The ADAS-cog is used to assess in a longitudinal way the evolution of cognitive function in patients with DAT (Rosen *et al.*, 1984). The ADAS is a comprehensive assessment battery used widely in clinical investigations of DAT. It comprises two components: the cognitive and non-cognitive subscales. The cognitive subscale is the most objective portion of the ADAS, since it relies solely on the patient's ability to perform specific tasks during the administration of the tests, and it captures the cardinal symptoms of DAT. The cognitive abilities evaluated in this subscale consist of memory, language and praxis. Scores on the ADAS-cog can range from 0 to 70 points, with higher scores indicating a greater degree of impairment. Normal elderly subjects score an average of 0–10 points on this subscale, whereas a sample of untreated patients with mild to moderate DAT score between 15 and 25 points. The average rate of decline over a 12-month period for untreated Alzheimer's disease patients is reported to be 6.8 points, while normal elderly subjects do not change significantly over this same period of time.

Global Symptomatology

Another measure used in the double-blind studies is the clinician interview-based impression of change (CIBIC) or its more recent variant, the clinician interview-based impression of change-plus (CIBIC-plus), both developed from the CGIC (Guy, 1976; Schneider *et al.*, 1997).

The CIBIC is an assessment of the patient's status by a semi-structured interview and provides a global rating score that reflects patient function in four areas: general, cognitive, behaviour, and activities of daily living. Using the results from baseline for reference, the rater then interviews the patient and caregiver

at specified times during the study to obtain an impression of change. After the interview, a seven-point scale is used for scoring, from very much improved to very much worse, as compared with baseline results. Another measure to estimate the caregiver's impression of change is the final comprehensive consensus assessment (FCCA) (Knapp *et al.*, 1994).

Behavioural and Psychological Symptomatology

A variety of reliable and sensitive scales have been applied in order to demonstrate clinical efficacy and safety of pharmacological agents in the treatment of BPSD (De Deyn and Wirshing, 2001).

The behavior pathology in Alzheimer's disease rating scale BEHAVE-AD is a 25-item scale that measures behavioural symptoms in a total of seven clusters: paranoid and delusional ideation, hallucinations, activity symptoms, aggressiveness, diurnal rhythm symptoms, affective symptoms and anxieties and phobias (Reisberg *et al.*, 1987). All items are scored on a four-point scale of increasing severity. The BEHAVE-AD global score is a measure of behaviour deemed to be disturbing or dangerous to the patient or to those in their environment.

The Cohen-Mansfield agitation inventory (CMAI) is a rating scale used in nursing homes to assess a total of 29 agitated behaviours on a seven-point scale of increasing frequency (Cohen-Mansfield *et al.*, 1989). The cluster scores include physical, verbal and total aggression, and physical, verbal and total nonaggressive scores. CGIC ratings by the investigator measure behavioural symptoms on a seven-point scale of increasing severity.

The neuropsychiatric inventory (NPI) scores a wide range of psychopathology and may help distinguish between different causes of dementia. It also records severity and frequency separately. The behavioural disturbances assessed by the NPI (Cummings *et al.*, 1994; Cummings, 1997) are delusions, hallucinations, agitation, dysphoria, anxiety, apathy, irritability, euphoria, disinhibition, aberrant motor behaviour, night-time behaviour disturbances, and appetite and eating abnormalities.

Cognitive Enhancers Based on Acetylcholinesterase Inhibitory Action Mechanisms

Five cholinesterase inhibitors have been marketed: tacrine, donepezil, rivastigmine, metrifonate and galantamine. At the time of writing, four are still available, one of which, tacrine, sees its use decreasing steadily. We will describe these products and discuss the findings from studies as well as the pros and cons of treating patients with these products.

Tacrine

Tacrine (tetrahydroaminoacridine) was the first drug to become available for the treatment of cognitive impairment in patients with DAT. Tacrine was compared with placebo in a 30-week, randomized, double-blind, controlled trial with four doses of tacrine (40 mg per day, 80 mg per day, 120 mg per day and 160 mg per day) (Knapp *et al.*, 1994). The patients receiving the highest-dose regimen fared better for the three primary outcome measures (ADAS-cog, CIBIC and FCCA) and for five of the eight secondary measures (Table XV-14.2). Placebo patients declined on the ADAS-cog by an average of 2.0 points compared with a mean improvement of 2.1 points for the patients receiving 160 mg per day of tacrine. Forty-two per cent of patients receiving 160 mg per day of tacrine were rated improved on the CIBIC compared with 18% of patients receiving placebo. Forty-two per cent of patients receiving 160 mg per day of tacrine were rated improved on the FCCA as compared with 16% of placebo patients. However, the evaluated patients represented only about 30% of the patients who had been part of the 160-mg per day tacrine group, the remainder being unable to complete the 30-week treatment period. The lower-dose groups also showed a significant proportion of drop-outs because of lack of efficacy, not-drug-related adverse events, and side effects. Treatment with tacrine was related with substantial side effects: elevation of transaminases, even in the lowest-dose group, requiring interruption and even suspension of tacrine treatment, and cholinergic side effects, especially gastrointestinal effects, such as nausea, vomiting, diarrhoea, anorexia, dyspepsia, abdominal pain and weight loss. Not all patients taking the higher doses showed improvement. This suggests that there must be subpopulations of patients who are responsive and others who are unresponsive to tacrine (and, to a wider extent, to cholinesterase inhibitors). Although apolipoprotein E (apoE) 4 is thought to be a prognostic factor of poor response to therapy with acetylcholinesterase inhibitors in DAT patients (Poirier *et al.*, 1995), a recently published study failed to show an influence of apoE genotype on the efficacy of galantamine in patients with DAT (Aerssens *et al.*, 2001). Gender is likely to be a more powerful determinant of outcome of anticholinesterase treatment than apoE genotype (MacGowan *et al.*, 1998). Indeed, female patients are more likely to fail to improve with tacrine (Farlow *et al.*, 1998; MacGowan *et al.*, 1998). Further prospective studies are needed to confirm these observations.

Tacrine also showed a more practical inconvenience, since it requires a time interval between food administration, and drug administration, and it has to be administered four times daily. The study patients were observed further in an unblinded open-label period to evaluate whether there was a long-lasting effect,

Table XV-14.2 Cholinesterase inhibitors: changes in ADAS-cog for the highest-dose patients versus placebo patients in double-blind, placebo-controlled randomized clinical trials

Drug	Reference	*N* patients with highest dose	Maximum daily dose (mg)	Duration (weeks)	ADAS-cog: difference from placebo
Tacrine	Farlow *et al.*, 1992	37	80	12	3.8
	Knapp *et al.*, 1994	64	160	30	4.1
Donepezil	Rogers and Friedhoff, 1996	34	5	12	3.2
	Rogers *et al.*, 1998	152	5	24 + 6	2.5*
		150	10	24 + 6	2.9*
Rivastigmine	Corey-Bloom *et al.*, 1998	145	6–12	26	4.9
Metrifonate	Cummings *et al.*, 1998	111	30–60	12	3.2
	Morris *et al.*, 1998	250	30–60	26	2.8
Galantamine	Tariot *et al.*, 2000	253	24	22	3.6
	Raskind *et al.*, 2000	197	32	26	3.4*
	Wilcock *et al.*, 2000	218	32	26	4.1

* Intent-to-treat analysis

especially on nursing-home placement and mortality. This study showed that patients treated with tacrine in doses of more than 80 mg per day had a decreased chance of admission to nursing home (Knopman *et al.*, 1996). However, there might be some difficulties with the interpretation of these results: since the study was neither randomized nor blinded after the first 30 weeks, it cannot be proven that there was a causal relationship between higher-dose treatment and more favourable outcomes.

Donepezil

Donepezil was the second cholinesterase inhibitor to become available for the treatment of cognitive dysfunction in DAT. The efficacy and safety of this agent was demonstrated initially in a randomized, double-blind, placebo-controlled trial involving 161 patients randomized to a once-daily treatment with either donepezil (1, 3 or 5 mg) or placebo during a 12-week study period, followed by a 2-week single-blind placebo wash-out (Rogers and Friedhoff, 1996). The primary efficacy parameters were the ADAS-cog and the CGIC. Improvements in ADAS-cog at week 12 were significant for the 3-mg per day ($P < 0.05$) and the 5-mg per day ($P < 0.01$) dose regimen as compared with placebo. The short treatment duration complicated the interpretation of CGIC results since the majority of patients were scored as being unchanged. The proportion of treatment failures was, however, significantly lower for the patients receiving the 5-mg per day dose regimen. The most frequent adverse events at the 5-mg per day dosage were nausea and vomiting (10% versus 5% for placebo). The use of donepezil was not associated with hepatotoxicity.

A second trial of similar design compared donepezil 5 mg per day and 10 mg per day with placebo during a 24-week treatment period (Rogers *et al.*, 1998). There was a significant dose-dependent improvement on ADAS-cog score, with 2.49 points for 5 mg per day and 2.88 for 10 mg per day, as well as a dose-dependent improvement on CIBIC-plus of 0.36 points for the low-dose group and 0.44 points for the 10-mg dose regimen as compared with the results of patients in the placebo group. Drop-out rates were 7% for placebo, 6% for 5 mg per day donepezil and 16% for 10 mg per day donepezil. As in the prior study, the most frequently observed side effects were of a cholinergic nature, and there was no hepatotoxicity. Donepezil is given as a once-daily administration, which is a favourable dosing regimen, especially in this patient population.

Longer treatment duration data are only provided by two open-label studies (Rogers *et al.*, 2000; Doody *et al.*, 2001a). A rather limited number of DAT patients ($n = 133$) were included and followed up for a period of 240 weeks in a US multicentre, open-label study (Rogers *et al.*, 2000). All patients were treated with 3, 5, 7 or 10 mg donepezil per day. During the first 6–9 months of the study, mean ADAS-cog scores showed evidence of improvement from baseline. Thereafter, the revealed decline was less than that estimated if this cohort of patients had not been treated (Rogers *et al.*, 2000). A much larger cohort of DAT patients ($n = 736$) was included in a multicentre, open-label extension study of several double-blind, placebo-controlled clinical trials (Doody *et al.*, 2001a). After a 3–6-week placebo wash-out, all patients were treated with 5 or 10 mg donepezil per day for up to 144 weeks. In contrast to the 3-week placebo wash-out patient group, all donepezil-associated benefits were lost after a 6-week wash-out period, and ADAS-cog scores declined below original baseline values for all patient groups. Although scores improved relative to the new open-label study baseline values, these patients remained below the original baseline values.

Rivastigmine

Rivastigmine is a pseudoirreversible cholinesterase inhibitor that binds to the enzyme but dissociates slowly, providing a much longer cholinesterase inhibition in the brain (about 10 h) than its plasma half-life (Anand and Gharabawi, 1996). Its efficacy and safety were assessed in the ADENA (Alzheimer's disease with ENA 713) project, which consisted of a real phase III study of mild to moderate DAT patients with admission of associated pathology. The study duration was 26 weeks, and participants received either rivastigmine 3–12 mg per day in two daily doses or placebo (Corey-Bloom *et al.*, 1998). The outcome parameters were ADAS-cog and CIBIC-plus. To evaluate the effect on activities of daily living, the progressive deterioration scale was used. Patients receiving rivastigmine showed significant improvements on ADAS-cog, CIBIC-plus and the progressive deterioration scale as compared with patients receiving placebo (see also Table XV-14.2). Side effects were again due to the cholinergic effects of the drug, and no liver abnormalities were observed. Patients who participated in this 26-week phase III trial and who returned for an assessment from week 26 onwards received open-label treatment for an additional period of 26 weeks (Farlow *et al.*, 2000). Patients were assigned randomly to receive treatment with rivastigmine 1–4 mg per day, rivastigmine 6–12 mg per day or placebo for the 26-week, open-label treatment. A greater percentage of patients from the original 6–12-mg per day group who received 12 mg per day during weeks 32–52 demonstrated improvement at week 52 from baseline in ADAS-cog scores compared with the original placebo-treated patients. This failure to 'catch up' in the group starting treatment later would be considered as an indication of disease-modifying effect of treatment. However, we must be cautious with this interpretation based on data of studies with the above-described design given, among other things, a potential bias and selection of patients who could tolerate rivastigmine for the open extension phase and the lack of randomization to treatment for the 52 treatment weeks at week 1. To our knowledge, there are no conclusive data available with regard to potential disease-modifying effects of acetylcholinesterase inhibitors.

Although not the focus of this chapter, we would like to refer to a recent paper indicating the beneficial effect of the acetylcholinesterase inhibitor rivastigmine on DLBD (McKeith *et al.*, 2000). In this paper, the authors demonstrated that rivastigmine 6–12 mg daily produces statistically and clinically significant behavioural effects in patients with Lewy body dementia, as demonstrated on the NPI-4 (a four-item subscore of the NPI, calculated as the sum of scores for delusions, hallucinations, apathy and depression, previously identified as the main Lewy body dementia cluster).

Metrifonate

Metrifonate is a drug that has been used for many years in the treatment of schistosomiasis. Since it is an acetylcholinesterase inhibitor, it was considered a possible DAT drug. It is unique among the cholinesterase inhibitors used in DAT since it binds irreversibly with cholinesterase. Its efficacy and safety were demonstrated recently in two large double-blind trials. The first trial evaluated patients after a treatment period of 12 weeks (Cummings *et al.*, 1998). Patients received placebo or one of three loading doses of metrifonate for 2 weeks, followed by one of three maintenance doses (0.2 mg kg^{-1} per day (10–20 mg per day), 0.3 mg kg^{-1} per day (15–25 mg per day) or 0.65 mg kg^{-1} per day (30–60 mg per day)) for 10 weeks. Metrifonate significantly improved cognitive ability, as assessed by the ADAS-cog (see Table XV-14.2). The CIBIC-plus results showed improved global functions. The agent was well tolerated, with 89% of the high-dose metrifonate group completing the study. Adverse events related to metrifonate included abdominal pain, diarrhoea, flatulence, nausea and leg cramps.

The second study observed patients for a 26-week double-blind treatment period, consisting of a 2-week loading dose phase (2 mg kg^{-1} per day or placebo) and a 24-week maintenance dose phase (0.65 mg kg^{-1} per day or placebo), followed by an 8-week follow-up period (Morris *et al.*, 1998). The efficacy outcome on the ADAS-cog was clinically significant, with a difference of 2.86

points (see Table XV-14.2). The difference for the mean CIBIC-plus score was also significant at 26 weeks. The study also showed beneficial effects on behaviour (Morris *et al.*, 1998).

Metrifonate was well tolerated in this trial, with an adverse-events-related discontinuation rate of 12% for metrifonate-treated patients. Most adverse events were of cholinergic nature. No hepatotoxicity was observed. Metrifonate was given in a once-daily dosing regimen. Unfortunately, a myasthenia-like syndrome has been reported recently as a side effect of metrifonate, and consequently the product has been withdrawn from the market.

In a post hoc analysis of pooled NPI data from two double-blind, placebo-controlled studies of metrifonate in DAT patients, an overall reduction of 15% on total NPI scores was revealed when compared with placebo (Cummings *et al.*, 2001). Metrifonate reduced or stabilized neuropsychiatric disturbances in 60% of symptomatic patients (Cummings *et al.*, 2001).

Galantamine

Galantamine is the most recently introduced antidementia agent. It is a competitive acetylcholinesterase inhibitor that also allosterically modulates nicotinergic acetylcholine receptors (Bores *et al.*, 1996; Albuquerque *et al.*, 1997). It is speculated that the latter mechanism of action may produce neuroprotective effects and may therefore slow down the disease progression. The efficacy and safety profile of galantamine has been the focus of several large phase III, placebo-controlled, randomized clinical trials. Efficacy measures included not only cognitive parameters but also activity of daily living and behavioural outcome measures.

A 5-month, randomized clinical trial testing galantamine 8, 16 or 24 mg per day versus placebo was performed in patients with mild to moderate Alzheimer's disease (Tariot *et al.*, 2000). It was concluded that galantamine 16 and 24 mg per day significantly benefits the cognitive (ADAS-cog), functional (Alzheimer's disease cooperative study activities of daily living inventory (ADAS/ADL)) and behavioural symptoms (NPI) of Alzheimer's disease as compared with placebo.

Raskind *et al.* (2000) reported on efficacy of galantamine over a 6-month treatment period followed by a 6-month extension. At 6 months, galantamine administered at either 24 or 32 mg per day significantly improved cognition and global function. Galantamine significantly improved cognitive function relative to placebo; the treatment effects were 3.9 points (lower dose) and 3.8 points (higher dose) on the ADAS-cog at month 6. Also, CIBIC-plus scores were significantly better in the active treatment groups. Moreover, cognitive and daily function were maintained for 12 months with the 24-mg per day dose. The most common adverse events, which were predominantly gastrointestinal, decreased in frequency during long-term treatment.

The last published phase III trial on galantamine in Alzheimer's disease considered 653 subjects with mild to moderate Alzheimer's disease. Patients assigned randomly to galantamine had their daily dose escalated over 3–4 weeks to maintenance doses of 24 or 32 mg in a twice-daily-dosing regimen. At 6 months, patients who received galantamine had a significantly better outcome on the 11-item subscale of the ADAS-cog than placebo-treated individuals (mean treatment effect of 2.9 points for the lower dosage, and 3.1 points for the higher dose) (Wilcock *et al.*, 2000). Also, at 6 months, patients on galantamine had a better outcome on the CIBIC-plus and had significantly better scores on the disability assessment for dementia scale than patients in the placebo group. ApoE genotype, however, had no effect on the efficacy of galantamine. Galantamine was tolerated well by most patients. The completion rates for the two galantamine groups were comparable to those reported for other cholinesterase inhibitors. More adverse events were reported with the higher dose, and more patients who received the higher dose discontinued treatment as a result of adverse events. The most common adverse event in the galantamine groups was nausea, which was mild for most patients and lasted a median of 5–6 days.

The effects of galantamine on cognitive, functional and behavioural symptomatology in vascular dementia have been studied recently. These promising data have not yet been the focus of international peer-reviewed publications.

Psychopharmacological Agents with Proven Efficacy on the Behavioural and Psychological Symptoms of Dementia

Until recently, the main focus of pharmacological research related to dementia was on the improvement of cognitive functioning. However, BPSD are among the most predominant and pervasive features of dementia and increasingly form a target for therapeutic intervention in this cognitively impaired population (Reisberg *et al.*, 1986; Reisberg *et al.*, 1987; American Psychiatric Association, 1994; Jost and Grossberg, 1996). Aggression and other behavioural symptoms of dementia (e.g. agitation, purposeless activity, wandering, pacing, psychotic symptoms) are important features of the illness and have a severe impact on the quality of life of patients and caregivers, thus complicating effective medical management (Reisberg *et al.*, 1987; Tariot *et al.*, 1994; Tariot *et al.*, 1995; Reisberg, 1992; Eastly and Wilcock, 1997). Behavioural symptoms have been described as the primary predictor of caregiver burden (Coen *et al.*, 1997; Donaldson *et al.*, 1997; McKhann *et al.*, 1984). In fact, the behavioural symptoms of dementia (in particular, aggression and agitation) may be the most common reason for patients being admitted to hospital or residential care (Ferris *et al.*, 1987).

A variety of pharmacological agents have been evaluated for the treatment of BPSD, including cholinergic agents, anticonvulsants, antidepressants, anxiolytics, hormonal preparations and neuroleptic drugs. Unfortunately, the reports often rely on anecdotal observations and/or open-label clinical trials (Porsteinsson *et al.*, 1997; Mintzer *et al.*, 1998). Neuroleptics have been studied more intensively than other agents. Although available evidence supports the efficacy of the conventional neuroleptics, side effects, including the risk of irreversible movement disorders, extrapyramidal symptoms (EPS), anticholinergic effects and adverse drug interactions, are particularly problematic in this elderly patient population (Stoppe *et al.*, 1999, Schneider *et al.*, 1990). Therefore, special efforts have been made to determine the efficacy and safety profile of some novel antipsychotic agents in the treatment of BPSD. Two atypical antipsychotic agents, risperidone and olanzapine, have been investigated in placebo-controlled, randomized clinical trials.

The utility of risperidone, a selective dopamine D_2 and serotonin $5\text{-}HT_{2A}$ receptor antagonist, in elderly patients with dementia and behavioural symptoms has been evaluated in two multicentre, placebo-controlled, double-blind clinical trials (Katz *et al.*, 1999; De Deyn *et al.*, 1999). The primary objective of both studies was to compare the efficacy and tolerability of risperidone with those of placebo in the treatment of behavioural symptoms in patients with dementia. The secondary objective of the latter trial (De Deyn *et al.*, 1999) was to compare the tolerability (particularly with regard to EPS) and the general safety of risperidone with those of haloperidol.

The European-Canadian study (De Deyn *et al.*, 1999) compared the effects of risperidone with those of placebo (efficacy and tolerability) and haloperidol (tolerability). This 13-week double-blind study involved 344 institutionalized elderly patients who had severe dementia (67% Alzheimer's disease, 26% vascular dementia, 7% mixed dementia), assigned randomly to receive either placebo or flexible doses (0.5–4 mg per day) of risperidone or haloperidol. Behavioural symptoms were assessed using the BEHAVE-AD, the CMAI and the clinical global impression (CGI) scale (Guy *et al.*, 1976, Reisberg *et al.*, 1987; Cohen-Mansfield *et al.*, 1989). Tolerability assessments included extrapyramidal symptom rating scale (ESRS) scores.

The Katz *et al.* (1999) trial was a USA-based study in which the effects of fixed doses of risperidone (0.5 mg per day, 1 mg per day, 2 mg per day) were compared with those of placebo. The study involved 625 institutionalized elderly patients with severe dementia (73% Alzheimer's disease, 15% vascular dementia, 12% mixed dementia).

In the flexible-dose regimen European-Canadian trial, the mean dose at endpoint was 1.1 mg per day in the risperidone group and 1.2 mg per day in the haloperidol group. The risperidone group showed significantly greater improvement in the mean BEHAVE-AD total score at week 12 than did the placebo group (completer analysis). The therapeutic effect was most evident for aggressive symptoms. Risperidone was associated with a significantly greater improvement than placebo in the BEHAVE-AD aggression cluster and CMAI aggressiveness scores (total, physical and verbal cluster) at both endpoint (intention-to-treat analysis) and week 12 (completer analysis). The superior effect of risperidone was seen as early as week 2 of treatment and was maintained over the next 10 weeks. Baseline scores for the paranoid and delusional ideation and hallucinations clusters were quite low, and thus there were no significant treatment-related changes in this parameter during the trial. Patients receiving risperidone also exhibited significantly greater global improvement (CGI severity rating) than placebo-treated patients at both week 12 and endpoint. A subanalysis was also performed, excluding patients with vascular dementia. The results (mean shifts on BEHAVE-AD and CMAI scores) were consistent with those observed in the mixed group of dementia patients. At endpoint and week 12, there were significantly greater improvements with risperidone than placebo on BEHAVE-AD aggressiveness score and CMAI total aggressiveness scores.

Significantly greater improvements were noted for haloperidol than placebo on BEHAVE-AD total and aggression cluster scores at endpoint. There were significantly greater improvements with risperidone than haloperidol on the BEHAVE-AD aggressiveness score and the CMAI total and verbal aggressive scores at week 12.

The severity of EPS (as measured by the ESRS total score) at endpoint was not significantly different between the risperidone and placebo groups, while EPS associated with haloperidol was significantly greater than with risperidone. There was no significant difference between the risperidone and placebo groups with respect to the change in mini-mental state examination (MMSE) score during the study. However, the decline in MMSE scores with haloperidol was greater than that with placebo, suggesting that haloperidol led to cognitive toxicity. There were no consistent changes or clinically relevant abnormalities in vital signs (blood pressure, heart rate), laboratory safety parameters, body weight or electrocardiogram.

In the USA trial (Katz *et al.*, 1999), treatment responses (defined post hoc as a 50% or greater reduction in BEHAVE-AD total score) occurred in more patients receiving risperidone 1 or 2 mg per day compared with those receiving placebo. At endpoint, patients receiving risperidone (1 or 2 mg per day) improved significantly more than placebo-treated individuals, as assessed by the BEHAVE-AD total score and the psychosis and aggressiveness scores. Effects of treatment on the CMAI score paralleled those on the BEHAVE-AD total score: patients receiving 1 or 2 mg per day of risperidone exhibited significantly greater improvements than did placebo-treated patients at week 12 and endpoint, according to the verbal, physical and total aggression scales of the CMAI. At the highest dose of risperidone (2 mg per day), there were significant increases in scores on the parkinsonism total and hypokinesia scales compared with placebo. Other dose-related events included mild somnolence and peripheral oedema.

Open-label follow-up studies to the above-mentioned phase III trials, involving a total of 413 patients, demonstrated sustained efficacy and safety of risperidone administered at approximately 1 mg per day in the treatment of BPSD. Jeste *et al.* (2000) reported the follow-up data from the patients enrolled after ending the Katz *et al.* (1999) trial. A total of 330 patients with Alzheimer's disease, vascular dementia or mixed dementia were enrolled in a 1-year, open-label study. The mean modal risperidone dose was 0.96 mg per day. The 1-year cumulative incidence of persistent emergent tardive dyskinesia among the 255 patients without dyskinesia at baseline was 2.6%. Patients with dyskinetic symptoms at baseline experienced significant reductions in the severity of dyskinesia. Patients who received 0.75–1.5 mg per day of risperidone showed a significant improvement in psychopathologic symptoms over the 1-year period. Although there was no control group, the observed incidence of persistent tardive dyskinesia with risperidone seems much lower than that seen in elderly patients treated with conventional neuroleptics, where yearly incidence rates up to 26% have been reported (Jeste *et al.*, 1995).

The second novel antipsychotic agent evaluated in a phase III trial involving a rather homogeneous population suffering from Alzheimer's disease is olanzapine. Street *et al.* (2000) conducted a multicentre, double-blind, placebo-controlled 6-week study in 206 elderly US nursing-home residents with DAT who exhibited psychotic and/or behavioural symptoms. Patients were assigned randomly to placebo or a fixed dose of 5, 10 or 15 mg per day of olanzapine. The primary efficacy measure was the sum of the agitation/aggression, hallucinations and delusions items (core total) of the NPI-nursing-home version. Low doses of olanzapine (5 and 10 mg per day) produced significant improvement compared with placebo, while 15 mg per day failed to do so. The occupational disruptiveness score, reflecting the impact of the patient's psychosis and behavioural disturbances on the caregiver, was reduced significantly in the 5-mg per day olanzapine group compared with placebo. Somnolence was significantly more common among patients receiving olanzapine (25.0–35.8%), and gait disturbance occurred in those receiving 5 or 15 mg per day (19.6% and 17.0%, respectively). No significant cognitive impairment, increase in EPS, or central anticholinergic effects were found at any olanzapine dose relative to placebo.

CONCLUSION

This chapter has focused on the growing number of possibilities for treatment of cognitive and behavioural symptoms in patients with DAT. Currently available drugs are more efficacious and show fewer side effects than first-generation drugs. However, the availability of these drugs may never justify treatment of memory problems without relying on a clear diagnosis.

The treatment presently available is a symptomatic treatment that does not modify the course of the disease significantly. Causal treatment is the subject of biochemical and animal studies. Once such disease-modifying treatment becomes available, a combination of this with symptomatic treatment of the cognitive and behavioural dysfunctions will possibly yield more important and longer lasting beneficial results.

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XVI

Substance-Related Disorders

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XVI-1

Animal Model of Substance Abuse and Dependence

Toni S. Shippenberg

INTRODUCTION

In 1968, the term 'substance dependence' replaced 'addiction' in the nomenclature of the World Health Organization and the American Psychiatric Association. Defined as a cluster of cognitive, behavioural and physiological symptoms indicative of an individual continuing substance use despite significant substance-related problems, this term is the accepted diagnostic term for compulsive use of a psychoactive substance (Table XVI-1.1). When defined as described, it is analogous to the term addiction. However, this term should not be confused with physical or psychic dependence, conditions in which the cessation or reduction of drug usage results in a withdrawal syndrome. Withdrawal and tolerance often are associated with compulsive drug use and are criteria used to define substance dependence. However, the diagnosis of dependence is based on the presence of a constellation of symptoms, and dependence can occur in the absence of either. Importantly, although individuals suffering from chronic pain may develop tolerance to the analgesic effects of an opiate and experience withdrawal symptoms, they do not exhibit compulsive drug-seeking behaviour.

Like substance abuse, substance dependence is defined as a maladaptive pattern of substance use that leads to clinically significant impairment or distress. However, the criteria for abuse do not include tolerance, withdrawal or a pattern of compulsive use but instead are based upon the adverse social consequences of drug use. Although a diagnosis of substance abuse is most often seen in individuals who have recently initiated drug use, not all individuals exhibiting substance abuse will progress to dependence. The identification of those factors that prevent the escalation of abuse to dependence in these individuals is an important focus in addiction research.

The concept of reinforcement has provided the cornerstone for current theories and animal models of substance abuse and addiction. A reinforcer is defined operationally as 'any event that increases the probability of a response' and often is used interchangeably with 'reward'. In general, drugs function as positive or conditioned reinforcers by virtue of their rewarding effects, and reward often connotes additional attributes of a drug (e.g. pleasure) that cannot be defined operationally.

In this chapter, animal models currently used to examine the neurobiological basis of substance dependence and the role of reinforcement processes in its initiation, maintenance and reinstatement are reviewed. The models are then evaluated with respect to the criteria of reliability, predictability and face validity. Limitations of these models that should be considered when interpreting data are also discussed.

Table XVI-1.1 Criteria for substance dependence. A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period

Criteria	Definition
1	Tolerance, as defined by either: (a) need for markedly increased amounts of the substance to achieve intoxication or desired effect (b) markedly diminished effect with continued use of the same amount of substance
2	Withdrawal as manifested by either: (a) characteristic withdrawal syndrome for the substance or (b) the same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms
3	Substance is often taken in larger amounts or over a longer period than was intended
4	A persistent desire or unsuccessful efforts to cut down or control substance use
5	A great deal of time spent in activities necessary to obtain the substance, use the substance or recover from its effects
6	Important social, occupational or recreational activities are given up or reduced because of substance use
7	Substance use is continued despite knowledge of having a persistent or psychological problem that is likely to have been caused or exacerbated by the substance.

CRITERIA FOR EVALUATING ANIMAL MODELS OF SUBSTANCE DEPENDENCE

The use of animal models in scientific research is based on the premise that there is homology among the physiology and behaviour of various species and, hence, extrapolations can be made from experimental animals to humans. Three criteria are typically used to evaluate the strength of a particular model: reliability, predictability and face validity. Reliability refers to the consistency and stability with which the variable is observed. An animal model should permit objective measurement of the variable of interest (e.g. reinforcing efficacy) and within-subject variability should be low. In addition, the phenomenon measured as well as manipulations that affect its development and/or expression should be reproducible. As discussed by Markou *et al.* (1993), the only meaningful criterion for evaluating an animal model concerns the ability of the model to accurately predict the modelled phenomenon (e.g. human condition) based on the behaviour of the model system (e.g. predictive validity). Predictive validity has often referred solely to the ability of a model to identify pharmacological agents with potential therapeutic value in humans. However, this definition does not take into account important ways in which a model can predict a particular behaviour. Although construct validity may be considered relevant to animal models, the process of

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construct validation can also be viewed as analogous to processes of model development, scientific investigation and hypothesis testing (Cronbach and Meehl, 1955; Ebel, 1961). Therefore, this chapter focuses upon predictability and face validity only.

Predictability refers to the ability of a model to identify effects of drugs (e.g. reinforcement, withdrawal) that are observed in human subjects, as well as pharmacological agents with potential therapeutic value. Face validity is evaluated with respect to the ability of a particular animal model to satisfy the DSM IV (American Psychological Association, 2000) criteria used for the diagnosis of substance dependence and abuse in humans (see Table XVI-1.1). In this regard, it should be noted that drug addiction, in humans, has been characterized as occurring in stages (e.g. initiation, maintenance and reinstatement). Furthermore, therapies effective in one stage of the addiction cycle may be ineffective in another. Therefore, the ability of a particular experimental paradigm to model these various stages should be considered.

ANIMAL MODELS OF THE POSITIVE REINFORCING EFFECTS OF DRUGS

A characteristic effect of drugs of abuse is their ability to function as positive reinforcing stimuli. This action has provided the framework for currently used animal models of substance dependence. Early models of drug reinforcement used operant paradigms in nonhuman primates; however, many of these same paradigms now are utilized in rodents. The use of these rodent models, together with molecular biological techniques as well as *in vivo* techniques for the quantification of neurotransmitter dynamics, has provided important insights regarding the neurobiology of addiction (Berke and Hyman, 2000; Hiroi *et al.*, 1997; Weiss *et al.*, 2000).

Operant Intravenous Drug Self-Administration

Experimental animals readily self-administer drugs of abuse via the intravenous route and, in general, drugs that are self-administered correspond to those that have high abuse potential in humans (Brady and Lukas, 1984; Fischman, 1989). Indeed, this relationship is so strong that intravenous drug self-administration is considered an animal model predictive of abuse potential. However, not all drugs abused by humans are self-administered by experimental animals (e.g. hallucinogens).

For intravenous self-administration studies, a chronic indwelling catheter is surgically implanted into a vein to permit drug infusion. During self-administration sessions, the subject acquires a drug infusion by performing a discrete response (e.g. pressing a lever or emitting a nose poke through a hole on an operant panel). The number and pattern of responding required for each infusion is determined by the schedule of reinforcement imposed by the experimenter. Drug availability usually is signalled by an environmental stimulus (e.g. light or tone). The number of infusions obtained for a given drug dose or the rate of responding during a session is typically assessed. Self-administration sessions are continued until animals exhibit stable rates of responding. Test sessions examining responding in response to other doses, test drugs or pharmacological manipulations are then conducted. From comparisons of dose–effect curves, the relative efficacies and potencies of drugs in maintaining self-administration can be determined. Alternatively, the number of sessions required to achieve stable rates of self-administration or the number of animals acquiring self-administration across sessions can be used to examine the influence of pharmacological or genetic manipulations on the acquisition of self-administration.

Factors affecting the amount of drug self-administered as well as the pattern and rate of responding include the schedule of reinforcement imposed, session duration (e.g. limited versus unlimited drug access), the presence of stimuli that predict drug availability, the drug itself, and the rate of drug infusion. Furthermore, there are species- and strain-related differences in the degree to which a drug is self-administered (Kuzmin and Johansson, 2000). In addition to the intravenous route of administration, the intragastric or oral route can be employed (see below).

Fixed-Ratio Schedule

In simple fixed-ratio schedules, the number of responses required for infusion of a drug is set at a fixed number. In rodents, these schedules generally will not maintain stable responding below a certain unit dose and, within the range of doses that do maintain stable responding, self-administration rate is inversely related to dose. Within the range of doses that maintain stable responding, animals increase their self-administration rate as the unit dose is decreased, apparently compensating for decreases in the unit dose. Conversely, animals reduce their rate of self-administration as the unit dose is increased, resulting in a characteristic inverted U-shaped dose–response curve (Figure XVI-1.1a; Brady and Lukas, 1984).

Fixed-Interval Schedules

In a fixed-interval schedule, the frequency of injections is determined primarily by the interval schedule imposed and not by the response rate (Corrigal and Koen, 1989). Therefore, the use of these different schedules can provide information regarding both the reinforcing effects of a drug and potential nonspecific motor effects that can confound data interpretation.

Progressive-Ratio Schedules

Under these schedules, the response requirements for successive drug infusions increase with sequential injections according to

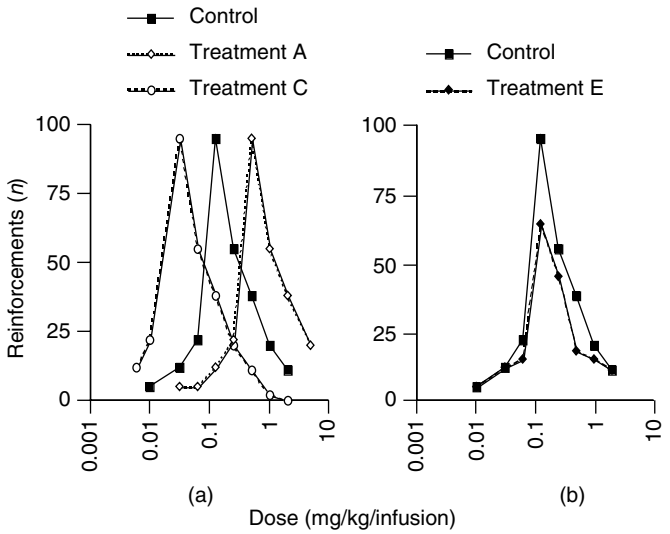

Figure XVI-1.1 Hypothetical data showing rates of responding for unit doses of drug under an FR schedule and the interactions between unit dose and pharmacological manipulation. (a) Response rates increase across low to moderate drug doses and then decrease across higher doses resulting in an inverted U-shaped function. Treatments can result in shifts to the left or right of the dose–response curve as well as upward or downward shifts (b). The failure to assess the effects of a particular treatment on multiple doses of a drug could lead to erroneous conclusions about the ability of a treatment to influence drug taking as well as the mechanisms mediating the treatment–drug interaction

some predetermined progression. The *breakpoint*, defined as the response requirement at which the subject ceases responding, is then determined. The breakpoint, defined as the largest ratio requirement that the subject completes (Hodos, 1961) or as the number of ratios completed by the subject per session (Depoortere *et al.*, 1993), represents the maximum work a subject will perform to receive an infusion of a drug and can be used to determine the reinforcing efficacy of drugs both within and across pharmacological classes as well as the neural basis of reinforcement (Griffiths *et al.*, 1978; Ranaldi and Roberts, 1996; for review see Stafford *et al.*, 1998).

Drug craving has been conceptualized as the incentive motivation to self-administer a drug that has been previously consumed. Therefore, this schedule can provide an animal model of drug craving. Since, however, the breakpoint reflects both the unconditioned (reinforcing) and conditioned incentive effects of drugs, it typically does not allow for the assessment of drug seeking in the absence of drug administration. Hence, the unconditioned effects of a drug (e.g. sedation, activation) can affect the dependent variable. This potential confound can, however, be minimized when the ratio progression is increased across rather than within sessions and/or when performance of the first ratio schedule presented during a trial is evaluated.

Multiple Schedules

Clinical definitions of substance dependence typically refer to the disruptive effects of drug use on non-drug-related activities. The use of multiple schedules of reinforcement enables the application of concepts of behavioural economics (e.g. commodities, consumption, price and demand) to operant behaviour. It also can provide a control for nonselective effects of drug reinforcement. In these procedures, self-administration of a drug is incorporated into a multiple component schedule with other reinforcers (e.g. other drugs, sucrose or water). Behaviour maintained by alternate presentation of natural reinforcers (e.g. food, water) and drugs of abuse in the same session and with the same reinforcement requirements has been reported for several species. Using these procedures, several groups have shown that the contingencies for concurrent reinforcers can affect behaviour asymmetrically and that the response requirement for reinforcers can affect drug self-administration (for review see Bickel *et al.*, 1995; Carroll *et al.*, 1989; Elsmore *et al.*, 1980). It is also apparent that drugs can substitute for, be complementary to, or be independent from, the 'price' of one another and that their effects can be interpreted in economic terms. In addition to evaluating the selectivity of manipulations that apparently reduce the reinforcing efficacy of a drug, these procedures can provide information regarding those factors affecting 'loss of control', as well as the effectiveness of behavioural and/or pharmacological interventions (Bickel *et al.*, 1995).

Second-Order Schedules

Under a second-order schedule, completion of an individual component (or part) of the schedule produces the terminal event (drug infusion) according to another overall schedule (Katz and Goldberg, 1991). Typically, completion of a unit schedule results in the presentation of a brief stimulus, and completion of the overall schedule results in the delivery of the stimulus and the drug. Second-order schedules have the advantage that they maintain high rates of responding and extended sequences of behaviour before any drug infusion occurs. Therefore, acute drug effects on response rates are minimized. High response rates are maintained even for doses that decrease rates when several injections are self-administered during a session. In addition, this schedule requires extended sequences of behaviour, thus, modelling the human condition in which drug taking is preceded by a series of behaviours (e.g. procurement, preparation). Second-order schedules of reinforcement can, when analysed appropriately, separate responses emitted for a drug from those affected by the drug and also enable assessment of the role of stimuli associated with drug administration upon responding. In contrast to the schedules described above that provide a measure of 'drug taking', by examining responding during the interval prior to any drug infusions, a measure of 'drug seeking' is obtained (Alderson *et al.*, 2000; Arroyo *et al.*, 1998). Furthermore, this measure, in contrast to others, is not obtained under extinction conditions (see below).

Oral Drug Self-Administration

Another approach to studying the motivation to consume a drug is to measure the volume consumed when a drinking bottle is available in the home cage. Typically the subject is offered a choice between a drug solution and alternative solutions, one of which is often water. The position of the solutions is random and alternated across self-administration session to avoid potential confounds of position bias. The proportion of drug intake relative to total intake is calculated as a preference ratio. Alcohol is most often studied with these procedures. However, similar studies have been done with cocaine (Jentsch *et al.*, 1998). This procedure has been used to characterize genotype-dependent differences in drug preference, most often alcohol preference (Li, 2000), and the effects of pharmacological treatments on drug intake and preference.

Using operant procedures, the reinforcing effects of drugs can be directly assessed by having animals work to obtain oral consumption of a drug. In this approach the effort to obtain the substance can be separated from the consummatory response (e.g. drinking) and intake can be easily charted over time. In addition, different schedules of reinforcement can be used to change baseline parameters. For operant self-administration of alcohol, rats can be trained to lever press for alcohol using a variety of techniques all designed to overcome the aversive taste and after-effects of initial exposure to alcohol. One approach involves using a sweetened solution fading procedure (Samson, 1986; Samson *et al.*, 1999). Alcohol concentrations are increased from 5% to 8% to a final concentration of 10% over time, with each concentration mixed first with saccharin or sucrose and then presented alone. Typically, rats are allowed to respond for 10% alcohol versus water for 4–6 weeks until stable self-administration is achieved. Using this approach, animals can be trained to lever press for concentrations of alcohol up to 40% (Samson, 1986). The animals will perform on fixed-ratio schedules and progressive-ratio schedules and obtain significant blood alcohol levels in a 30 min session (Samson *et al.*, 1999). Similar procedures have been applied to other drugs of abuse (Macenski and Meisch, 1998; Meisch *et al.*, 1992; Stewart *et al.*, 1994).

Evaluation of Self-Administration Techniques

Drug self-administration techniques have both reliability and predictive validity. The dependent variable provides a measure of the motivation to obtain drugs (e.g. the amount of work an animal will perform to obtain drug) and has demonstrated that drugs function as powerful reinforcers. Responding maintained by drugs as reinforcers is stable across sessions and can be altered predictably by neurotransmitter antagonists. Intravenous drug self-administration also has predictive validity in that drugs having high reinforcement potential in experimental animals have reinforcing effects in humans as measured by both operant and subjective reports (Fischman, 1989; Johanson and Balster, 1978; Schuster and Thompson, 1969).

Drug self-administration can vary as a function of the dose available as well as the species or strain tested. It is influenced by the

presence of alternate reinforcers, environmental stimuli that signal drug infusions, post-reinforcement interval, and prior history of the subject (Carroll *et al.*, 1981; Comer *et al.*, 1996; Glowa and Williams, 1992; Nader and Woolverton, 1992). These variables should be considered when interpreting self-administration studies.

Self-administration under a fixed ratio schedule of reinforcement typically results in an inverted U-shaped curve. Since both leftward and rightward shifts in the dose–effect function will decrease self-administration of some unit doses but simultaneously increase self-administration of other doses (Figure XVI-1.1b,c), construction of full dose–effect functions is essential. Without this data, it is unclear as to whether a particular pharmacological manipulation results in a shift of the dose–response function to the left or to the right or is unique to the dose tested. Since a given pretreatment may decrease self-administration by having nonspecific effects on behaviour (e.g. sedation), the influence of a pretreatment on non-drug reinforcers should be assessed regardless of the schedule employed.

The duration of drug access as well as the inter-infusion interval should also be considered when attempting to model the human condition. An important feature of unlimited-access cocaine self-administration paradigms is that they provide an animal model of the 'binge' abstinence patterns of cocaine use seen in humans (Fitch and Roberts, 1993; Gawin, 1991). As such, unlimited-access conditions can also enable examination of the variables controlling reinstatement of drug use following a period of abstinence. Even when limited-access conditions are imposed, dramatic differences in cocaine self-administration are seen depending upon whether self-administration sessions are 1 or 6 hours in duration (Ahmed and Koob, 1998). In a recent study, rats with 1-hour access to cocaine exhibited lower intake that remained stable for several weeks as compared to rats with longer access (e.g. 6-hour sessions), which exhibited a progressive increase in intake over the same number of sessions. Interestingly, after a period of abstinence the short-access animals resumed stable intake whereas the rate of escalation of intake of the longer-access animals was greater than that observed previously.

Brain Stimulation Reward Thresholds

Electrical self-stimulation of certain brain areas is rewarding for animals and humans as demonstrated by the fact that subjects will readily self-administer the stimulation (Olds and Milner, 1954). The high reward value of intracranial self-stimulation (ICSS) has led to the hypothesis that ICSS directly activates neuronal circuits that are activated by conventional reinforcers (e.g. food, water, sex) and that are modulated by drugs of abuse. Although ICSS is an operant procedure, it differs significantly from drug self-administration in that subjects work to directly stimulate presumed reinforcement circuits in the brain, and the effects of test drugs on ICSS are determined. Various drugs of abuse decrease thresholds for ICSS, and there is a good correspondence between the ability of drugs to decrease ICSS thresholds and their abuse liability (Kornetsky and Esposito, 1979). More recently, ICSS has been used in conjunction with *in vivo* voltammetry, a technique that permits rapid determination of changes in neurotransmitter release in discrete regions of the brain, to examine the neurochemical basis of reward (Garris *et al.*, 1999).

Many ICSS procedures have been developed (for review see Gallistel, 1983; Stellar and Stellar, 1985). Two, however, provide a measure of stimulation thresholds that are not confounded by influences on motor and performance capability. These are the rate-frequency curve-shift procedure, and the discrete-trial, current-intensity procedure (Markou and Koob, 1993; Miliaressis *et al.*, 1986).

Rate-Frequency Procedure

In the rate-frequency procedures, a frequency threshold measure is obtained by the generation of a stimulation input–output function (Gallistel, 1983; Miliaressis *et al.*, 1986). Rate-frequency curves are collected by allowing the rats to press a lever for a series of pulse frequency stimuli delivered through an electrode in a rewarding brain site (e.g. medial forebrain bundle). A runway apparatus also can be used with running speed as the dependent measure. Frequencies can be presented in an ascending, descending or random order or in alternating descending and ascending series. The locus of rise, defined as the frequency at which the function rises from zero to an arbitrary criterion-of-performance level, is determined and provides a measure of ICSS reward threshold (Gallistel, 1983). The behavioural maximum measure is the asymptotic maximal response rate, and changes in this variable are thought to reflect motor or performance effects. Changes in the reward efficacy of stimulation (i.e. intensity manipulations) shift the rate-frequency functions laterally, resulting in marked changes in the locus of rise value. However, they do not alter the asymptote or the shape of the function. In contrast, performance manipulations or changes in motivation (as may occur after priming), alter the value of the behavioural maximum and the shape of the function.

Discrete-Trials Procedure

In the discrete-trials procedure the current-intensity threshold is determined (Kornetsky and Esposito, 1979; Markou and Koob, 1993). This procedure consists of a series of discrete trials in which the subject is expected to emit a single response to receive the electrical stimulus, the current-intensity of which is varied between trials. At the start of each trial, rats receive a non-contingent, experimenter-administered electrical stimulus and then must turn a wheel manipulandum to obtain a contingent stimulus identical to the previously delivered non-contingent stimulus (positive reinforcer). The threshold value is defined as the midpoint between the current-intensity level at which the animal makes two or more positive responses out of the three stimulus presentations and the level at which the animal makes less than two positive responses at two consecutive intensities. Response latency is defined as the time that elapses between the delivery of the non-contingent electrical stimulus and the animal's response on the wheel. Manipulations that increase the reward value of stimulation lower thresholds whereas increases in threshold are indicative of decreases in reward value (Bespalov *et al.*, 1999; Panagis *et al.*, 2000). Increases in response latency can be interpreted as a motor or performance deficit. Since, however, the discrete-trials threshold procedure is designed to minimize behavioural response requirements, it is less sensitive to manipulations that produce motor and performance deficits (Markou and Koob, 1993).

Evaluation of ICSS

ICSS permits direct assessment of the effects of drugs on reward circuits in the brain and, as such, eliminates the influences of consummatory-like behaviours on responding. Since electrodes can be placed in various structures, it permits mapping of those brain areas mediating the rewarding effects of drugs. It can also be used to examine the neuroanatomical basis of drug withdrawal and relapse (Vorel *et al.*, 2001).

Many of the same limitations described above apply to ICSS studies. In addition, it should be noted that ICSS can be elicited in many brain regions and certain brain areas support higher rates of stimulation reward than others. In addition, stimulation of particular brain regions may activate different circuits.

ANIMAL MODELS OF THE CONDITIONED REINFORCING EFFECTS OF DRUGS

Drug-seeking behaviour in humans is maintained not only by the primary reinforcing effects of a drug but also by environmental stimuli that have signalled drug use (O'Brien *et al.*, 1977, 1992). Through the processes of classical conditioning, these stimuli can then function as conditioned reinforcers that maintain drug-seeking behaviour and exacerbate craving and/or withdrawal (O'Brien *et al.*, 1992; Wikler and Pescor, 1967).

Conditioned Place Preference

The conditioned place preference paradigm is a classical conditioning procedure that has been used to model the conditioned reinforcing (or aversive) effects of drugs in experimental animals. In this paradigm, administration of a drug (the unconditioned stimulus, UCS) is paired with one distinct environment and administration of placebo with another. After several environmental pairings, non-injected animals are allowed access to both environments and the time spent in each is measured. The animal's choice to spend more time in either environment provides a direct measure of the conditioned reinforcing effect of a drug.

The apparatus used in conditioning experiments consists of two or three environments that are differentiated from each other on the basis of visual, tactile and/or olfactory cues. The distinctiveness of the environments is essential for the development of conditioning. In the unbiased design, the environments are manipulated so that animals differentiate one from the other but do not exhibit an innate preference for either of the place cues. Pairing of drug with a particular environment is counterbalanced and change in the time spent in the drug-paired environment can be directly attributed to the conditioned reinforcing effects of a drug. Control groups, which receive saline in each of the environments, are run to ensure the unbiased nature of the conditioning procedure. In contrast, however, to the biased procedure (see below), a preconditioning phase, to assess pretest preferences, is not necessary, thus preventing the potential confound of latent inhibition (Lubow, 1989) and decreasing the time necessary for a particular experiment.

In the biased design, animals exhibit a preference for one of the place cues prior to the commencement of conditioning. A preconditioning phase, in which animals are allowed access to both environments, is necessary to determine the innate preference of each animal. The drug then is paired with the preferred or non-preferred environment depending upon whether the drug is assumed to produce aversive or positive reinforcing effects, respectively. The difference in time spent in a particular environment during the preconditioning and conditioning phases is then used to determine drug effect. Although this design is used often, data interpretation can be problematic since place preferences may indicate either incentive motivational effects of a drug or a decrease in the aversive properties of the least-preferred environment.

Studies in rodents have shown that drugs that function as positive reinforcers in other paradigms produced conditioned preferences for an environment associated with their administration (Cunningham *et al.*, 1999; Mucha and Herz, 1985; for review see Bardo and Bevins, 2000; Shippenberg and Elmer, 1998). In contrast, drugs that produce dysphoric or aversive effects in humans produce conditioned aversions for the drug-associated place. This procedure, thus, permits assessment of the conditioning of drug reinforcement and can provide indirect information regarding the positive and negative reinforcing effects of drugs. More recently, place conditioning has been used in conjunction with gene transfer and homologous recombination techniques to delineate the neural basis of drug-induced reinforcement (Carlezon *et al.*, 1998; Risinger *et al.*, 1996). It is particularly useful in the former case since the effects of virally mediated gene transfer are transient and require a procedure that can be completed within several days after gene transfer (Carlezon *et al.*, 1998).

Evaluation of Conditioned Place Preference Procedures

The conditioned place preference paradigm has reliability and predictive validity. Drugs that produce conditioned preferences for the drug-associated environment are those that function as positive reinforcers in other paradigms. Conditioned place aversions also are observed in response to drugs that are negative reinforcers or those that produce aversive or dysphoric states in human subjects (Mucha and Herz, 1985). In contrast to many other procedures, place conditioning is assessed in the absence of drug. Therefore, the potential confound of drug-induced alterations in locomotor activity on performance are alleviated. Finally, it was initially argued that the conditioned place preferences produced by drugs may result from their ability to increase locomotor activity. This, in turn, would increase exploration within a particular environment and decrease neophobia. Subsequent studies, have, however, clearly shown that this is not the case (Shippenberg and Elmer, 1998).

Several issues must be considered when interpreting data from place conditioning studies. First, the drug is administered non-contingently and there is evidence that the behavioural and neurochemical effects of abused drugs differ depending upon whether drug administration is controlled by the subject (Hemby *et al.*, 1997). Although the conditioned place preference paradigm has been validated as a protocol for measuring the rewarding effects of drugs in non-human primates self-administering a drug (Foltin and Evans, 1997, 2001), studies in human subjects are lacking. The issue of whether humans or non-human primates would exhibit conditioning in response to non-contingent drug administration has not been examined.

Route of drug administration and number of environmental pairings, as well as the number of conditioning sessions, can profoundly affect place conditioning (Bechara and van der Kooy, 1985; Shippenberg and Heidbreder, 1995). Strain-related differences in the conditioned response to opiates and alcohol have also been observed (Cunningham *et al.*, 1999; Shoaib *et al.*, 1995). Since tests of conditioning are usually conducted in the absence of drug, the issue of state-dependency (Overton, 1974) must also be considered. Finally, place conditioning now is used in many studies assessing genotype-dependent differences in drug sensitivity. However, a lack of a conditioned response may indicate a loss of the reinforcing effects of a drug or a generalized impairment of learning or memory processes required for the acquisition or performance of a conditioned response, or differences in state-dependent learning. In addition, genotype-dependent differences in the saliency of environmental cues used for conditioning may occur. These factors are rarely controlled for and should be considered before concluding that a particular manipulation impacted on reinforcement.

Conditioned Reinforcement Paradigm

The conditioned reinforcement paradigm allows characterization of the incentive value imparted on formerly neutral environmental stimuli that have been repeatedly associated with drug self-administration. In a conditioned reinforcement paradigm, subjects are trained in an operant chamber containing two levers. Responses on one lever, denoted as the active lever, result in the presentation of a brief stimulus (termed the conditioned stimulus or CS) followed by presentation of a primary reinforcer (e.g. drug) whereas responses on the other lever have no consequences (inactive lever) (Davis and Smith, 1976). The ability of the previously neutral CS to maintain responding in the absence of the primary reinforcer provides a measure of the reinforcing value of these stimuli. This

procedure provides a test for the conditioned incentive effects of drugs because responding for drug-associated stimuli occurs under extinction conditions (e.g. in the absence of drug). It also provides an animal model of drug craving since the incentive motivational effects of a stimulus are examined in the absence of drug taking.

Evaluation of Conditioned Reinforcement Procedures

This procedure has validity. Humans will work to obtain stimuli previously associated with drug use, and drug-associated stimuli produce drug craving. Many of the same limitations discussed for the conditioned place preference paradigm apply to the conditioned reinforcement paradigm. Interpretation of the data is limited by the fact that animals do not control their intake and it remains unclear as to whether similar effects would be observed with contingent drug injections. A potential interpretational advantage of this paradigm over that of place preference conditioning is that operant responding, rather than approach or avoidance, is quantified.

Second-Order Schedules

Second-order schedules also can be used to evaluate the conditioned reinforcing effects of drugs. As described above, completion of the first component or unit of the schedule rather than an individual response produces the terminal event according to another overall schedule (Goldberg and Gardner, 1981). In some versions of a second-order schedule, completion of the first component or unit produces a stimulus and then completion of a fixed number of first components produces the stimulus and drug. Manipulation of these stimuli can modify acquisition, maintenance, resistance to extinction, and recovery from extinction. To assess the effects of conditioned reinforcement, the number of responses with the paired stimulus can be compared to the number of responses with a non-paired stimulus. For example, substitution of drug-paired stimuli with non-drug-paired stimuli actually can decrease response rates.

Evaluation of Second-Order Schedules

Second-order schedules are reliable and have predictive validity. Until recently, this procedure was limited to non-human primates. However, several investigators have effectively implemented this procedure in the rat. The maintenance of performance in second-order schedules with drug-paired stimuli may be analogous to the maintenance and reinstatement of drug seeking in humans with the presentation of drug-paired stimuli. Drug craving in the absence of drug can also be assessed by terminating sessions immediately after the first drug infusion that occurs after completion of the terminal schedule (see below).

MODELS OF THE SUBJECTIVE EFFECTS OF DRUGS: DRUG DISCRIMINATION

The drug discrimination paradigm is based on the capacity of animals to use the interoceptive effects produced by drug or placebo administration as discriminative cues to control responding maintained by food or other stimuli that reinforce behaviour. Currently, it is the only animal model used to evaluate the subjective effects of drugs. This usage is based on studies showing: (i) a high correlation between patterns of cross-generalization in animal drug discrimination experiments and subjective effects in human subjects and (ii) that, in many instances, there is a correlation between the measured subjective effects of drugs in human subjects and their discriminative responses (Dykstra *et al.*, 1997; Preston and Bigelow, 1991). Although considerable controversy exists as to which, and to what extent, subjective effects of drugs contribute to the maintenance of drug taking, it has been hypothesized that the stimulus effects of drugs may contribute to bouts of drug taking in intermittent users and to relapse to dependence in former drug addicts (Meyer and Mirin, 1979; Stolerman, 1992). In this view, discriminative stimuli signal the availability of a reinforcer and therefore set the occasion to engage in those behaviours that enable consumption of the reinforcing drug. Consistent with hypothesis, self-administration studies have shown that stimuli predictive of drug administration elicit drug-seeking and drug-taking behaviour and also retard the extinction of responding for psychostimulants and opiates (Katner *et al.*, 1999; McFarland and Ettenberg, 1997; See *et al.*, 1999; Weiss *et al.*, 2000).

For drug discrimination studies, animals are trained to emit a particular response following administration of a fixed drug dose (e.g. depression of one lever) and to press another lever following administration of saline under a fixed schedule of reinforcement. Most commonly, an appetitively motivated operant procedure is used in which animals are food or water deprived. Responding on the training-condition-appropriate lever results in the delivery of food or water. Training is continued until the animal reliably selects the appropriate lever after drug or saline administration. Once trained, tests of stimulus generalization or antagonism are interposed between training sessions to determine whether other doses of the training drug or a specific drug treatment produce stimulus effects qualitatively similar to or different from those of the training drug. As with other operant paradigms, various reinforcement schedules (e.g. fixed-ratio, fixed-interval, differential reinforcement of low response rate) and response measures (e.g. nose poking, maze running) can be used. Dose 1 versus dose 2 and drug 1 versus drug 2 versus saline discriminations also can be employed (Colpaert and Janssen, 1982; Gauvin and Holloway, 1991; Picker and Cook, 1997).

Drug discrimination studies in non-human primates and rodents have shown that the discriminative stimulus effects are homologues to the subjective effects they produce in humans, and that comparison of the subjective and discriminative stimulus effects of a test drug to those produced by a known drug of abuse can predict abuse liability (for review see Colpaert, 1999). They have also been used to model the subjective effects of drug withdrawal (Emmett-Oglesby *et al.*, 1990; Gellert and Holtzman, 1979) and drug tolerance (Sannerud and Griffiths, 1993).

Evaluation Of Drug Discrimination Procedures

Drug discrimination offers both reliability and predictive validity. Stimulus generalization gradients are stable once drug discrimination is acquired and the stimulus effects of various drugs are altered predictably by neurotransmitter antagonists. Drug discrimination also has predictive validity in that drugs that produce discriminative stimulus effects that generalize to drugs of abuse are those with known abuse liability.

Generalization gradients are dependent upon the dose of drug used for training. Certain neurotransmitter antagonists attenuate the discriminative stimulus effects of a drug when a low training dose is employed. However, these same antagonists fail to modify the discriminative stimulus effects of the same drug when a higher training is employed. Therefore, the use of multiple training doses is essential.

Different test procedures (extinction versus reinforced responding on the lever on which the first schedule requirement is completed) may yield different results depending upon the variable that is used to measure generalization. As with all animal models, species and strain differences as well as the experimental history of an animal can alter the discriminative stimulus effects of a drug (Morgan *et al.*, 1999).

MODELS OF THE NEGATIVE REINFORCING EFFECTS OF DRUG WITHDRAWAL

One criterion used to define substance dependence in human subjects is the presence of a withdrawal syndrome following the cessation of drug use. Although many of the overt physical signs associated with withdrawal from drugs are easily quantified, abstinence from chronic drug use is also associated with long-lasting alterations in motivation and affect that can occur even in the absence of somatic withdrawal signs. It has been postulated that the negative affective states precipitated by drug withdrawal contribute to continued drug use (Koob and Le Moal, 2001). Furthermore, according to the theory of Wikler and Pescor (1967), the high rate of relapse observed in addicts is due in part to conditioned responses to stimuli that have been paired either with the aversive effects of drug withdrawal or the positive reinforcing effects of drug taking.

Operant Drug Self-Administration

Drug self-administration can be conducted in animals rendered physically dependent upon the drug (e.g. abstinence from drug use results in a withdrawal syndrome). Although it is clear that animals will self-administer drugs in the absence of withdrawal, the reinforcing values of drugs may change depending upon the presence of absence of a withdrawal state. Indeed, evidence that physical dependence can increase the reinforcing efficacy of drugs has been presented. Monkeys made physically dependent upon morphine show increases in their progressive-ratio performance compared to animals that do not exhibit withdrawal sympomatology (Yanagita, 1973). Rats with a history of self-administration of alcohol will self-administer alcohol during withdrawal in sufficient quantities to prevent withdrawal symptoms (Roberts *et al.*, 1996).

Conditioned Place Preference

The conditioned place preference paradigm has become an important tool with which to examine the conditioned motivational responses that develop as a consequence of withdrawal and their neurobiological basis. For such studies, subjects are exposed to one environment while undergoing withdrawal and to another in the absence of a withdrawal state. During tests of conditioning, animals are allowed access to both environments and the time spent in each is determined. Rodent studies (Bechara and van Der Kooy, 1995; Funada *et al.*, 1996; Hand *et al.*, 1988) have shown that individuals rendered physically dependent upon morphine exhibit dose-related conditioned aversions for an environment associated with spontaneous or opioid receptor antagonist-precipitated withdrawal. These conditioned responses can be observed after only a single conditioning session and are not observed in opiate naïve animals. Importantly, these conditioned effects are extremely long-lasting and persist for some weeks following the cessation of opiate administration. They can be observed in the absence of a quantifiable somatic withdrawal syndrome. Recent studies using the conditioned place preference paradigm in mice in conjunction with gene knockout techniques have provided new information regarding the neural substrates mediating the somatic versus the affective components of opiate withdrawal (Matthes *et al.*, 1996; Murtra *et al.*, 2000).

Brain Stimulation Reward

ICSS thresholds have been used to assess changes in systems mediating reinforcement processes during the course of drug dependence. In contrast to acute administration of psychostimulant drugs that lowers ICSS threshold, withdrawal from chronic administration of these same drugs elevates ICSS thresholds (Kokkinidis and McCarter, 1990; Wise and Munn, 1995). Similar results have been observed following withdrawal from ethanol and nicotine (Epping-Jordan *et al.*, 1998; Schulteis *et al.*, 1995) and precipitated withdrawal in opiate-dependent rats.

Drug Discrimination

Drug discrimination can be used to characterize both specific and nonspecific aspects of withdrawal. Generalization to an opiate antagonist provides a more general nonspecific measure of opiate withdrawal intensity and time course (Gellert and Holtzman, 1979). Examples of a more specific aspect of withdrawal are animals that have been trained to discriminate pentylenetetrazol, an anxiogenic-like substance, from saline in alcohol-, diazepam- and opiate-dependent animals. During withdrawal, generalization to the pentylenetetrazol cue has suggested an anxiogenic-like component to the withdrawal syndrome (Emmett-Oglesby *et al.*, 1990; Gauvin and Holloway, 1991).

MODELS OF DRUG-SEEKING AND RELAPSE

As discussed above, environmental cues repeatedly paired with primary reinforcers can acquire incentive properties via classical conditioning processes (Davis and Smith, 1976; Katner *et al.*, 1999; Weiss *et al.*, 2000; Wikler and Pescor, 1967). It has been postulated that the development of these conditioned reinforcing effects plays an important role in drug craving and relapse to addiction. Indeed, human studies have shown that the presentation of stimuli previously associated with drug delivery increases the likelihood of relapse as well as self-reports of craving and motivation to engage in drug taking (Ehrman *et al.*, 1992; O'Brien *et al.*, 1977, 1992). Recently, several techniques have been developed to model drug seeking (e.g. craving) and relapse in experimental animals.

Reinstatement of Self-Administration After Extinction

The reinstatement procedure has been used extensively in recent years to model relapse in the experimental animal. Individuals are trained to self-administer a drug, usually under a fixed ratio schedule of reinforcement, until stable rates of responding are achieved. Responding (e.g. lever pressing) for drug is then extinguished by substituting saline for drug for several sessions. Once responding is extinguished, test sessions are conducted in which the ability of non-contingent administration of the training drug or a test drug (e.g. drug priming) to reinstate responding is determined (deWit and Stewart, 1981). Various laboratories have shown that non-contingent drug administration leads to the reinitiation of responding for opiates as well as psychostimulants. Furthermore, compounds that are in a different pharmacological class from that of the training drug are generally less effective in reinstating responding that those of the same pharmacological class (deWit and Stewart, 1981, 1983; Gerber and Stretch, 1975). The ability of stimuli that have previously signalled drug infusions or of other manipulations (e.g. stress) to reinstate self-administration behaviour can be assessed (Ranaldi and Roberts, 1996; Shaham and Stewart, 1995).

Second-Order Schedules

In contrast to other schedules of reinforcement, in second-order schedules, completion of the first component or unit of a second-order schedule, rather than an individual response, produces drug infusion according to another overall schedule. Therefore, it has

been argued that responding during the first interval that occurs prior to any drug delivery, in contrast to other procedures or during subsequent intervals of the second-order schedule, provides a measure of drug seeking in the drug-free state (Everitt and Robbins, 2000). Analysis of responding for cocaine has indicated that in the first interval, a monotonic relationship between dose and responding is seen (Arroyo *et al.*, 1998). This contrasts with later intervals in which self-administration increases for lower doses and decreases for higher doses. These data indicate that responding during the first interval may provide a measure of the reinforcing efficacy of a drug. To date, however, this relationship has not been observed with other classes of abused drugs.

Using second-order schedules, the ability of cues associated with drug administration to reinstate self-administration behaviour can also be assessed (for review see Everitt and Robbins, 2000). Studies in non-human primates have shown that responding for morphine decreased when presentation of a light that served as the conditioned stimulus was omitted. When, however, the light was reinstated, responding resumed (Goldberg and Tang, 1977). Rats exhibit a decrease in responding and an increased latency to initiate responding when a stimulus paired with cocaine self-administration is withheld (Alderson *et al.*, 2000; Arroyo *et al.*, 1998). In animals in which the stimulus was still present, a greater number of responses are seen, indicating that presentation of the conditioned stimulus retarded extinction (Arroyo *et al.*, 1998; Ranaldi and Roberts, 1996).

Extinction with and Without Cues Associated with Intravenous Drug Self-Administration

Extinction procedures assess the persistence of drug-seeking behaviour in the absence of response-contingent drug availability. In this paradigm, subjects first are trained to self-administer a drug until stable self-administration patterns are exhibited. Extinction sessions are identical to training sessions for self-administration except that no drug is delivered after the completion of the response requirement. The probability of reinstating responding under extinction conditions with drug-paired stimuli or even stimuli previously paired with drug withdrawal can be examined (Weiss *et al.*, 2000). Discriminative stimuli that are paired with infusions of cocaine or vehicle can also be employed to examine reinstatement of responding. Using this method, it has been shown that a discriminative stimuli associated with self-administration of cocaine elicits responding for drug for several months following the termination of extinction sessions (Ciccocioppo *et al.*, 2001).

Evaluation of Models of Drug-Seeking and Relapse

Each of the techniques described has reliability and predictive validity. Presentation of stimuli associated with drug injection induces drug craving in humans and maintains responding in the extinction and second-order schedules (Ehrman *et al.*, 1992; O'Brien *et al.*, 1992). Re-exposure to drugs increase self-reports of craving in human addicts (O'Brien *et al.*, 1991). Human drug users, especially those who abuse psychostimulants, often go through periods of drug use and abstinence (e.g. extinction). Therefore, it would appear that extinction paradigms provide a particularly useful model for examining the neurobiology of abstinence. Second-order schedules can be used to examine extinction, and, by examining responding during the first interval of the schedule, can also provide a measure of drug seeking that does not require the imposition of extinction by the experimenter. This distinction is important since it is likely that the motivational effects that drive responding in these two conditions differ profoundly.

Finally, as mentioned previously, increasing evidence indicates that the neurochemical and behavioural responses to a drug can differ depending upon whether it is administered contingently or non-contingently (Di Ciano *et al.*, 1998; Hemby *et al.*, 1997). Therefore, interpreting the effects of drug priming in self-administration and, in particular, in extinction–reinstatement procedures is not without difficulty. The extent to which 'priming', which can entail contingent administration of relatively large doses of drug, models the human condition is unclear.

SUMMARY AND CONCLUSIONS

To what extent do the procedures described above model the human condition? Table XVI-1.1 summarizes the DSM IV criteria used for the diagnosis of substance dependence. Criteria 1 (tolerance) and criteria 3 (withdrawal) can be assessed using a number of techniques discussed here as well as with others that provide a more physiological approach to these phenomena (e.g. quantification of somatic withdrawal signs or tolerance to opiate-induced antinociception). Mice, rats and non-human primates readily self-administer drugs that have high abuse liability in humans. When unlimited-access conditions are employed, an escalation of drug self-administration and a binge-like pattern of consumption is seen (Ahmed and Koob, 1998; Fitch and Roberts, 1993). In rats and non-human primates, progressive-ratio schedules can be used to determine reinforcing efficacy (Griffiths *et al.*, 1978; Stafford *et al.*, 1998). Therefore, it would appear that criterion 3 (the substance is often take in larger amounts or over a longer period than was intended) can be modelled in experimental animals. Criterion 6 (important social, occupational or recreational activities given up because of substance use) has been demonstrated in animal models involving choice procedures and behavioural economics paradigms (Carroll *et al.*, 1989; Elsmore *et al.*, 1980). It is, however, apparent that existing procedures can only model particular components of substance dependence and that a battery of animal models must be used to understand the factors leading to the initiation and maintenance of compulsive drug use.

Drug addiction in humans has been characterized as occurring in several stages, although progress from one stage to the next is not inevitable. The first stage is initiation or acquisition, which may lead to habitual use, physical or psychic dependence, and loss of control. An individual may stop taking a drug at any stage. However, relapse to drug taking after a period of abstinence is common. Although the factors responsible for relapse are not well understood, accumulating evidence suggests that drug-associated environmental stimuli, acute re-exposure to drug, and stress can act as triggers of craving leading to relapse in humans (Jaffe *et al.*, 1989; Robbins *et al.*, 1997). Animal models of drug craving and relapse continue to be developed and refined. Second-order schedules can be used as a measure of the conditioned reinforcing properties of drugs (Goldberg and Gardner, 1981). Recent work suggests that reliable responding for cocaine can be obtained with a second-order schedule in rats as well as in non-human primates (Arroyo *et al.*, 1998; Katz and Goldberg, 1991; Markou *et al.*, 1999; See *et al.*, 1999). The conditioned place preference paradigm also provides a measure of conditioned reinforcement that is conceptually similar to the measures provided by the operant paradigms (see above). More recently, stimuli that predict drug availability have been shown to be powerful cues for reinstating drug-seeking behaviour in studies using second-order schedules or extinction procedures (McFarland and Ettenberg, 1997; Weiss *et al.*, 2000). Priming injections of cocaine or heroin have also been shown to reinstate drug-seeking behaviours in extinction models and similar effects have been observed in response to certain stressors (deWit and Stewart, 1981; 1983; Shaham and Stewart,

1995; Spealman *et al.*, 1999). The extent to which the procedures currently available model the human condition with reliability and predictive validity requires additional study.

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XVI-2

Amino Acid Transmitter Systems in Substance-Related Disorders

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INTRODUCTION

There is increasing evidence suggesting that ethanol, psychostimulants, nicotine and opioids may affect the central nervous system (CNS) by interfering with amino acid neurotransmitter systems. This review aims to clarify recent knowledge that has accumulated over the last decade in the understanding of the pharmacological, cellular and behavioural basis of such substances of abuse.

AMINO ACID TRANSMITTERS

Among the amino acid neurotransmitter systems are excitatory amino acids (aspartate, glutamate) which activate postsynaptic cells, the inhibitory amino acid GABA which depresses the activity of the postsynaptic cells, as well as the sulphonated amino acid taurine to which both neurotransmitter and neuromodulator functions have been assigned.

Glutamate

Glutamic acid (glutamate) is the major excitatory amino acid neurotransmitter in the CNS. Its excitatory effect on neurones is mediated by activating receptors, which are gated to ion channels, or to a protein that mediates a second messenger. This neurotransmitter has been reported to play an important role in alcoholism (Tsai *et al.*, 1995, Tsai and Coyle, 1998). Glutamate receptors in the mammalian CNS are divided into ionotropic (NMDA, kainate, AMPA) (Nakanishi, 1994; Hollmann *et al.*, 1994; Schoepfer *et al.*, 1994; Westbrook, 1994) and metabotropic receptors (Collingridge and Lester, 1989). The NMDA receptor, which is coupled to a voltage-sensitive ion channel, is permeable to calcium and monovalent cations Na^+ and K^+, and has been implicated in many physiological and pathological processes including synaptic plasticity, learning and memory, epileptiform seizures (Dingledine *et al.*, 1986) as well as neurotoxicity (Lovinger, 1993).

γ-Amino Butyric Acid

γ-Aminobutyric acid (GABA) is the major inhibitory amino acid neurotransmitter in the vertebrate CNS (Krnjevic, 1974, 1997; Curtis, 1995; McKernan and Whiting, 1996). The GABA receptors are divided currently into two types. The classical GABA receptor and its ion channel are specifically stimulated by muscimol and antagonized by bicuculline and termed the $GABA_A$ receptor or $GABA_A$ receptor channel. $GABA_A$ receptors belong to the ligand-gated ion channel family of receptors. The $GABA_A$ receptor consists of several protein subunits (at least five), which form a picrotoxin-sensitive anion channel and a number of allosteric regulatory sites for various drugs such as barbiturates, benzodiazepines, convulsants, neurosteroids, and possibly even anaesthetics and ethanol (Olsen, 1981; Sieghart, 1992, 1995; Morrow, 1995). Stimulation of these receptors usually brings about hyperpolarization of the cell membrane by opening of the Cl^- channel and entry of Cl^- into the neurone (Krnjevic, 1997; Perkins and Wong, 1997; van den Pol *et al.*, 1996). For more detail see chapter XIX-3, particularly the paragraph on the molecular biology of $GABA_A$ receptors.

GABA receptors which are specifically activated by baclofen and antagonized by phaclofen are the $GABA_B$ receptors. $GABA_B$ receptors are functionally coupled to K^+ and Ca^{2+} channels via G protein, and their stimulation causes an increase in the K^+ permeability or a decrease in Ca^{2+} currents in the target cell, which can result in decreased neurotransmitter release (Dolphin, 1990; Nicoll *et al.*, 1990; Gage, 1992; Malcangio and Bowery, 1995; Bowery and Brown, 1997; Kerr and Ong, 1995; Nishikawa *et al.*, 1997).

Taurine

Taurine (2-aminoethane sulphonic acid, $H_2NCH_2CH_2SO_3H$) is a β-non-essential sulphonated amino acid, which is synthesized from cysteine, and present in many mammalian cells at relatively high concentrations (millimolar). Although a multitude of studies of its possible function within the cell have been suggested, which include stabilization of membranes, osmoregulation of the cell and modulation of neurotransmitters, many of its biological effects remain unclear. However, recent studies have suggested that taurine is able to exert a multitude of functions in the CNS such as modulation of ion movements at both the calcium and chloride channels while interacting with many neurotransmitter systems to combat ethanol effects.

Interaction of Taurine with Neurotransmitters

The functions of taurine within the brain remain obscure although an increasing literature has identified a variety of roles. It has been proposed that taurine may play an important role either as an inhibitory neurotransmitter (Davison and Kaczmarek, 1971) or neuromodulator in the CNS (Lombardini, 1976; Scheibel *et al.*, 1981; Oja *et al.*, 1990) modulating synaptic activities, stabilizing and regulating cell volume (Oja and Kontro, 1983; Huxtable, 1989). It is clear that one of taurine's principal roles is to modulate calcium homeostasis, by reducing calcium accumulation within the cell

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(Li and Lombardini, 1991; Vislobokov *et al.*, 1992), stimulating the pumping rate of the Ca^{2+}-activated ATPase pump when necessary (Huxtable, 1992). Taurine alters chloride movements (Haas and Hösli, 1973) by increasing the chloride conductance, and as a consequence of this modulation of ion channels taurine could alter neurotransmission by interacting with many neurotransmitter systems and receptors. Taurine interacts with the GABA recognition site at [^{35}S]*t*-butylbicyclophosphorothionate (TBPS) of the GABA receptor complex (Quinn and Harris, 1995) and modulates GABA release by its binding to both $GABA_A$ and $GABA_B$ receptors (Kontro *et al.*, 1990; Williams *et al.*, 1980; Bureau and Olsen, 1991; Malminen and Kontro, 1986). Such modulation of neurotransmission could be mediated by taurine's action on brain GABA and glycine (Huxtable, 1989, 1992; Kontro *et al.*, 1990) receptors. Furthermore, taurine could stimulate glutamic acid decarboxylase (GAD) in the brains of genetically bred epileptic rats (Bonhaus and Huxtable, 1985) influencing the conversion of glutamate to GABA. Taurine has also been suggested to modulate the dopaminergic transmission in the striatum (Kontro, 1987) by modifying taurine release in this brain region (Kontro and Oja, 1988).

Taurine interacts with glutamate and its receptors. As such, taurine has the ability to inhibit NMDA, kainate and quisqualate receptors (Kurachi *et al.*, 1983); both *in vitro* and microdialysis studies of various brain regions have shown that agonists of glutamate receptors, namely NMDA, kainate or quisqualic acid, induced taurine release (Lehmann *et al.*, 1985; Menéndez *et al.*, 1990; Shibanoki *et al.*, 1993; Saransaari and Oja, 1994). This interaction of taurine with the glutamatergic system could be of interest regarding the mechanism underlying the protective effects of taurine against toxic agents acting via excitatory amino acids. Many studies showed that taurine may protect cells from cytotoxicity (French *et al.*, 1986; Waterfield *et al.*, 1994; Oja and Saransaari, 1996; Saransaari and Oja, 1997; Obyrne *et al.*, 1997).

ETHANOL DEPENDENCE AND AMINO ACID TRANSMITTERS

Effect of Ethanol on Glutamate

Acute Effect of Ethanol

Electrophysiological and neurochemical studies showed that ethanol at pharmacologically relevant concentrations (50–100 mM) inhibits or antagonizes the action of agonists at the NMDA receptor in several neuronal preparations (Dildy Mayfield and Leslie, 1989; Hoffman *et al.*, 1989; Lovinger *et al.*, 1989, 1990; Woodward and Gonzales, 1990; Simson *et al.*, 1991; Hoffman, 1995). Furthermore, a decrease in glutamate extracellular striatal concentration occurs in an *in vivo* microdialysis study after administration of $2\,g\,kg^{-1}$ ethanol (Carboni *et al.*, 1993), while another microdialysis study showed a biphasic response with either $0.5\,g\,kg^{-1}$ (increase) or $2\,g\,kg^{-1}$ (decrease) ethanol on the glutamate release in both hippocampus and nucleus accumbens regions (Moghaddam and Bolinao, 1994). The inhibitory effect of ethanol on the NMDA receptor was not selective. Indeed, electrophysiological studies in *Xenopus* oocytes expressing mRNA from rat hippocampus and cerebellum demonstrated that there was a different degree of inhibition by ethanol on NMDA and non-NMDA receptors (Dildy Mayfield and Harris, 1992a, 1992b; Lovinger, 1993). However, ethanol appears to be less potent in inhibiting the function of non-NMDA ionotropic glutamate receptors (Hoffman *et al.*, 1989; Lovinger *et al.*, 1989).

Acute ethanol-induced inhibition of the NMDA receptor leads to many serious consequences on developmental, learning and memory processes. Alteration of NMDA receptor function during development can lead to severe and potentially permanent brain dysfunction (Weaver *et al.*, 1993), as well as ethanol-prevented long-term potentiation (LTP), which is a process that underlies learning (Maren and Baudry, 1995), particularly in hippocampus (Morrisett and Swartzwelder, 1993). Moreover, data from *in vivo* hippocampal studies showed that the decrease in hippocampal glutamate transmission induced by ethanol or benzodiazepines closely correlated with spatial memory deficits (Shimizu *et al.*, 1998). Interestingly, this phenomenon was blocked by NMDA antagonists (Coan *et al.*, 1987).

Conclusion 1

- Acute ethanol induced a decrease in intracellular glutamate level.
- Acute ethanol inhibits the NMDA receptor. This could have many serious consequences on developmental, learning and memory processes.

Chronic Effect of Ethanol

Chronic ethanol intoxication results in an upregulation of NMDA receptor number and function caused by the physiological response to the depressant effects of ethanol (Michaelis *et al.*, 1990; Chandler *et al.*, 1993a; Snell *et al.*, 1993; Trujillo and Akil, 1995; Whittington *et al.*, 1995). This upregulation may be explained by the enhancement of receptor expression and exchanger-associated proteins (Trevisan *et al.*, 1994; Follesa and Ticku, 1995; Snell *et al.*, 1996; Chen *et al.*, 1997). For example, an increase in NMDA receptor subunit NR1 and NR2 polypeptide levels was reported by Ticku (1998), and an upregulation of the NMDA receptor NR2B subunit mRNA without altering NR1 mRNA in rat brain and cortical neurones was reported by Hu *et al.* (1996) and Ticku (1998). Furthermore, Western blot analysis indicates that chronic ethanol treatment upregulates NMDA receptor subunits NR1, NR2A and NR2B in the rat cerebral cortex and hippocampus; these returned to almost control levels 48 h after the last dose of ethanol administration (Kalluri *et al.*, 1998).

These results are supported by the finding that NMDA antagonists such as dizocilpine (MK-801) or CGP 39551 administered during ethanol withdrawal significantly attenuated ethanol-induced deficit in spatial reversal memory (Riaz and Faingold, 1994; Thomas *et al.*, 1997). Such antagonists are useful in curbing ethanol withdrawal, seizure and tremors (Grant *et al.*, 1990, 1992; Liljequist, 1991; Ripley and Little, 1995). Exposure to MK-801 during ethanol withdrawal will also protect against alcohol-related disruption in spatial learning (Thomas *et al.*, 1997). In addition, during ethanol withdrawal, calcium channels are upregulated and become hyperexcited (Morton *et al.*, 1992; Whittington *et al.*, 1995) and neuronal calcium effects are amplified (Whittington *et al.*, 1995; Molleman and Little, 1997). The use of calcium channel antagonists (e.g. nitrendipine, nimodipine, isradipine and darodepine) prevented or suppressed behavioural signs of ethanol withdrawal (Littleton *et al.*, 1990; Colombo *et al.*, 1995).

Chronic ethanol treatment (CET) enhances NMDA-stimulated, but not kainate-stimulated, intracellular calcium levels. Seven days after treatment with 100 mM ethanol, the magnitude of cell death mediated by NMDA, but not AMPA, increased (Smothers *et al.*, 1997). In addition, recent studies suggested that nitric oxide may also play a part in the neuroadaptation that develops during chronic ethanol intoxication which could be mediated by changes in Ca^{2+} flux. Four days of CET (100 mM ethanol) enhanced NMDA-stimulated, but neither kainate nor AMPA-stimulated, nitric oxide formation (Chandler *et al.*, 1997), which is reputed to be an important biological messenger and regulator of the CNS (Bredt and Snyder, 1994). Nitric oxide may play an important role in alcohol dependence and withdrawal as well as alcohol-induced brain damage. Studies where nitric oxide synthase inhibitors, such

as L-NAME and 7-NI, are administered to chronically alcoholized rats at the cessation of alcoholization show reduction or alleviation of the signs of ethanol withdrawal (Adams *et al.*, 1995; Uzbay *et al.*, 1997). However, in all of these studies no results for nitric oxide production or nitric oxide synthase activity have been reported.

Ethanol Withdrawal Effect

Cessation of chronic ethanol treatment leads to seizure, hyperexcitability and ethanol withdrawal. Animal studies have revealed that overactivation of glutamate receptors contributes to the generation of these symptoms (Grant *et al.*, 1990; Gulya *et al.*, 1991) and could lead to excitotoxic death (Iorio *et al.*, 1993; Davidson *et al.*, 1995). Human studies have indicated that excitatory neurotransmitters were elevated in the cerebrospinal fluid (CSF) of alcohol-dependent patients and positive correlation between excitatory neurotransmitters and oxidative markers were identified (Tsai and Coyle, 1998). The increase of c-fos mRNA in alcohol withdrawal may result from activation of NMDA receptors (Morgan *et al.*, 1992) which appears to be independent of withdrawal seizures (Morgan and Linnoila, 1991; Morgan *et al.*, 1992).

Recent microdialysis studies showed that ethanol withdrawal was associated with increases in glutamate in the striatum (Rossetti and Carboni, 1995), the nucleus accumbens (Dahchour and De Witte, 1996) and hippocampus (Dahchour and De Witte, 1998) approximately 5–8 h after cessation of ethanol inhalation, which reached a maximum value at 12 h in the nucleus accumbens.

Glutamate-Induced Excitotoxicity and Ethanol

Glutamate receptor-induced excitotoxicity (Michaelis, 1990; Lovinger, 1993; Hoffman and Tabakoff, 1994; Hoffman, 1995) may be one mechanism by which ethanol induces behavioural teratogenicity, i.e. foetal development (Tsai *et al.*, 1995). It is known that an increase in the excitatory amino acid glutamate will have a dramatic effect on neurones by the process of excitotoxicity with associated depolarization of the membrane. Neurotoxic effects are observed after application of glutamate or its agonist to neurones in tissue culture (Choi, 1988, 1992) while even 5 min exposure of glutamate (10–1000 μM) induces neuronal swelling followed by degeneration over the proceeding 24 h (Peterson *et al.*, 1989; Regan and Choi, 1991). Furthermore, addition of NMDA (25–100 μM) to cultured cerebral neurones for 25 min induced excitotoxicity, while prior exposure to chronic ethanol (100 mM for 96 h) further sensitized these neurones to excitotoxic NMDA receptor activation (Chandler *et al.*, 1993b). In conclusion, ethanol administered acutely disrupts glutamatergic neurotransmission by inhibiting the response of the NMDA receptors. Prolonged inhibition of NMDA receptors by ethanol results in development of hypersensitivity; cessation causes marked augmentation of activity in postsynaptic neurones and, in the extreme, induces glutamate-induced excitotoxicity.

Conclusion 2

- Upregulation of the NMDA receptor is a consequence of a physiological response to the depressant effects of ethanol.
- Chronic ethanol treatment yields many changes in NMDA receptor subunits, which could be responsible for their hyperexcitability.
- Cessation of chronic ethanol treatment leads to many symptoms, including seizure and hyperexcitability, which are characteristic of ethanol withdrawal.
- During ethanol withdrawal, an increase in glutamate and hypermotility was reported. Antagonists of glutamate receptors were able to block many withdrawal symptoms.

Effect of Ethanol on GABA

Acute Effect of Ethanol on GABA

Increasing evidence indicates that the effects of acute ethanol are mediated by its effects on GABA or its receptors. Indeed, many studies have documented that ethanol induced increases in $GABA_A$ responses (Nestoros, 1980; Suzdak *et al.*, 1986; Allan and Harris, 1986, 1987; Mehta and Ticku, 1988; Deitrich *et al.*, 1989; Aguayo, 1990; Nishio and Narahashi, 1990; Engblom and Akerman, 1991; Ueha and Kuriyama, 1991; Mihic and Harris, 1996). However, several other groups have failed to detect any robust ethanol-induced enhancement of $GABA_A$ responses in either biochemical (Uusi-Oukari and Korpi, 1989; Mihic *et al.*, 1991) or electrophysiological (White *et al.*, 1990; Osmanovic and Shefner, 1990; Korpi and Seeburg, 1993; Morrisett and Swartzwelder, 1993) experiments. These conflicting results could be explained by the differences in ethanol doses, material preparations, animal strains and animal lines. For example, studies of ethanol effects on $GABA_A$ receptors in mice specifically bred for their differences in sensitivity to ethanol have reported that ethanol enhanced GABA-mediated chloride uptake into brain microsacs in long sleep (LS) but not in those of short sleep (SS) mice (Mihic and Harris, 1996).

Chronic Effect of Ethanol on GABA

Biochemical and electrophysiological studies have reported that chronic ethanol exposure reduces $GABA_A$ receptor-mediated chloride channel function in rats and mice (Allan and Harris, 1987; Morrow *et al.*, 1988; Buck and Harris, 1991; Sanna *et al.*, 1993; Morrow, 1995). Such 'downregulation' occurs after chronic ethanol treatment and may represent a mechanism for tolerance to ethanol. In addition, studies have clearly demonstrated that chronic ethanol treatment differentially alters $GABA_A$ receptor subunit (See chapter XIX-3 for more detail on molecular biology of $GABA_A$ receptors) expression in the cerebral cortex (Morrow *et al.*, 1990; Montpied *et al.*, 1991; Devaud *et al.*, 1995) and cerebellum (Morrow *et al.*, 1992). After CET, a benzodiazepine inverse agonist (Ro 15-4513) binding was selectively upregulated (Becker and Jarvis, 1996; Ticku, 1998) with a decrease in $GABA_A$ receptor α_{1-3} subunit and a decrease of α_6 (in cerebellum) and β_{2-3} subunit without alteration in γ_2 subunit (Ticku, 1998). The level of $GABA_A$ receptor α_1 subunit mRNA is reduced, while α_4 subunit mRNA is increased, both in the cerebral cortex and cerebellum (Devaud *et al.*, 1996), while decreases in $GABA_A$ receptor α_1 subunit mRNAs and increases in α_6 subunit mRNA levels are detectable (Morrow *et al.*, 1992). The levels of α_1 and α_5 subunit mRNA are also decreased in cortex, cerebellum and the ventral tegmental area (VTA), while the level of hippocampal α_5 mRNA was increased (Charlton *et al.*, 1997). Long-term ethanol exposure decreased immunoreactivity levels of α_l subunit of the $GABA_A$ receptor complex in the VTA (Ortiz *et al.*, 1995). However, many studies have suggested that there are selective alterations of the $GABA_A$ receptor subunit after chronic ethanol treatment (Mahmoudi *et al.*, 1997). These findings support the hypothesis that tolerance to ethanol involves reduction in $GABA_A$ receptor numbers and/or function, which may result from a decrease in receptor levels or changes in the protein composition of the receptor leading to decreased sensitivity to neurotransmitters. Such alterations would contribute to ethanol withdrawal symptoms such as hyperexcitability, seizures and tremors.

Ethanol Withdrawal Effect on GABA

Withdrawal from chronic ethanol treatment has been shown to lead to increased seizure susceptibility (Kokka *et al.*, 1993). This is believed to be caused by decreases in GABAergic function

(McQuilkin and Harris, 1990; Mehta and Ticku, 1992). Human studies indicate that GABA concentrations were lower in both the CSF (Tsai and Coyle, 1998) and blood (Adinoff *et al.*, 1995) of alcohol-dependent patients by comparison to those of controls. This may contribute to the symptoms of ethanol withdrawal and associated neurotoxicity. Changes in $GABA_A$ receptor subunit during ethanol withdrawal are also observed. $GABA_A$ receptor α_1 α_4 and γ_1 subunit mRNA levels return to control levels, while β_{2-3} subunit mRNA level increases compared to both controls and ethanol-dependent rats (Devaud *et al.*, 1996, 1997). The reduced activity of inhibitory GABA neurotransmission might contribute to the hyperexcitability and withdrawal seizures. GABA agonists are used to alleviate these symptoms during ethanol withdrawal. Flumazenil (Ro15-1788) a benzodiazepine receptor antagonist, reduces seizure severity and reverses anxiogenic response in mice and rats during acute ethanol withdrawal (Buck *et al.*, 1991a, 1991b, 1991c; File *et al.*, 1992) and suppresses or ameliorates symptoms of tremors (Gerra *et al.*, 1996; Nutt *et al.*, 1993).

Outcomes of the Effects of Ethanol on GABA

LTP requires activation of glutamate receptors and inhibition of $GABA_A$ receptors, and plays a fundamental role in learning and memory formation (Bliss and Collingridge, 1993; Maren and Baudry, 1995). The finding that ethanol enhances $GABA_A$ receptor function in the hippocampus (Weiner *et al.*, 1994; Peris *et al.*, 1997) suggests that ethanol may have an inhibitory effect on memory by its effect on both glutamate and GABA systems (Weiner *et al.*, 1997; Valenzuela and Harris, 1997).

Conclusion 3

- Evidence from many studies has documented that ethanol induced an increase in $GABA_A$ receptor function.
- Chronic ethanol treatment differentially alters $GABA_A$ receptor subunits and this may represent a mechanism for tolerance to ethanol.
- During ethanol withdrawal both concentration and function of GABA and $GABA_A$ receptors were reduced. This could exacerbate withdrawal symptoms.

Effect of Ethanol on Taurine

Implication for Taurine in Ethanol Effects

The interaction of taurine and ethanol has been demonstrated in many physiological experiments where ethanol-induced behavioural activity such as hypnosis (sleep time) in mice (Boggan *et al.*, 1978; McBroom *et al.*, 1986; Ferko, 1987; Ferko and Bobyock, 1988) and rats (Mattucci-Schiavone and Ferko, 1985), locomotion activity in the open field (Aragon *et al.*, 1992) and conditioned taste aversion (Aragon and Amit, 1993) have been modified after taurine co-administration. It should also be remembered that the osmoregulatory and electrical activities of cells are interdependent, and depolarization will induce ionic changes and cell swelling (Huxtable, 1989). Therefore, the taurine modulatory action on membrane excitability and neurotransmitter processes could be mediated by ionic changes resulting from osmotic regulation of the cells. Exposure of primary astrocyte cultures to iso-osmotic ethanol from 10 to 100 mM leads to cell swelling and the release of [^{3}H]taurine and D-[^{3}H]aspartate. In contrast, exposure to hyperosmotic ethanol, at the same concentrations, caused neither swelling nor release (Kimelberg *et al.*, 1993). Moreover, many studies showed increases in taurine after the administration of agents which induce osmolarity changes within the cells, such as water intoxication (Wade *et al.*, 1988) and glutamate analogues (Menéndez *et al.*, 1990). Membrane depolarization also induces taurine release from brain cells (Oja and Saransaari, 1992).

The action of taurine on ethanol metabolism will also be considered in the context of its modulatory effects. Previous studies have clearly demonstrated that taurine, when administered either intraperitoneally or intragastrically at various concentrations, has no effect on peripheral ethanol metabolism (Aragon and Amit, 1993; Watanabe *et al.*, 1985). However, taurine may enhance the metabolism of acetaldehyde, the major metabolite of ethanol, by activating the hepatic enzyme aldehyde dehydrogenase and thereby reducing blood acetaldehyde levels. Indeed, taurine ($0.5\,g\,kg^{-1}$) given orally significantly reduced the elevated blood and liver acetaldehyde concentrations following ethanol loading ($1.5\,g\,kg^{-1}$ body weight; Watanabe *et al.*, 1985). Whether brain acetaldehyde levels would be decreased after a taurine supplementation is unknown, since levels of this major metabolite of ethanol oxidation in the brain are extremely low (Ward *et al.*, 1997) and difficult to assay. In addition, the activities of brain acetaldehyde dehydrogenase isoenzymes remain undefined. However, the demonstration of the rewarding effects of acetaldehyde after intraventricular injection (Smith *et al.*, 1984) clearly indicates a role for this major ethanol metabolite in the rewarding action of ethanol.

One of our contributions to the knowledge of alcohol research is that administration of acute ethanol to rats increases the level of the sulphonated amino acid taurine in many brain regions, including nucleus accumbens (Dahchour *et al.*, 1994, 1996), hippocampus, frontal cortex (Dahchour, 1998) and amygdala (Quertemont *et al.*, 1998, 1999). To our knowledge this is the first time that such increases were identified using the microdialysis technique combined with electrochemical detection. No changes in either GABA or glutamate were identified in any of these brain regions. Furthermore, an increase in taurine microdialysate level was also detected after intraperitoneal acetaldehyde injection (Ward *et al.*, 1997).

Conclusion 4

- Acute ethanol increases extracellular taurine concentration in different brain regions.
- The action of taurine has been demonstrated in ethanol-induced behavioural activity such as hypnosis, locomotor activity and conditioned taste aversion.
- The action of taurine on ethanol metabolism has also been reported.

PSYCHOSTIMULANT DEPENDENCE AND AMINO ACID TRANSMITTERS

Sensitization is a phenomenon which refers to the progressive augmentation of behavioural response to repeated administration of psychomotor stimulants such as cocaine and amphetamine. Although behavioural sensitization to psychostimulants appears to be mediated by dopaminergic system (for review, see Kalivas and Stewart, 1991), there is increasing evidence that excitatory amino acids play a role in this phenomenon (for review, see Wolf, 1998).

The acute systemic administration of a high dose of cocaine ($30\,mg\,kg^{-1}$ intraperitoneally) increases glutamate and aspartate microdialysate content in the nucleus accumbens (Smith *et al.*, 1995; Reid *et al.*, 1997), and this increase in glutamate was much higher in rats that have been sensitized to cocaine (Pierce *et al.*, 1996). Furthermore, the ability of glutamate receptor antagonists to prevent behavioural sensitization to cocaine or morphine in mice and rats (Karler *et al.*, 1989, 1990, 1994; Kalivas and Alesdatter, 1993; Li *et al.*, 1999) has demonstrated the implication of excitatory amino acids in the mechanism underlying behavioural sensitization. In addition, co-administration of glutamate receptor

antagonists with psychostimulants interferes with the development of behavioural sensitization in mice and rats (Kalivas and Alesdatter, 1993; Karler *et al.*, 1994; Li *et al.*, 1999; Wolf and Jeziorski, 1993). Whether the effects of psychostimulants are directly linked to excitatory amino acids or act indirectly by interfering with the dopaminergic system (or other transmitter system) raises an important question concerning the interaction between neurotransmitter systems in behavioural sensitization. In fact, a microdialysis study showed that agonists of dopamine receptors administered by reverse dialysis in the nucleus accumbens increased extracellular glutamate and stimulated locomotor activity (Dalia *et al.*, 1997). On the other hand, other studies showed that glutamate agonists administered in the nucleus accumbens increased the dopamine microdialysate content (Ohno and Watanabe, 1995; Svensson *et al.*, 1994). As such, both dopaminergic and glutamatergic systems are required for locomotor activity and thereby behavioural sensitization to psychostimulants. In addition, the GABAergic system is linked anatomically and functionally to the dopaminergic and glutamatergic systems particularly in brain regions that are implicated in drug dependence. Evidence from many studies demonstrated that the GABAergic system is also implicated in the motor stimulatory effects induced by psychostimulants, and administration of both $GABA_A$ (muscimol) (Austin and Kalivas, 1991; Mogenson and Nielsen, 1983) and $GABA_B$ (baclofen) (Kalivas *et al.*, 1990; Johnson *et al.*, 1996) agonists prevent the locomotor activity induced by psychostimulants. Furthermore, Karler *et al.* (1995) described an amphetamine- and cocaine-induced interaction between dopaminergic, glutamatergic and GABAergic systems in the striatum and suggested that the motor stimulatory effects of amphetamine and cocaine are mediated by dopaminergic activation of glutamatergic and GABAergic pathways in the striatum. There is also direct evidence that acute administration of psychostimulants, particularly amphetamine, produced an increase of extracellular GABA and taurine concentrations in the striatum (Porras and Mora, 1993; Del Arco *et al.*, 1997). In contrast, both GABA concentration and release were reduced in many brain regions, including ventral pallidum, nucleus accumbens and striatum, following repeated administration of amphetamine (Bourdelais and Kalivas, 1990; Linderfords *et al.*, 1992; Jung *et al.*, 1999).

Conclusion 5

- Excitatory amino acids are implicated in the development of behavioural sensitization to psychostimulants and glutamate receptor antagonists were able to prevent this behavioural sensitization.
- High doses of cocaine induced increases in glutamate and aspartate in the nucleus accumbens.
- Acute administration of psychostimulants, particularly amphetamine, produced an increase of extracellular GABA and taurine concentrations in the striatum.

OPIOID DEPENDENCE AND AMINO ACID TRANSMITTERS

There is increasing evidence suggesting that glutamate and or its receptors are implicated in the genesis of opioid tolerance and dependence. The NMDA receptor antagonist MK-801 has been reported to inhibit both the development of tolerance to the analgesic effect of morphine and the expression of morphine (μ-opioid receptor agonist) withdrawal (Trujillo and Akil, 1991, 1994). In addition, glutamate administration was able to precipitate the withdrawal signs from chronic morphine or butorphanol (a mixture of $\mu\delta/\kappa$-opioid receptor agonist) and this withdrawal sign was blocked by pretreatment with MK-801 (Tokuyama *et al.*, 1995). Moreover, naloxone, which is an opioid receptor antagonist also, precipitated withdrawal from morphine and studies showed that this effect was associated with increased glutamate concentration in the locus coeruleus (Aghajanian *et al.*, 1994; Zhang *et al.*, 1994).

Regarding the effect of opioids on GABA, it has been reported in a microiontophoresis study that morphine administration into the ventral pallidum attenuated the GABA-evoked response (Johnson and Napier, 1997). This results are in agreement with another electrophysiological study which reported that local administration of morphine in the dorsal raphe nucleus and periaqueductal grey area of freely moving rats decreased GABA release (Stiller *et al.*, 1996). However, chronic morphine increased GABA tone on 5-HT neurones of dorsal raphe nucleus (Jolas *et al.*, 2000).

Conclusion 6

- The NMDA receptor antagonist MK-801 has been reported to inhibit both the development of tolerance to the analgesic effect of morphine and the expression of morphine withdrawal.
- The acute administration of morphine attenuates GABA function and decreases GABA release. However, chronic morphine increased GABA.

NICOTINE DEPENDENCE AND AMINO ACID TRANSMITTERS

There is also evidence that nicotine dependence is associated with perturbation in many neurotransmitter systems, including amino acid transmitters. An acute dose of nicotine produced an increase of glutamate in the nucleus accumbens and in different forebrain regions (Reid *et al.*, 2000; Toth *et al.*, 1993; Vidal, 1994), and stimulated the release of GABA in various brain regions, including striatum (Lu *et al.*, 1998). Glutamate receptor antagonists attenuated the development of sensitization after chronic administration of nicotine (Shoaib *et al.*, 1997).

Conclusion 7

- An acute dose of nicotine produced an increase of glutamate in the nucleus accumbens and in different forebrain regions, and stimulated the release of GABA in various brain regions including striatum.

CONCLUSIONS

Substance-related disorders induced by ethanol, psychostimulants, opioids, nicotine and other substances are a major health problem in which several neurotransmission systems are involved, including the dopaminergic, serotonergic, glutamatergic and GABAergic systems. However, the most relevant question of such a multifunctional mechanism found in different substance-related disorders is whether the action of such substances is directly upon neurotransmitters and their receptors or whether it functions indirectly by altering the neurotransmission network in the brain. It was clear from this chapter that two major amino acid systems, i.e. excitatory (glutamate) and inhibitory (GABA) amino acids, were involved in the action of different addictive substances and could be responsible for tolerance and dependence related to these substances. Furthermore, long-term exposure to these substances induced wide and specific changes in the molecular biology of different receptor subunits of both glutamate and GABA receptor amino acids.

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XVI-3

Endocrinology of Drug Dependence

Pier Vincenzo Piazza and Bruno Aouizerate

AIMS AND SCOPE

This chapter will analyse the relationships between glucocorticoid hormones and drug abuse, focusing principally on clinical studies. The reason for this choice is that a growing body of evidence from investigations in animals suggests that glucocorticoids play an important role in mediating behavioural and neurochemical responses to drugs of abuse (for review see Piazza and Le Moal, 1996, 1997). Unfortunately, studies in humans on the relationships between drugs of abuse and glucocorticoids are still at an early stage and many of the available investigations are performed on small groups of subjects. Furthermore, many studies addressing the same issue are difficult to compare because they often use subjects who differ in drug history, current medication or withdrawal and abstinence periods. The goal of this chapter is therefore not to draw firm conclusions but to try to generate a working hypothesis that could lead future research.

First we will brief by review the interaction between glucocorticoids and the behavioural responses to drugs of abuse. Then we will summarize the principal methods used for studying the activity of this hormonal system in humans; this could be a useful companion for readers not familiar with the field. The following four sections will analyse the relationships between glucocorticoids and the abuse of drugs belonging to four different classes: opiates, cocaine, alcohol and nicotine. Finally, we will try, through a synthesis of the available information, to extract a working hypothesis of the potential role of glucocorticoids in drug abuse. Therapeutic implications and future directions will conclude the chapter.

GLUCOCORTICOIDS AND THE BEHAVIOURAL RESPONSES TO DRUGS OF ABUSE

Glucocorticoid hormones (cortisol in humans and corticosterone in rodents) are the final step in the activation of the hypothalamic–pituitary–adrenal (HPA) axis, one of the major systems implicated in responding to environmental modifications and stress (see previous chapters for a description of the physiology of this neuroendocrine system).

During the last decade, a large amount of experimental evidence has suggested that glucocorticoids may play an important role in modulating the behavioural effects of drugs of abuse. This research has been prompted by the discovery (Piazza *et al.*, 1991) that animals spontaneously vulnerable to drug abuse had a longer stress-induced secretion of glucocorticoids and that administration of these hormones to drug-resistant subjects could increase the likelihood of their self-administering psychostimulant drugs. Since this initial observation, several reports using complementary approaches have shown that the suppression of glucocorticoid secretion decreases stimulant and reinforcing effects of drugs of abuse (for review see Piazza and Le Moal, 1996, 1997, 1998). In particular, glucocorticoids seem to increase the motivation of the individual to self-administer drugs. This effect is probably mediated by a stimulation of drug-induced dopamine release in the nucleus accumbens (for review see Piazza and Le Moal, 1996), one of the principal neurobiological changes mediating the reinforcing effects of drugs of abuse (Wise and Rompre, 1989; Piazza and Le Moal, 1996).

Some investigations have also been devoted to understanding the origin of the interaction between glucocorticoids and drugs of abuse (for review see Piazza and Le Moal, 1997). This research has led to the discovery that glucocorticoids, in the range of concentrations induced by stress, have positive reinforcing effects and stimulate dopamine release (Piazza *et al.*, 1993, 1996). Consequently, it has been hypothesized that these hormones are part of the endogenous reward system and are secreted during stress in order to counteract the aversive effect of stressors (for review see Piazza and Le Moal, 1997). In this respect, the action of glucocorticoids in the central nervous system would be similar to the effect of these hormone in the periphery, where they are supposed to help in controlling the primary response of the organism to stress (for review see Munck *et al.*, 1984).

METHODS FOR STUDYING THE ACTIVITY OF THE HPA AXIS IN HUMANS

Glucocorticoid Secretion

One of the most used approaches to evaluate the functional activity of the HPA axis is measurement of glucocorticoid concentration in biological fluids. Glucocorticoid levels are generally measured by radioimmunoassay (RIA) in plasma and in saliva. These two measurements evaluate different fractions of circulating glucocorticoids. In the blood, glucocorticoids are distributed in three fractions. The largest one (89.5%) is bound to a corticosterone-binding globulin (CBG), a smaller fraction is bound to albumin (6.5%) and the remaining portion (4.0%) is free. Only free and albumin-bound glucocorticoids can pass the blood–brain barrier (Pardridge and Mietus, 1979a, b), and only free glucocorticoids can enter the cell and have biological effects. Measurement of plasma glucocorticoids evaluates the total levels of the hormone, including the fractions binding to CBG and albumin. In contrast, saliva glucocorticoids reflect only the free fraction of the hormone, i.e. the biologically active one. Finally, levels of glucocorticoids can also be estimated by measuring urinary excretion of 17-ketosteroids.

Glucocorticoid Negative Feedback

Two major tests are used to determine the functional status of glucocorticoid negative feedback: the dexamethasone suppression test and the metyrapone test.

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Dexamethasone is a potent synthetic glucocorticoid agonist which inhibits the secretion of adrenocorticotrophic hormone (ACTH) and of cortisol for at least 24 h after a single dose of 1–2 mg. This test is usually utilized to study a decrease in glucocorticoid feedback revealed by a lower or absent suppression of endogenous glucocorticoids by dexamethasone (Liddle, 1960).

Metyrapone decreases glucocorticoid biosynthesis by inhibiting the steroid $P450_{11\beta}$, the enzyme that allows 11β-hydroxylation, which is the final step of cortisol production by the adrenal cortex. The blockade of cortisol biosynthesis by metyrapone releases the HPA axis from the inhibitory influence of glucocorticoids, resulting in a large increase in plasma ACTH during the 2–8 h following a single administration (Kreek, 1987, 1996, 2000). Higher levels of ACTH after metyrapone are considered an index of an enhanced glucocorticoid feedback. Conversely, a blunted ACTH response to metyrapone can be an index of a decreased glucocorticoid feedback.

Stimulation Tests

Infusion of corticotrophin-releasing factor (CRF) and ACTH-derived peptides is used to evaluate the functional activity of the HPA axis at different levels. The CRF stimulation test evaluates the sensitivity and functional capacity of pituitary corticotrophic cells to secrete ACTH. The ACTH stimulation test evaluates the sensitivity of the system at the level of the adrenal gland, measuring its sensitivity and functional capacity to produce glucocorticoids.

HPA FUNCTION IN OPIATE-RELATED DISORDERS

Synopsis

Opiate drugs induce a significant suppression of the activity of the HPA axis, decreasing the secretion of ACTH and cortisol. They also induce a blunted response to CRF, ACTH and to stress challenges, as well as a decreased response to metyrapone administration. These effects are observed after acute and chronic exposure to opiates without clear signs of tolerance except after long periods of methadone treatments at high doses (60–120 mg per day). Abrupt opiate withdrawal is, in contrast, accompanied by a strong activation of the HPA axis that occurs several days after the last drug intake. However, during abstinence, glucocorticoid levels appear to normalize. In contrast, a blunted response to stress and an increase in glucocorticoid feedback seem to persist for years after the last drug consumption.

Opiate Abuse and the Secretion of Glucocorticoids

Acute and Chronic Exposure to Opiate Drugs

In subjects non-addicted to opiates, reduction in circulating concentrations of cortisol has been reported in response to a single injection of various opiate agonists, such as morphine (McDonald *et al.*, 1959; Delitala *et al.*, 1983; Zis *et al.*, 1984; Banki and Arato, 1985; Facchinetti *et al.*, 1985), codeine (Garland and Zis, 1989) or methadone (Gold *et al.*, 1980; Delitala *et al.*, 1983) and also in response to the mixed agonist–antagonist buprenorphine (Rolandi *et al.*, 1983). A similar decline in cortisol levels has also been observed after chronic treatment with morphine (3–27 months) in patients with pain syndromes (Palm *et al.*, 1997; Abs *et al.*, 2000). Only two studies have failed to demonstrate an effect of opiates on the secretion of glucocorticoids (Tolis *et al.*, 1975; Oyama *et al.*, 1977).

Opiates also reduce the secretion of glucocorticoids in opiate-addicted subjects. A decrease in 17-ketosteroid excretion (Eisenman *et al.*, 1958, 1961) and in plasma cortisol levels (Higgins *et al.*, 1992) was observed after a single injection of morphine to former heroin-dependent patients. Decrements in both urinary 17-ketosteroid excretion and plasma levels of 17-hydroxycorticosteroids were found in narcotic addicts during both a 3-week period of morphine induction and a 1- to 5-month period of dose stabilization (Eisenman *et al.*, 1958, 1961). Similarly, during the active phase of heroin addiction, in which addicts were exposed daily to multiple high-amplitude fluctuations of this short-acting opiate, ACTH and cortisol levels were decreased, with reduced or flattened circadian diurnal rhythms (Facchinetti *et al.*, 1984, 1985; Kreek, 1972, 1973, 1987, 1996, 2000; Cami *et al.*, 1992). Only one study reported no significant change in plasma cortisol in opiate-dependent patients during a phase of heroin acquisition compared to the drug-free baseline condition (Mendelson *et al.*, 1975). The persistence of an inhibitory effect of opiates on glucocorticoid secretion even after chronic exposure is also confirmed by studies in methadone-treated subjects. Methadone is a synthetic agonist of the μ-opioid receptors characterized by a long half-life and largely used as a substitutive treatment in opiate addicts. During methadone-aided detoxification, decreased plasma (Mendelson *et al.*, 1975; Hellman *et al.*, 1975) and saliva cortisol (Cami *et al.*, 1992) have been reported. Only when methadone is administered as a long-term maintenance treatment at high doses (60 mg per day) is partial tolerance to the inhibitory effects of opiates observed (Kreek, 1972, 1973, 1984, 1987, 1996, 2000; Woody *et al.*, 1988).

Opiate Withdrawal and Abstinence

Abrupt withdrawal from opiates is followed by a striking rise in urinary 17-ketosteroid excretion (Eisenman *et al.*, 1958, 1961) and an increase in plasma (Hellman *et al.*, 1975) and saliva cortisol concentrations (Cami *et al.*, 1992). Similar effects are observed when withdrawal is precipitated by the administration of the opiate antagonist naloxone (Higgins *et al.*, 1992; Rosen *et al.*, 1995). This hypercortisolism seems to persist at least for the first 2 weeks following the last opiate consumption (Cami *et al.*, 1992). However, long-term abstinence from opiates results in a normalization of the HPA axis activity. Former heroin addicts, tested after 2 years or longer drug-free periods, show plasma cortisol levels that do not differ from controls (Woody *et al.*, 1988).

Opiate Abuse and Sensitivity to Stimulation Tests

Although information on this issue is scarce, the entire cascade leading to glucocorticoid secretion seems blunted in subjects exposed to opiate drugs. In healthy subjects morphine was observed to reduce the CRF-induced increase in plasma ACTH and cortisol levels (Rittmaster *et al.*, 1985; Allolio *et al.*, 1987). Similarly, in subjects under a long-term methadone maintenance treatment a significant blunted cortisol secretion was found after administration of 1-24αACTH (Dackis *et al.*, 1982).

Opiate Abuse and Glucocorticoid Negative Feedback

The ACTH response to metyrapone has been shown to be blunted during active cycles of heroin addiction (Kreek, 1972, 1973), during the first 3 months of the induction phase of methadone treatment (Kreek, 1987, 1996, 2000), and after longer methadone treatments up to a dose of 40 mg per day of methadone (Vescovi *et al.*, 1990). However, when higher doses of methadone are used (60–120 mg per day), a normal response to metyrapone was found (Cushman *et al.*, 1970; Kreek *et al.*, 1983). Similarly, in subjects receiving

long-term high-dose methadone, dexamethasone produced normal suppression of cortisol secretion (Kreek, 1972, 1973). These data suggest a decreased glucocorticoid feedback during exposure to opiates. However, a blunted ACTH response to metyrapone could also result from the hyposensitivity of pituitary corticotrope cells to CRF that is also induced by opiates (Rittmaster *et al.*, 1985; Allolio *et al.*, 1987). In this case, the results of the metyrapone test could just be another index of a blunted functional status of the HPA axis.

During abstinence from opiate drugs, quite the reverse results have been found. Thus, an enhanced response to metyrapone, suggesting an increased glucocorticoid feedback, has been reported in drug-free or medication-free former opiate addicts. This enhanced response seems to persist for at least one year following the last exposure to opiates (Kreek, 1987, 1996, 2000).

Opiate Abuse and Sensitivity to Stress

An inhibition of the activation of the HPA axis induced by a psychological performance stress has been found in opiate addicts who had stopped heroin use during the 12-h period preceding the study (Folli *et al.*, 1992). Although this finding could be attributed to the effect of an early withdrawal, they are more likely due to an inhibitory effects of opiate drugs on stress response. Thus, the increase in plasma cortisol levels induced by surgical stress has been found to be suppressed when morphine is administered during general anaesthesia to non-addict subjects (Reier *et al.*, 1973; George *et al.*, 1974). A blunted response to stress has also been found in opiate addicts after short- and long-term abstinence. In heroin-dependent patients tested after 14–24 days of abstinence from heavy heroin abuse (Vescovi *et al.*, 1989), the increase in ACTH secretion induced by sauna-elicited hyperthermia is suppressed. Suppression of the activation of the HPA axis by a psychological performance stress has also been described after a 4-year period of abstinence from heroin use (Mutti *et al.*, 1992). This suppression of stress-induced activation of the HPA axis is consistent with the enhanced glucocorticoid negative feedback that seems to persist after long periods of abstinence (Kreek, 1987, 1996, 2000).

HPA FUNCTION IN COCAINE-RELATED DISORDERS

Synopsis

Acute administration of cocaine increases the secretion of glucocorticoids and a reduction of this effect appears after chronic exposure to the drug. Abrupt withdrawal from protracted heavy cocaine use has been associated with an increased glucocorticoid secretion. However, after a short period of abstinence, glucocorticoid secretion normalizes. In parallel, in former cocaine addicts without comorbidity with depression, enhanced glucocorticoid negative feedback has been described after short and long periods of abstinence. Abstinent cocaine addicts also show suppression of stress-induced cortisol secretion except when a stressor inducing craving for cocaine is used.

Cocaine Abuse and the Secretion of Glucocorticoids

Acute and Chronic Exposure to Cocaine

Acute administration of cocaine has been found largely to increase cortisol levels in different populations of subjects: in normal healthy volunteers (Heesch *et al.*, 1995), in patients with concomitant opiate and cocaine dependence (Mendelson *et al.*, 1992; Teoh *et al.*, 1994) and in cocaine abusers (Wilkins *et al.*, 1992; Baumann *et al.*, 1995). Furthermore, exposure to cocaine *in utero* has also been found to elevate cortisol levels in the neonate (Scafidi *et al.*, 1996).

A certain degree of tolerance to cocaine-induced activation of the HPA axis has been reported (Mendelson *et al.*, 1998). Acute cocaine injection in cocaine- and opiate-dependent patients causes a blunted elevation in ACTH levels when compared to occasional cocaine users. Heart rate, systolic and diastolic blood pressure, perception of 'high' and 'euphoria' were also attenuated in cocaine and opiate addicts compared to episodic cocaine users.

Cocaine Withdrawal and Abstinence

Cocaine-addicted patients, tested after 24 hours of cessation from cocaine abuse, show significantly higher diurnal plasma ACTH and cortisol levels when compared to normal healthy controls (Vescovi *et al.*, 1992a). Another study, however, within the same time window, suggested no effects (Mendelson *et al.*, 1988). It is noteworthy that in the latest study no control group was included; cortisol levels were considered normal since they were within the high normal limits for healthy adult men (between 20 and $23\,\mu g\,dl^{-1}$). Nevertheless, abstinence from continuous cocaine use seems to be associated with a normalization of glucocorticoid secretion. Thus, cocaine abusers tested after 15 days of abstinence did not differ from normal volunteers for plasma ACTH and cortisol levels (Vescovi *et al.*, 1992a).

Cocaine Abuse and Glucocorticoid Negative Feedback

The dexamethasone suppression test, when performed in cocaine-dependent patients between 4 and 10 days after the last cocaine intake, revealed 42% non-suppressors, as defined by post-dexamethasone cortisol levels above $5\,\mu g\,dl^{-1}$ (Gawin and Kleber, 1985). After 4 weeks of abstinence a normal response to dexamethasone was detected (Gawin and Kleber, 1985). Resistance to dexamethasone in some cocaine-dependent patients may reflect concomitant symptoms of depression, which has been associated with non-suppression of cortisol by dexamethasone (Asnis *et al.*, 1981, 1982a, 1982b, 1986; Carroll *et al.*, 1968, 1981). Thus, in another study 37.5% of cocaine abusers who were treated with antidepressants for their self-reported dysphoric mood failed to suppress cortisol secretion after dexamethasone (Boza *et al.*, 1986). The influence of concomitant depression on the activity of the HPA axis in cocaine abusers is also suggested by the observation that the severity of depressive symptoms has been found to be positively correlated with the cocaine-induced elevation in ACTH and cortisol concentrations (Elman *et al.*, 1999).

In non-depressed cocaine-dependent subjects, conversely, there seems to be an increase in glucocorticoid feedback. Thus, cocaine addicts tested with metyrapone over a period of 1–155 days following the last cocaine exposure have shown an enhanced response of the HPA axis to metyrapone (Schluger *et al.*, 1997).

Cocaine Abuse and Sensitivity to Stress

Cocaine abusers studied after a 14-day period of abstinence from cocaine (Vescovi *et al.*, 1992b) failed to show activation of the HPA axis induced by sauna-elicited hyperthermia in healthy volunteers. Also cocaine exposure *in utero* has been found to attenuate the pituitary–adrenal axis response to stress. After an invasive stressor heel-stick procedure, cocaine-exposed premature infants were observed to exhibit lowered cortisol levels when compared to infants not exposed to cocaine (Magnano *et al.*, 1992).

Although the response to stress seems to be decreased by cocaine, an elevation in cortisol levels has been found in cocaine-dependent subjects when an acute psychological stress inducing a significant increase in craving was used (Sinha *et al.*, 1999).

HPA FUNCTION IN ALCOHOL-RELATED DISORDERS

Synopsis

Acute and chronic alcohol ingestion increases the secretion of glucocorticoids. During alcohol withdrawal, there is a further increase in cortisol concentration that rapidly normalizes if abstinence is maintained. However, one study has reported reduced concentrations of cortisol after prolonged abstinence. During early abstinence, there is also an attenuated response to CRF or stress and a blunted suppression of cortisol secretion in response to dexamethasone. More prolonged periods of abstinence appear to allow a complete normalization of the HPA axis activity. Finally, risk for alcohol abuse seems to be associated with a deregulation of the HPA axis function.

Alcohol Abuse and the Secretion of Glucocorticoids

Acute and Chronic Exposure to Alcohol

A dose-dependent increase in plasma cortisol levels has been observed in response to acute ethanol administration in normal volunteers with significant effects for plasma concentrations of ethanol above $100\,mg\,100\,ml^{-1}$ (Fazekas, 1966; Jenkins and Connolly, 1968; Merry and Marks, 1969; Bellet *et al.*, 1970; Linkola *et al.*, 1979; Välimäki *et al.*, 1984a). However, gastrointestinal symptoms after ethanol drinking may be involved in these effects of ethanol. In a study that controlled for this factor (Inder *et al.*, 1995), enhanced plasma ACTH and cortisol concentrations were found only in subjects who experienced significant gastrointestinal side effects (nausea, vomiting) after ethanol intake. This observation could explain why several studies have failed to reveal a significant effect of alcohol on cortisol secretion (Leppäluoto *et al.*, 1975; Jeffcoate *et al.*, 1980; Davis and Jeffcoate, 1983; Prinz *et al.*, 1980; Ida *et al.*, 1992; Waltman *et al.*, 1993). In normal volunteers, cortisol secretion is also increased during repeated exposure to ethanol (4-day period of ethanol intoxication) and this for ethanol blood concentrations below $100\,mg\,100\,ml^{-1}$. Also in this case, gastrointestinal symptoms were present during ethanol ingestion (Mendelson and Stein, 1966). In alcohol-dependent subjects an increase in plasma cortisol levels has been reported during drinking periods ranging from 4 to 31 days (Mendelson and Stein, 1966; Mendelson *et al.*, 1971; Stokes, 1973; Wilkins *et al.*, 1992). However, an attenuation of the stimulant effects of ethanol seems to occur after 10 days of consumption (Wilkins *et al.*, 1992). This persistent elevation in adrenocortical secretion during chronic heavy drinking of alcohol may contribute to the clinical picture of 'pseudo-Cushing's syndrome' reported in some chronic alcoholics and usually reversible after cessation of drinking (Smals *et al.*, 1977; Ylikahri *et al.*, 1980; Cobb and Van Thiel, 1982; Elias *et al.*, 1982; Kirkman and Nelson, 1988; Jeffcoate, 1993).

Alcohol Withdrawal and Abstinence

Acute alcohol withdrawal syndrome has been extensively associated with an enhanced secretion of glucocorticoids (Mendelson and Stein, 1966; Mendelson *et al.*, 1971; Bannan *et al.*, 1984; Välimaki *et al.*, 1984b; Risher-Flowers *et al.*, 1988; Bardeleben *et al.*, 1989; Adinoff *et al.*, 1991; Wilkins *et al.*, 1992). A marked rise in plasma cortisol levels has been found on the first day of withdrawal from alcohol along with suppression of the diurnal rhythm. Furthermore, plasma cortisol levels seem positively correlated with the severity of the alcohol withdrawal syndrome. Reversal of these hormonal elevations and restoration of diurnal rhythm appear during the first week of ethanol withdrawal. Normal ACTH and cortisol levels have also been reported in studies examining longer periods of abstinence (from 1 week to 6 months) (Margraf *et al.*, 1967; Bardeleben *et al.*, 1989; Adinoff *et al.*, 1990; Vescovi *et al.*, 1992c). However, one study describes a reduced cortisol secretion after 4 weeks of abstinence from alcohol (Bailly *et al.*, 1989).

Alcohol Abuse and Sensitivity to Stimulation Tests

Early abstinence from chronic alcohol exposure seems to be associated with an attenuated response of the pituitary–adrenal axis to CRF stimulation. A significantly blunted ACTH (Bardeleben *et al.*, 1989; Adinoff *et al.*, 1990; Costa *et al.*, 1996) and cortisol (Bardeleben *et al.*, 1989; Bailly *et al.*, 1989; Costa *et al.*, 1996) responses to CRF were found in alcohol-dependent subjects when tested within 12 hours to 3 weeks after the last ethanol drink. However, after a more prolonged abstinence from alcohol (from 3 weeks to 6 months) normal ACTH and cortisol responses to CRF have been observed (Adinoff *et al.*, 1990).

Alcohol Abuse and Glucocorticoid Negative Feedback

When tested during the first week of abstinence from chronic alcohol consumption, 10–50% of alcoholics did not suppress plasma cortisol in response to the standard 1 mg or 2 mg dose of dexamethasone (Oxenkrug, 1978; Swartz and Dunner, 1982; Newsom and Murray, 1983; Dackis *et al.*, 1984; Targum *et al.*, 1984; Del Porto *et al.*, 1985; Burov *et al.*, 1986; Majumdar *et al.*, 1988; Bailly *et al.*, 1989; Costa *et al.*, 1996). Association with the diagnosis of major depression, family alcoholism or disturbances of the liver's metabolic capacity has been described in some of these studies (Swartz and Dunner, 1982; Newsom and Murray, 1983; Burov *et al.*, 1986). Nevertheless, after 4 weeks of cessation of alcohol intake the response to dexamethasone seems to normalize (Newsom *et al.*, 1983; Dackis *et al.*, 1984; Del Porto *et al.*, 1985; Bailly *et al.*, 1989).

Alcohol Abuse and Sensitivity to Stress

ACTH and cortisol activation produced by various stress procedures (mental arithmetic, cold pressure, isometric handgrip task and sauna) have been found blunted after a period of cessation of alcohol drinking varying from 3 to 5 weeks (Errico *et al.*, 1993; Bernardy *et al.*, 1996; Vescovi *et al.*, 1997). However, after 12 weeks of controlled abstinence (Ehrenreich *et al.*, 1997) a normal ACTH response to stress has been reported.

HPA Axis Activity in Subjects with High and Low Risk of Alcoholism

Over the past decade, a series of studies have shown that the sons of alcoholics have a three- to four-fold elevated risk of developing alcoholism in late adolescence and adulthood (Goodwin, 1985; Schuckit, 1985). A deregulation of the pituitary–adrenal axis appears to be associated with a high risk of future development of alcoholism although the nature of this perturbation seems influenced by sex. A decreased intensity of ACTH and cortisol responses to ethanol in subjects with a positive family history (FHP) for alcoholism was found when compared with subjects with a negative family history (FHN) (Schuckit, 1984; Schuckit *et al.*, 1987, 1988, 1996; Schuckit and Gold, 1988). However, opposite findings have been described when the response to an acute ethanol challenge was examined in females (Lex *et al.*, 1991). In this pilot study, plasma cortisol levels after ethanol drinking were observed to be higher in the FHP females than in the FHN controls. In contrast, similar responses were found in male and female FHP/FHN when the effects of acute ethanol on the

secretion of the peptide β-endorphin were studied (Gianoulakis *et al.*, 1989, 1996). β-Endorphin shares with ACTH the same precursor, pro-opiomelanocortin (POMC) (Akil and Morano, 1995). In response to ethanol, there were higher β-endorphin circulating levels in both male and female FHP groups compared to the FHN control groups (Gianoulakis *et al.*, 1989, 1996). Finally, a lower response to CRF stimulation appears to be associated with a familial history of alcoholism. A blunted ACTH response to CRF in FHP compared to FHN subjects was found, although cortisol levels were comparable in the two groups (Waltman *et al.*, 1994).

HPA FUNCTION IN NICOTINE-RELATED DISORDERS

Synopsis

Acute and chronic administration of nicotine through intense cigarette smoking has been shown to increase the secretion of glucocorticoids although signs of tolerance to this effect appear over prolonged periods of cigarette smoking. Acute withdrawal from heavy cigarette smoking is associated with an increase in cortisol levels followed by a progressive decrease and normalization. However, cortisol levels seem to drop below baseline after a prolonged period of abstinence from nicotine. Dexamethasone and CRF stimulation tests seem unaltered in nicotine abusers. Controversial data exist on the effects of nicotine on stress-induced glucocorticoid secretion.

Nicotine Abuse and the Secretion of Glucocorticoids

Acute and Chronic Exposure to Nicotine

A dose-dependent increase in plasma ACTH and cortisol levels has been found after intravenous nicotine infusion in normal healthy volunteers without history of regular cigarette smoking (Newhouse *et al.*, 1990). Similarly, when examined in nicotine-addicted subjects, acute nicotine administration (mainly through smoking) was found to cause a dose-dependent elevation in plasma 11-hydroxycorticosteroid (Kershbaum *et al.*, 1968), ACTH and cortisol concentrations (Wilkins *et al.*, 1982; Seyler *et al.*, 1984; Gossain *et al.*, 1986; Meliska and Gilbert, 1991; Gilbert *et al.*, 1992; Baron *et al.*, 1995; Coiro and Vescovi, 1999). Subjective distress (nausea, sickness and unpleasantness) after smoking cigarettes containing high concentrations of nicotine has been found to be correlated with the increase in plasma cortisol concentrations (Gilbert *et al.*, 1992). Furthermore, plasma cortisol levels after cigarette smoking in laboratory conditions have been found to be significantly higher in smokers compared to non-smokers (Gossain *et al.*, 1986; Baron *et al.*, 1995), but no differences have also been reported (Meliska and Gilbert, 1991).

Although it seems clear that acute exposure to nicotine in laboratory conditions increases cortisol levels, this does not appear to be the case when smokers are studied during their usual smoking conditions. Indeed, several studies investigating cortisol concentrations in subjects who were asked to follow their habitual pattern of smoking did not find any significant increase in glucocorticoids (Tucci and Sode, 1972; Cherek *et al.*, 1982; Benowitz *et al.*, 1984; Yeh and Barbieri, 1989; Kirschbaum *et al.*, 1994). Therefore, the elevation in cortisol induced by nicotine in the laboratory setting may be the result of an interaction between nicotine and the stress of the experimental condition or the result of rapid smoking procedures. This possibility is also suggested by the absence of changes in cortisol and ACTH concentrations as a function of the dose of nicotine when the latest is delivered transdermally (Pickworth *et al.*, 1996).

Nicotine Withdrawal and Abstinence

Nicotine withdrawal seems to be associated with an enhanced adrenocortical secretion. After 24 h of abstinence, plasma cortisol concentrations were found to be higher than those observed during smoking (Hughes *et al.*, 1988). However, this increase seems short lasting since there were no significant changes in plasma ACTH and cortisol levels after 3 days of cessation from *ad lib* smoking, and this despite a significant increase in several subjective and physiological measures of the severity of nicotine abstinence (Pickworth *et al.*, 1996). After a longer period of abstinence (2–6 weeks), plasma cortisol concentrations were observed to decrease below control levels (Puddey *et al.*, 1984; Frederick *et al.*, 1998). The magnitude of the drop in cortisol was strongly related to post-quit distress (Frederick *et al.*, 1998).

Nicotine Abuse and Negative Glucocorticoid Feedback

The few studies that have analysed glucocorticoid feedback in nicotine abusers by using the dexamethasone suppression test have not found any significant modification. No changes in post-dexamethasone cortisol levels were observed after 1–2 days of abstinence when compared to the *ad lib* smoking period (Hughes *et al.*, 1988). Scores for withdrawal symptoms or heart rate were not correlated with dexamethasone-induced suppression (Hughes *et al.*, 1988). Also the percentage of cortisol suppression after dexamethasone before, and 2 or 4 weeks after, quitting smoking has not been found to be related to abstinence success (Frederick *et al.*, 1998).

Nicotine Abuse and Sensitivity to Stimulation Test and Stress

There have been few investigations on the response of the HPA axis to stimulation tests or stress in smokers. Conflicting findings have been reported. Kirshbaum *et al.* (1994) studied the response of smokers to CRF stimulation and psychological stress. Smokers exhibit a trend toward smaller ACTH and cortisol responses in both stimulation conditions. However, these results failed to reach statistical significance. In another study conducted in females, Baron *et al.* (1995) failed to reveal differences between smokers and non-smokers in CRF-induced release of cortisol. An attenuation of the response to stress by nicotine smoking is, in contrast, suggested by the study of Gilbert *et al.* (1997). These authors found no effect of a noise stress in smokers exposed to nicotine. However, in another study, Pomerleau and Pomerleau (1990) observed an additive effect of smoking and stress (competitive mental arithmetic) on plasma cortisol concentrations.

SUMMARY OF CHANGES IN ACTIVITY OF THE HPA AXIS OBSERVED DURING DIFFERENT PHASES OF DRUG ABUSE

Exposure to Drugs

The data analysed in the previous sections indicate that exposure to drugs of abuse profoundly modifies the secretion of glucocorticoids, although not in a homogeneous way. Acute exposure to cocaine, alcohol and nicotine increases the secretion of glucocorticoids. These effects persist over chronic exposure to the drugs although a partial tolerance is observed after long periods of very high drug regimens. Opposite effects have been observed for opiate drugs that reduce the secretion of glucocorticoids upon acute and chronic exposure.

Much scarcer data are available on other functional indices of the HPA axis activity during periods of drug exposure. Opiate intake or

administration of substitutive therapies has been associated with a reduced response to CRF, ACTH and stress stimulation as well as to a blunted response to metyrapone. Similar investigations conducted on nicotine addicts have failed to reveal a decrease in the response to CRF and stress in an unequivocal way. Unfortunately, there is no information available on the other drugs of abuse.

Withdrawal

During withdrawal from drug taking, more homogeneous changes in glucocorticoid secretion have been observed. Indeed, during withdrawal from opiates, cocaine, alcohol and nicotine, an increase in the level of glucocorticoids has convincingly been reported. This increase seems of longer duration for opiates than for the other drugs.

Data on other functional analyses of the activity of the HPA axis during withdrawal are scarcer. A decrease in glucocorticoid feedback, as studied with the dexamethasone suppression test, has been reported for cocaine and alcohol but there are no available data for opiates and no modifications have been reported for nicotine. For alcohol, a decrease of sensitivity to CRF stimulation has been described.

Abstinence

During abstinence from different drugs of abuse, the secretion of glucocorticoids does not seem to change in a homogeneous way. A normalization of glucocorticoid levels has been reported in former opiate and cocaine abusers. In contrast, in abstinent nicotine addicts glucocorticoid concentrations seem to decrease below baseline levels. In former alcoholics, normalization but also a decrease below baseline of glucocorticoids levels, similar to that observed for nicotine, have been described.

During abstinence from opiates, cocaine and alcohol, a reduction of the glucocorticoid response to stress has been observed. Unfortunately, we do not have available data for nicotine. This decrease in stress response, at least in former opiate and cocaine addicts, seems to be maintained even after long periods (years) of abstinence. This blunted response to stress is accompanied by an increase in glucocorticoid feedback, as suggested by results obtained using the metyrapone test in abstinent opiate and cocaine addicts. Again, we do not have available data in nicotine and alcohol addicts. Finally, stimulation tests during abstinence have been studied only in former alcohol addicts showing a decreased response to CRF that normalizes after longer period of abstinence.

POSSIBLE RELATIONSHIPS BETWEEN CHANGES IN THE ACTIVITY OF THE HPA AXIS AND DRUG ABUSE

Changes in Glucocorticoid Secretion During Drug Exposure

Although most of the drugs of abuse increase the secretion of glucocorticoids, it is unlikely that these changes can be involved in mediating the positive reinforcing effects of drugs. First, this idea is not supported by the observation that opiates decrease glucocorticoid secretion. Second, in the case of psychostimulant drugs or alcohol, which increase glucocorticoid levels, research in animals does not suggest that drug-induced glucocorticoid secretion plays a role in mediating the behavioural effects of drugs. Thus, although behavioural effects of drugs are reduced by the suppression of endogenous glucocorticoids, most of them are completely restored by replacement of basal glucocorticoid levels or by levels of glucocorticoids that are lower than that observed after drug administration (for review see Piazza and Le Moal, 1996, 1997). Finally, biological effects of glucocorticoids are principally mediated by modifications of protein transcription which occur in a time window that is much longer than the one within which the rapid reinforcing effects of drugs take place. This idea is also supported by a recent observation in humans (Wachtel *et al.*, 2001) showing that an increase in glucocorticoid levels before the administration of psychostimulant drug does not modify its subjective effects.

Drug-induced secretion of glucocorticoids could instead be involved in the mediation of some drug effects that appear after repeated exposure, such as sensitization (Robinson and Berridge, 2001). Thus, it has been shown that behavioural sensitization, induced by drugs and in particular by stress, is at least in part dependent on an increase in glucocorticoid secretion (for review see Piazza and Le Moal, 1998). For example, repeated treatment with glucocorticoids can increase the behavioural response to psychostimulants or morphine (Deroche *et al.*, 1992; Stohr *et al.*, 1999). Furthermore, blockade of stress-induced glucocorticoid secretion totally suppresses the stress-induced sensitization (for review see Piazza and Le Moal, 1998). A sensitized state can also be reversed by the administration of glucocorticoid antagonist (De Vries *et al.*, 1996) or by synthesis inhibitors such as metyrapone (Marinelli *et al.*, 1996).

Changes in Glucocorticoid Secretion During Withdrawal

The increase in glucocorticoids observed during withdrawal could have two effects. The first one could be to counteract the negative state associated with withdrawal. Thus, it has been shown that glucocorticoids have positive reinforcing effects that could at least partially substitute for that of drugs (for review see Piazza and Le Moal, 1997). The second potential effect of an increase in glucocorticoid concentrations during withdrawal could be to increase drug craving. Thus, it has been shown that injections of glucocorticoids can reinstate drug seeking in animals (Deroche *et al.*, 1997). This effect is dose dependent, and shows an inverted U-shaped curve. Up to doses of corticosterone that induce plasma levels of the hormones similar to that observed during stress, an increase in reinstatement is produced. However, when doses of glucocorticoids are above the physiological range, a dose-dependent decrease in reinstatement is observed (Deroche *et al.*, 1997).

Changes in Glucocorticoid Secretion During Abstinence

Although changes during abstinence could seem disparate, they can be interpreted as an increase in the central effects of glucocorticoids. Indeed, an increase in the central effects of these hormones is consistent with: (1) a decrease of basal glucocorticoid secretion, which has been reported for alcohol and nicotine; (2) an increase in glucocorticoid negative feedback, which has been reported for cocaine and opiate; (3) a blunted response to stress, which has been reported for cocaine, opiate and alcohol.

A higher sensitivity to the central effects of glucocorticoids in former drug abusers could be a long-lasting modification induced by prolonged exposure to drugs or a pre-existing phenotype related to vulnerability to drugs. Data from animal research support the latest hypothesis. Studies in rats that differ for vulnerability to drugs (for review see Piazza and Le Moal, 1996) have shown that drug-vulnerable subjects (HR) have a higher sensitivity to glucocorticoids than more drug-resistant individuals (LR) (Piazza *et al.*, 1993, 1996). Thus, before any exposure to drugs, HR animals are more sensitive to the reinforcing and stimulant effects of glucocorticoids and show a higher release of dopamine in the nucleus accumbens in response to the administration of these hormones (Piazza *et al.*, 1993, 1996). It is important to remember that the mesoaccumbens

dopamine system is considered one of the major substrates of the addictive effects of drugs of abuse.

Therapeutic Implications

On the basis of the data and hypothesis described above, it is suggested that pharmacological strategies modifying the activity of glucocorticoid hormones could be a useful companion for the treatment of drug abuse. Given the scarcity of available treatments, this hypothesis needs to be taken into account. Two opposite therapeutic strategies could be foreseen. The first strategy could consist in using glucocorticoid agonists during withdrawal. Indeed, the data analysed above suggest that relatively high doses of glucocorticoid agonists could have a double effect. These drugs, by their positive reinforcing effects (Piazza *et al.*, 1993), could help to reduce the negative state associated with withdrawal and at the same time, by bringing glucocorticoid levels above the physiological range, they could also reduce drug craving (Deroche *et al.*, 1997). The second strategy could consist in using antagonists of glucocorticoid receptors during abstinence. These antagonists could help in preventing relapse by two action mechanisms. First, they could counteract the high sensitivity to glucocorticoids observed in former drug abusers and that probably contributes to their vulnerability to drugs. Second, they could prevent the relapse that seems to be prompted by an increase in glucocorticoid levels (Deroche *et al.*, 1997).

Two studies have analysed the effects of manipulations of the activity of the HPA axis on cocaine self-administration in humans. The results are not encouraging since both the blocker of cortisol synthesis ketoconazole and the glucocorticoid agonist dexamethasone have shown no effects (Ward *et al.*, 1998, 1999). However, some caution should be used in the interpretation of these negative results. First, the treatments were short lasting. In fact, since glucocorticoids mainly act through modification of transcriptional activity, it is probable that longer treatments are needed to obtain an effect. Second, ketoconazole and dexamethasone were tested under an active phase of drug taking and using a schedule of self-administration that allows the subject to readily obtain high drug doses. On the basis of results obtained in animals, it is unlikely that blockade of glucocorticoid secretion or glucocorticoid agonists can have effects under such self-administration schedule. Indeed, animals totally deprived of glucocorticoids still self-administer cocaine (Deroche *et al.*, 1997). What seems to be reduced by manipulations of glucocorticoid levels is the amount of work the animal is ready to do to obtain drugs (for review see Piazza and Le Moal, 1997). In other words, the motivation to seek drugs seems to be reduced. For this reason, it is not surprising that in conditions in which the drug is readily available manipulations of the HPA axis do not show significant effects. These treatments, which could decrease the motivation to seek drugs and the probability of relapse, have a higher chance of being effective in a phase in which the individual is actively trying to discontinue drug intake.

FUTURE DIRECTIONS

In our opinion, one of the most interesting avenues for future investigations is to study more deeply if in addicts there is an increase in the central effects of glucocorticoids. In parallel, it would be important to study in animals the molecular mechanisms of such an enhanced functional state of the HPA axis. In humans, this can be done by using the metyrapone test but also by using a modified version of the dexamethasone suppression test. This test is currently used principally to investigate a decrease in glucocorticoid negative feedback. For this purpose, it is performed by giving high dexamethasone doses and measuring the lack of suppression. The opposite strategy should be used in order to search for an increase in glucocorticoid feedback. In this case, low doses of dexamethasone should be used in order to evaluate whether certain subjects suppress endogenous glucocorticoids more readily than others. A lower threshold for suppression would certainly be convincing evidence of a higher sensitivity to glucocorticoid central effects. Animal studies on hypersensitivity to glucocorticoids will certainly benefit from new transgenic approaches. In particular, the development of reporter animals enabling measurement of changes in the transcriptional activity of the glucocorticoid receptor will be very important in order to identify the anatomical and cellular targets of such a phenomenon.

Finally, there has not been an active effort to develop agonists or antagonists of the glucocorticoid receptors that act efficiently at the level of the central nervous system. This development is certainly a priority if we want to analyse the possible relevance of manipulations of the activity of the HPA axis for the treatment of drug abuse.

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XVI-4

Neuroimmunology

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Bidirectional communication of the immune system between the central nervous system (CNS) and the periphery appears to be essential for appropriate antigen response and restoration of homeostasis. Immune activation induced by bacteria and virus proteins is associated with the production of various cytokines from monocytes and macrophages. These cytokines activate immune cells and regulate the production of glucocorticoids and β-endorphin from the hypothalamic–pituitary–adrenal (HPA) axis, may activate the autonomic nervous system (ANS) or may influence the CNS. Stimuli that alter this communication can potentially threaten homeostasis (Sarkar, 1996). Drugs represent a threat to this system because on one hand they can alter the activity of the neuronal circuitries that are important for appropriate immune responses, and on the other they may expose the individual to other risks, e.g. subsequent infections or organ dysfunction, which may impose secondary immune changes and interfere with different neuronal pathways. This may also limit the interpretation, in particular of the central effects of all drugs as receptor subtypes, in many different neuronal tissues are usually involved in the immune response. Previous studies reflect the net effects of global receptor activation. However, compensatory or synergistic effects may cloud the interpretation of specific tissue involvement in the drug modulation of these immune parameters.

ALCOHOL

Clinical Relevance

Alcohol is the most abused drug worldwide (Lieber, 1995). Alcohol-associated health problems are a major social and medical problem in the United States and other Western countries. In the United States, over 50% of adults consume alcohol regularly, and approximately 15 million people are chronic alcoholics (Lieber, 1995; O'Connor and Schottenfeld, 1998). In a recent survey of the Substance Abuse and Mental Health Services Administration in the United States, heavy drinking was reported by 5.6% of the population aged 12 and older, or 12.6 million people (Substance Abuse and Mental Health Services Administration, 2001). Alcoholism has been associated with over 100 000 deaths in the United States and results in more than $100 billion in annual cost (Secretary of Health and Human Services, 1997).

Immune Modulation

Alcohol has profound effects on the expression and function of immune system mediators and the brain. Among the most intensely investigated cytokines related to alcohol are tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, which are collectively grouped as pro-inflammatory cytokines. Most of our understanding about the impact of alcohol on these mediators comes from studies of the peripheral immune system, where cytokines play a pivotal role in the regulation of immune and inflammatory responses. At present much less is known about how ethanol affects the protein networks in the brain. Alcohol freely enters the brain and it is likely to have similar effects on the function of cytokines in the CNS as it does on those in the periphery. However, in the brain, cytokines impart their effect via interactions with different target cells such as astrocytes and neurones, as well as through novel mechanisms such as modulating neurotransmitter receptor function (Gahring *et al.*, 1999, 1997; Miller and Fahey, 1994; Miller *et al.*, 1991). Therefore, cytokine-mediated effects must be evaluated in terms of their function within the CNS which, overall, may not be equivalent to those in the periphery.

Alcohol and Lipopolysaccharide

Heavy alcohol use results in endotoxaemia, which is a systemic inflammatory response caused by the interaction of intestinal-derived endotoxin/lipopolysaccharide (LPS) with Kupffer cells and other cells in the liver (Fleming *et al.*, 2001; Lands, 1995; Bhagwandeen *et al.*, 1987; Bode *et al.*, 1987). Experimentally, LPS treatment increased adrenocorticotropic hormone (ACTH) and corticosterone levels both in alcohol- and non-alcohol-treated rats, but corticosterone levels were significantly higher in alcohol-treated rats (Rivier, 1999).

A gut leak was observed in chronic alcoholics upon examination of gastroduodenal and intestinal permeability through the measurement of the urinary excretion of sucrose, after oral administration of lactulose and mannitol (Keshavarzian *et al.*, 1999). Endotoxin concentrations in the plasma of alcoholics were more than five-fold increased as compared to non-alcoholic controls (Parlesak *et al.*, 2000).

Pro-/Anti-Inflammatory Immune Modulation

Rapid changes in the production of several cytokines can be evoked by acute exposure to alcohol in LPS-stimulated rats (Nelson *et al.*, 1995) and in normal isolated human monocytes (Mandrekar *et al.*, 1996; Szabo *et al.*, 1996a). Pro-inflammatory cytokines such as TNF-α and IL-6 send important signals, resulting in peripheral immune stimulation of the brain. Specifically, their ability to increase the neuronal activity of hypothalamic perikarya involved in the release of ACTH and corticosteroids (Tilders *et al.*, 1994; Rivier, 1995; Chrousos, 1995; Ericsson *et al.*, 1994; Rivier, 1999) appears essential in mediating an appropriate endocrine response to immune challenges (Turnbull and Rivier, 1999). At present, there is still a fair amount of controversy regarding the role of specific cytokines involved in the HPA axis response to immune signals.

Regardless of the mechanism involved, the ability of alcohol to alter the HPA axis response to central cytokines has important

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implications. While originally only thought to occur following CNS infections or trauma, increases in brain cytokines are now known to take place in response to stress (Minami *et al.*, 1991) or during periods of elevation in blood-borne cytokines (Lee and Rivier, 1994; vanDam *et al.*, 1992; Schoebitz *et al.*, 1993; Hagan *et al.*, 1993; Fontana *et al.*, 1984), such as those that occur during immune activation (Lee and Rivier, 1994; Shalaby *et al.*, 1989; Ertel *et al.*, 1991; Bristow *et al.*, 1991; Dinarello and Cannon, 1993). Brain cytokines probably play an important role in regulating the activity of neuronal circuits in subjects whose homeostasis is threatened. Indeed the appropriate secretion of corticotropin-releasing factor (CRF), ACTH and corticosteroids upon exposure to cytokines plays a necessary role in the mechanisms that mediate the survival of an organism exposed to an antigen, which includes monitoring the number and activity of immune cells (Munck and Guyre, 1986; Irwin and Hauger, 1988; Irwin *et al.*, 1990).

TNF-α

Because TNF-α is the first such protein to be released during endotoxaemia (Rivier, 1999), it has been considered important in modulating at least the early phase of LPS-induced ACTH release (Ebisui *et al.*, 1994), most likely through an increased release of CRF by nerve terminals in the median eminence (Watanobe and Taskebe, 1992). However, studies conducted with TNF-α antagonists have not always provided clear results, which has raised the possibility that secretagogues such as prostaglandins, produced independently of TNF-α, might also play a predominant role (Rivier, 1999).

TNF-α provides a particularly striking example of the potential contrast between the function of a cytokine in the periphery versus the CNS. In the periphery, engagement of the TNFR1/p55 receptor by TNF-α often results in cell death through apoptosis (Ware *et al.*, 1996). In the brain, however, under non-chronic pathological conditions, it was demonstrated that TNF-α functions as a neuroprotective agent (Gahring *et al.*, 1999). TNF-α mediates its neuroprotective effect against an excitotoxic challenge by glutamate receptor agonists such as *N*-methyl-D-aspartate (NMDA) primarily through the TNFR1/p55 receptor, which is present on neurones both *in vivo* and *in vitro*. Exposure to the equivalent of an intoxicating level of alcohol on cultured murine cortical neurones did not quantitatively alter NMDA-excitotoxic neuronal death. However, it inhibited the neuroprotective properties of TNF-α, suggesting that the effect of ethanol at this concentration is specific to the TNF-α pathway and not due to altered NMDA receptor function in this excitotoxicity assay. This was further supported by the fact that neuroprotection against NMDA induced by IL-1α IL-1β, IL-6 or nicotine was not affected by the presence of 20 mM alcohol (Gahring *et al.*, 1999). However, alcohol can inhibit NMDA receptors at 100 mM concentrations (Gahring *et al.*, 1999). At these concentrations the addition of human tumour necrosis factor (hTNF)-α had no further neuroprotective effect nor did it antagonize the ethanol-induced neuroprotection (Gahring *et al.*, 1999).

The nuclear factor κB (NFκB) is a major intracellular mediator of TNF-α-induced effects. Inhibition of the NFκB translocation to the nucleus has been reported to inhibit TNF-α-induced neuronal protection (Barger *et al.*, 1995). The effects of alcohol on NFκB translocation and the composition of the complex (p50/p65 heterodimers vs. p50 and p52 homodimers) have been investigated (Mandrekar *et al.*, 1997). Alcohol (25 mM) induced the activation of p50/p50 homodimers that suppressed the induction of cytokine transcripts.

Events downstream of TNF-α-mediated activation of NFκB, including the production of nitric oxide (NO), may also be affected by the presence of alcohol. Chronic alcohol exposure seems to enhance NMDA-mediated increases in NO production by neurones, but in contrast greatly reduced NO synthesis by glial cells (Syapin, 1998). Furthermore, acute alcohol treatment decreased cytokine-stimulated NO synthesis in glia and neurones in cortical cell cultures but enhanced cytokine-stimulated NO synthesis in other cells such as the endothelial cells lining the blood–brain barrier (Syapin, 1998). In C6 glial cells, ethanol also suppressed cytokine-induced inducible nitric oxide synthase (iNOS) expression and reduced IL-1β and TNF-α potency without affecting interferon (IFN)-γ potency. Ethanol-mediated reductions in cytokine-induced iNOS messenger ribonucleic acid (mRNA) and immunoreactive protein levels suggested an effect on gene transcription. IFN-γ-responsive elements did not participate in the acute ethanol-induced inhibition of rat iNOS gene transcription (Syapin *et al.*, 2001). In a previous report supporting these results it was found that ethanol markedly blocked the TNF-α-induced increase in intercellular adhesion molecule (ICAM)-1 and mRNA levels, whereas IFN-γ did not inhibit this response (DeVito *et al.*, 2000).

Several studies showed that polymorphism in TNF-α and TNF-β genes affect TNF-α and TNF-β protein production *in vivo* (Messer *et al.*, 1991; Pociot *et al.*, 1993). Individuals who carry $TNFB^2/TNFB^2$ have a higher TNF-α secretory capacity than those who carry the $TNFB^1/TNFB^1$ genotype. According the reports of Bruce *et al.* (1996), who showed that neurones from TNF receptor knock-out mice exhibited reduced survival in culture compared to those from wild-type mice, individuals with $TNFB^1/TNFB^1$ may be susceptible to alcohol-induced brain damage. A multivariate analysis showed that the $TNFB^1/TNFB^1$ genotype was an independent risk factor for the development of alcoholic brain atrophy (Yamauchi *et al.*, 2001).

IL-1β

The cytokine IL-1β increases ACTH release by stimulating the synthesis of CRF and vasopressin in the paraventricular nucleus (PVN) of the hypothalamus and the release of these peptides from nerve terminals in the median eminence (Rivier, 1995, 1996). Alcohol, like IL-1β, stimulates ACTH secretion by activating the synthesis and release of CRF and vasopressin in the PVN neurones in adult rats. Not surprisingly, it was found that alcohol altered IL-1β-stimulated ACTH secretion (Rivier, 1996). However, alcohol effects were dependent on the dose and duration of alcohol treatments. Acute treatment with high doses of ethanol, but not low doses, blunted IL-1β-stimulated ACTH release through increased glucocorticoid feedback (Rivier, 1993). Long-term treatment with ethanol using an ethanol liquid diet also decreased the ACTH response to IL-1β (Lee and Rivier, 1993), possibly by reducing PVN neuronal activity and by blunting pituitary ACTH response to vasopressin (Lee and Rivier, 1995). Acute ethanol exposure has been shown to reduce the IL-1β inhibitory influence on β-endorphin release, while chronic exposure potentiated the cytokine action on β-endorphin release (Sarkar, 1996). Furthermore, the cytokine action on β-endorphin release may be regulated by endogenous NO. The ability of NO blockers to prevent IL-1β action on β-endorphin release was increased following acute treatment with ethanol, but was reduced following long-term treatment with ethanol (Sarkar, 1996).

IL-6

Another important pro-inflammatory cytokine is IL-6, which, despite the fact that it is usually secreted after the initial peak ACTH secretion has taken place (Besedovsky and Del Rey, 1991), also plays a role in the HPA axis response to immune stimulation (Perlstein *et al.*, 1993). After LPS treatment, IL-6 is released into the general circulation but it is also manufactured in tissues such as the pituitary and adrenal glands, where it may play paracrine/autocrine roles in regulating ACTH and corticosterone secretion (Rivier,

1999). On the basis of these data, it seems probable that circulating TNF-α and IL-6 play different roles at different times, and that their appearance in various tissues supports a participatory role in the endocrine responses to endotoxaemia (Rivier, 1999). In a study by Rivier (1999) in intact rats, the alcohol-induced plasma ACTH and corticosterone responses had returned to basal levels 3 h after LPS was injected, and alcohol pretreatment increased the corticosterone but not the ACTH response after LPS treatment. In contrast, in adrenalectomized corticosterone-replaced animals the alcohol-induced ACTH response was still elevated at the time of LPS injection. However, the overall ACTH response of rats pretreated with alcohol was statistically similar. As expected, LPS also significantly stimulated both TNF-α and IL-6 release into the general circulation. The IL-6, but not the TNF-α, response was inhibited by alcohol pretreatment in intact rats, a phenomenon that was not present in adrenalectomized rats. Finally, LPS also augmented the TNF-α and/or IL-6 content of the pituitary, adrenal glands and spleen, and it was shown that these responses were not altered by alcohol pretreatment. Therefore, it was concluded that acute alcohol treatment increased LPS-induced corticosterone response, while it blunted the IL-6 response. LPS also significantly elevated the pituitary, adrenal and splenic content of TNF-α and IL-6, and alcohol did not influence these changes (Rivier, 1999).

IL-10

The balance between the monocyte production of cytokines IL-12 and IL-10 was determined in the presence and absence of both alcohol and LPS. Alcohol alone, and in combination with LPS, stimulates monocyte production of IL-10 at the expense of IL-12 and TNF-α monocytes (Mandrekar *et al.*, 1996; Szabo *et al.*, 1996a). These results are very important for understanding the immune response in alcoholics since IL-10 inhibits some of the cellular immune reactions that are dependent on IL-12 for their initiation and continuation. These observations, including *in vitro* exposure and/or alcohol feeding, suggest that alcohol enhances IL-10 production and decreases IL-12 secretion (Irwin and Miller, 2000; Szabo *et al.*, 1996b; Waltenbaugh *et al.*, 1998; Zisman *et al.*, 1998; Girouard *et al.*, 1998).

IL-12

Shortly summarized, IL-12 production was increased or unchanged after acute *in vitro* ethanol treatment of human monocytes (Girouard *et al.*, 1998), was increased after alcohol challenge in human volunteers (Szabo, 1998) and in the serum of alcoholics without cirrhosis (Laso *et al.*, 1998), and was decreased after physiological ethanol doses in splenic cell cultures in mice (Friedman, 1998) and in ethanol-fed mice (Waltenbaugh *et al.*, 1998; Zisman *et al.*, 1998). The variability in results makes it unclear whether the immune alterations associated with alcoholism occur with relatively uniform risk or tend to affect individuals whose susceptibility is higher than average (Irwin and Miller, 2000).

Th1/Th2 Subsets

The specific effects of ethanol on T helper cell (Th)1 and Th2 cytokines are still unclear (Irwin and Miller, 2000). An increase in Th2 responses was reported in alcohol-dependent rats (Na *et al.*, 1997), whereas an increase in Th2 responses and a decrease in Th1 responses was observed in mouse models (Waltenbaugh *et al.*, 1998; Zisman *et al.*, 1998). It has been shown that alcohol enhances IL-10 production and decreases IL-12 secretion, therefore shifting the Th1/Th2 ratio towards a decreased cellular immune response (Starkenburg *et al.*, 2001; Irwin and Miller, 2000; Szabo *et al.*, 1996; Waltenbaugh *et al.*, 1998; Zisman *et al.*, 1998; Girouard *et al.*, 1998, Cohen *et al.*, 1995). Yet in contrast, Laso *et al.* (1998, 1999) found an increased Th1 response and a decreased Th2 response.

In chronic alcoholics an increased number of Cluster of differentiation (CD)4+ T cells ('helper cells'), CD8+ T cells ('cytotoxic and suppressor cells'), and natural killer (NK) cells (Laso *et al.*, 1996a, 1997) were found. The ratio of CD4+ T cells and CD8+ T cells was normal or elevated (Cook, 1998). Cook *et al.* (1995) demonstrated an increased expression of CD57 in alcoholics. CD57 is commonly associated on both CD8+ T cells and in CD4+ T cells during chronic increased antigenic exposure (Cook *et al.*, 1995). Cook *et al.* concluded that these and other T cell alterations in alcoholics are cytokine driven in part, and result in T cell differentiation states that are functionally inappropriate. It was reported recently that IL-6 derived from antigen-presenting cells is able to polarize naive Th cells into effector Th2 cells by inducing the initial production of IL-4 in CD4 T cells (Rincon *et al.*, 1997). The inducing effect of IL-4 is dominant such that if IL-4 levels reach a necessary threshold, then differentiation of the T cells to the Th2 subset occurs (Romagnani, 1997).

B Cells

Investigations into the B cell count in chronic alcoholics have not yielded uniform results. The B cell count in patients without liver diseases tends to be normal (Cook *et al.*, 1996) or slightly reduced (Sacanella *et al.*, 1998). In chronic alcoholics the major classes of immunoglobulins (Ig), IgA, IgG and IgM, may all be elevated (Cook, 1998). Typically, IgA is also increased in alcoholics without liver disease, and tissue deposits of IgA are often observed, especially in skin, liver and kidney (Paronetto, 1993; Cook, 1998). Alcoholic patients with elevated immunoglobulin levels are often immunodeficient (Cook, 1998). It was also shown that ethanol exposure reduced B cells more than T cells in the spleen of normal adult mice (Meadows *et al.*, 1992). Serum IgE was found to be increased in alcoholic patients compared to healthy volunteers (Gonzalez-Quintela *et al.*, 1999). Acute ethanol administration decreases the basal level of major histocompatibility complex (MHC) class Ia expression on murine splenic B cells by a glucocorticoid mechanism, as well in immunized as in non-immunized mice (Weiss *et al.*, 1996, 1998). Reduced expression of Ia decreases Th-dependent responses to antigen (Bekkhoucha *et al.*, 1984; Matis *et al.*, 1983). Short-term ethanol administration provoked a decrease in the total number of T and B cells in the Peyer's patches, but changes were detected either in the total number of cells or in the absolute number of T and B cells studied in the mesenteric lymph nodes (Lopez *et al.*, 1997). Long-term ethanol administration decreased the number of T and B cells in the Peyer's patches but not in the mesenteric lymph nodes (Lopez *et al.*, 1997). The authors concluded that ethanol impaired the mucosal immune system at the level of the Peyer's patches, which is the site for antigen presentation and, therefore, for the induction of a mucosal immune response (Lopez *et al.*, 1997).

Prenatal Ethanol Exposure

Maternal ethanol consumption impacts the endocrine and immune functions of the offspring (Sarkar, 1996). The maternal HPA axis appears important for the development of immunocompetency in 'Fetal Alcohol Effects' (FAE) animals, since maternal adrenalectomy abolished the suppression of T cell-dependent function in the male offspring (Redei *et al.*, 1993). These data also support the notion that alcohol-induced changes in the mother's adrenal function produce an altered thymic development in the foetus, leading to immune suppression in the offspring (Sarkar, 1996).

FAE animals have an HPA hyper-responsiveness to immune signals, in contrast to adult rats exposed to ethanol. The enhanced

HPA response in these animals may be related to the increased ability of PVN neurones to secrete CRF and vasopressin in response to IL-1β challenge (Lee *et al.*, 1996). FAE animals have a reduced lymphocyte proliferative response to concanavalin A (ConA) and a reduced lymphoblast response to IL-2. Importantly, FAE males showed more vulnerability to the adverse effects of foetal ethanol exposure on immune function (Weinberg and Peterson, 1991). In a separate study, the determination of immune responsiveness (lymphocyte proliferation to mitogens ConA and pokeweed mitogen) following a chronic metabolic stressor (1 day of cold exposure) revealed that FAE females also had an altered immune response (Giberson and Weinberg, 1995).

FAE attenuates the febrile response to the systemic administration of an endotoxin, and the febrile and anorectic responses to IL-1 (Yirmiya *et al.*, 1993, 1996). Hypothalamic levels of IL-1 after intraperitoneal administration of LPS and fever induced by intracerebroventricular IL-1 was significantly lower in FAE rats (Taylor *et al.*, 1999). Whereas FAE does not affect central thermoregulatory mechanisms *per se*, FAE alters the kinetics of hypothalamic IL-1 production/appearance and decreases the responsiveness of central mechanisms which mediate the febrile response to IL-1 (Taylor *et al.*, 1999).

Phenotypic analysis of spleen and bone marrow cells, taken between 1 and 5 weeks after birth from animals exposed to ethanol *in utero*, has shown quantitative alterations in the developmental intermediates in the B cell lineage. Cells from the ethanol-exposed animals showed decreased numbers of pre-B cells in the bone marrow and total B cell counts in both the bone marrow and spleen were also low (Moscatello *et al.*, 1999). B cell numbers were reduced as well in ethanol-exposed neonatal mice (Giberson and Blakley, 1994).

Alcohol and Liver Disease

Clinical Relevance

It was suggested that host or environmental factors may play a role in the genesis of alcohol-related liver disease (Tilg and Diehl, 2000). Alcohol can injure the liver even in the absence of dietary deficiencies because of its intrinsic toxicity (Lieber, 1995), and long-term consumption of alcohol results in a spectrum of liver abnormalities (MacSween and Burt, 1986). Despite the possible mild systemic effects of alcohol, severe effects may be seen resulting primarily from a complex syndrome called alcoholic liver disease (ALD), which has three increasingly destructive forms: steatosis (fatty liver), alcoholic hepatitis (AH) and cirrhosis (AC) (Fleming *et al.*, 2001). Steatosis can be followed by early fibrosis, which in turn can be associated with AH, leading to irreversible damage caused by severe fibrosis and, subsequently, to cirrhosis (Lieber, 1995). Alterations in the immune system occur in ALD (Paronetto, 1993). Whether these alterations are a consequence or a cause of the liver injury has yet to be determined (Lieber, 1995).

Alcohol and LPS/Intestinal Permeability

Tilg and Diehl (2000) concluded that intestinally derived endotoxin and endotoxin-induced cytokines play an important role in the pathogenesis of steatohepatitis because it was observed that long-term alcohol abuse increases intestinal permeability (Fukui *et al.*, 1991), and that patients with the highest serum cytokine concentration had the highest rate of in-hospital mortality (Felver *et al.*, 1990; Hill *et al.*, 1992). LPS derived from the cell walls of bacteria commonly found in the intestine is a well-known and powerful activator of many immune system cells. It could be suggested that Kupffer cell activation and liver damage in chronic alcoholics are accentuated by the interaction of alcohol and this bacterial product (Schenker and Bay, 1995; Cook, 1998). Therefore, the gut leak was suggested as a necessary cofactor for development of ALD in chronic alcoholics (Keshavarzian *et al.*, 1999).

Pro-/Anti-Inflammatory Immune Modulation

The production of TNF-α is one of the earliest events in many types of liver injury. It triggers the production of other cytokines that together recruit inflammatory cells, kill hepatocytes, and initiate a healing response including fibrinogenesis (Tilg and Diehl, 2000). Comparing TNF-α response in patients with alcoholic fatty liver (AF), AH, AC and healthy controls after LPS stimulation of monocytes, only patients with AC had elevated TNF-α plasma levels, and the release of IL-6 from monocytes was increased only in AF (Schafer *et al.*, 1995). Regional and systemic production of TNF-α and other pro-inflammatory cytokines is stimulated by the translocation of bacterial products from the intestinal lumen to the mesenteric circulation and its lymphatics (Tilg and Diehl, 2000). Initially increased serum levels of TNF-α (Bird *et al.*, 1990; Felver *et al.*, 1990) as well as several TNF-inducible cytokines, such as IL-1 (McClain *et al.*, 1986), IL-6 (Hill *et al.*, 1992) and IL-8 (Sheron *et al.*, 1993; Hill *et al.*, 1993), were reported in hospitalized patients with AH. Because TNF-α is toxic to many cells, and is known to induce apoptosis, it is thought that excessive secretion of TNF-α by monocytes and Kupffer cells contributes to the death of cells such as liver cells (Cook, 1998).

Patients with severe acute AH have a worse outcome when they have elevated TNF-α serum levels (Bird *et al.*, 1990; Felver *et al.*, 1990). The presence of elevated TNF-α was more significantly associated with death than recovery (Bird *et al.*, 1990; Felver *et al.*, 1990). IL-6 levels showed independent associations with the presence of ascites, encephalopathy and low mean arterial pressure (Genesca *et al.*, 1999). Neopterin and IL-8 plasma levels were raised in patients with alcohol-induced cirrhosis and were predictive of mortality associated with infections and upper gastrointestinal bleeding, respectively (Homann *et al.*, 2000).

Th1/Th2 Subsets

The balance of Th1/Th2 in alcoholics suffering from late-stage chronic cirrhosis may be different from alcoholics during an early episode of acute AH, or during an episode of infection due to immune deficiency (Cook, 1998).

The increased production of both IL-12 and Th-1-associated cytokines and the expansion of the peripheral blood cytotoxic cell compartment can contribute to the understanding of the pathogenetic mechanisms causing ALD, since self-reactive clones of cytotoxic cells may contribute to hepatocyte necrosis (Laso *et al.*, 1999).

B Cells

In ALD B cell numbers are often decreased, despite the fact that they produce abnormally large amounts of immunoglobulins (Cook, 1996, 1998; Laso *et al.*, 1996a). Typically, IgA is increased in alcoholics without liver disease as well as in patients with ALD. IgG is elevated in ALD, and IgM is increased only in patients with ALD and active disease, such as AH (Cook, 1998).

NK Cells

Various studies on NK lymphocytes in chronic alcoholics have yielded different results. In contrast to the findings of Laso and colleagues (1996a, 1997), who demonstrated a significant increase of NK in patients with alcoholic hepatitis and cirrhosis, Cook *et al.*

(1997) observed a reduced NK count and loss of NK activity in some patients with ALD.

Alcohol and Hepatic Encephalopathy

Liver diseases, and in particular alcoholic liver diseases, are conditions characterized by high circulating levels of cytokines, namely IL-1, IL-6 and TNF-α. Interactions between cytokines and CNS endothelial cells may trigger a cascade of events including enhanced blood–brain barrier permeability, brain oedema, astrocyte alterations and gliosis. Cytokine-induced production of nitrogen-reactive molecules by endothelial cells may also lead to further cellular damage and neuronal dysfunction (Duchini, 1996). Brain tissue has a remarkable ability to accumulate glutamate. This ability is due to transporter proteins present in the plasma membranes of both glial cells and neurones. The transporter proteins represent the only significant mechanism for removal of glutamate from the extracellular fluid (Danbolt, 2001). TNF-α- and IL-1β-potentiated glutamate-mediated oxidative stress results in a decrease in glutamate transporter activity (Liao and Chen, 2001). During hepatic encephalopathy ammonium ions penetrate the blood–brain barrier, where an astroglia-specific enzyme, glutamine synthetase, catalyses the formation of glutamine from glutamate. In hepatic encephalopathy, the osmotic action of glutamine appears to be largely responsible for cerebral oedema, oedema-associated disturbances of cerebral blood flow and ionic homeostasis. In chronic hepatic encephalopathy not accompanied by cerebral oedema, glutamine contributes to the impairment of cerebral energy metabolism. The increased transport of glutamine from the brain to the periphery accelerates the blood-to-brain transport of aromatic amino acids, resulting in the conversion of tryptophan to metabolites which are directly implicated in hepatic encephalopathy (Albrecht and Dolinska, 2001). Tryptophan was also shown to be increased before ethanol withdrawal occurred (Badawy *et al.*, 1998). The metabolism of tryptophan to quinolinic acid is activated by the pro-inflammatory cytokine TNF-α (Fuegener *et al.*, 2001). Therefore, pro-inflammatory cytokines due to liver disease, as well as due to infections, may make patients more prone to, or may further aggravate, encephalopathy.

Alcohol and Traumatic Stress

Clinical Relevance

Chronic alcohol misuse is at least as common in surgical patients as in neurological or psychiatric patients (Moore *et al.*, 1989; Spies *et al.*, 2001). The rate of infections after surgery is increased three to four times in chronic alcoholics compared to non-alcoholic patients (Spies *et al.*, 1996; Tønnesen and Kehlet, 1999) Wound infections, urinary tract infections, tracheobronchitis and pneumonia are two to four times more frequent among alcoholics compared with controls (Spies *et al.*, 2001; Rantala *et al.*, 1997; Tønnesen and Kehlet, 1999). Due to the increased post-operative complications in chronic alcoholics, the median hospital stay is significantly prolonged (Spies *et al.*, 1995; Jensen *et al.*, 1988).

Preoperative immune suppression and altered response to surgical stress were found in patients with chronic alcohol abuse (Spies *et al.*, 2001; Spies and Rommelspacher, 1999; Tønnesen, 1999; Tønnesen and Kehlet, 1999; Tønnesen *et al.*, 1992). The rate of post-operative infections in chronic alcoholics may be related to preoperative immune suppression (Christou, 1985).

Immune Modulation

Surgical injury induces a generalized immune response associated with decreased LPS responsiveness (Kawasaki *et al.*, 2001) and an increased stress response (Tønnesen *et al.*, 1992). Recently it was shown that ether laparotomy resulted in a robust increase in [^{125}I]IL-1α binding and IL-1R1 mRNA levels in the pituitary 2 h after the onset of stress. Therefore, CRF plays a pivotal role in the regulation of IL-1 receptors under stress conditions (Narzaloo *et al.*, 2001). In patients undergoing pancreatoduodenectomy, increased plasma levels of ACTH and cortisol were associated with stimulated production of cytokines. The appearance of endotoxin in the blood circulation was followed by an increase in plasma TNF-α and IL-6 levels. In contrast, after gastrectomy plasma TNF-α remains undetectable but an increase in plasma concentrations of IL-10 and cortisol was observed (Ogata *et al.*, 2000).

Pro-/Anti-Inflammatory Immune Modulation

The reasons for post-operative immunodeficiency in alcoholics are complex. High levels of glucocorticoids have inhibitory effects on the immune system, while levels that are too low can lead to inflammation (Sarkar, 1996). TNF-α and IL-6 are considered important signals in the peripheral immune stimulation of the brain. These pro-inflammatory cytokines are especially able to enlarge the neuronal activity of hypothalamic perikarya that are involved in the release of ACTH and corticosteroids (Rivier, 1999; Tilders *et al.*, 1994; Chrousos, 1995). TNF-α is one of the first proteins released during endotoxaemia (Givalois *et al.*, 1994), and it was thought that this cytokine plays an important role in modulating at least the early phase of LPS-induced ACTH release through the release of CRF by nerve terminals (Ebisui *et al.*, 1994; Watanobe and Taskebe, 1992; Rivier, 1999). IL-6 also plays a role in the response of the HPA axis to immune stimulation (Perlstein *et al.*, 1993). In a recent investigation Sander *et al.* (2002) showed that the IL-6/IL-10 ratio in chronic alcoholic patients dropped immediately following surgery, which was predictive for infections during the ICU course.

Th1/Th2 Subsets

Experimentally an alcohol-induced reduction of the delayed-type hypersensitivity (DTH) response in mice was reported together with a profound inhibition of this immune response. This indicates that an alcohol-induced inhibition of cell-mediated immunity occurs independently of antigen priming (Schodde *et al.*, 1996). In a clinical study the skin response of DTH was decreased in patients who underwent colorectal surgery (Tønnesen *et al.*, 1992). Among chronic alcoholics the reduced DTH response was already present preoperatively and, compared with non-alcoholics, was enhanced to a significantly lower extent post-operatively (Tønnesen *et al.*, 1992; Spies *et al.*, 2001).

Summary (See Tables XVI-4.1 and XVI-4.2)

Taken together, chronic alcohol exposure increased endotoxin release, decreased the ratio of pro- to anti-inflammatory cytokines, decreased the Th1/Th2 ratio and B cells, but increased immunoglobulin levels peripherally, whereas it decreased pro-inflammatory release centrally. In ALD, responses with respect to pro-inflammatory release, NK cell activity and immunoglobulins were exaggerated.

NICOTINE

Clinical Relevance

An estimated 65.5 million people in the United States reported current use of a tobacco product in 2000, which accounts for 29.3%

Table XVI-4.1 Central immune effects of alcohol

Stimulus: Alcohol	Central	
	Cytokines	Cells
Acute, experimental	IL-1β down in hypothalamus cells (Lee and Rivier, 1993) TNF-α down in murine cortical cells (Gahring *et al.*, 1999)	
Chronic, experimental	IL-1β down in hypothalamus cells (Lee and Rivier, 1993)	
+ Stimuli, acute, experimental	Stimulus LPS: IL-1β, TNF-α down in C6 glia cells (Syapin *et al.*, 2001) Stimulus LPS: IL-6, TNF-α no change in pituitary cells (Rivier, 1999) Stimulus TNF-α: ICAM-1 down in astrocytes (DeVito *et al.*, 2000) Stimulus IFN-γ: ICAM-1 no change in astrocytes (DeVito *et al.*, 2000)	

Intercellular adhesion molecule (ICAM); interferon (IFN); interleukin (IL); lipopolysaccharide (LPS); natural killer (NK) cell; tumour necrosis factor (TNF).

Table XVI-4.2 Peripheral immune effects of alcohol

Stimulus: Alcohol	Peripheral	
	Cytokines	Cells
Chronic, human	Endotoxin up (Parlesak *et al.*, 2000) IL-12 up (Laso *et al.*, 1998) IL-6, IL-8, IL-10, IL-12, and IL-13 up (Gonzalez-Quintala *et al.*, 1999)	Th1/Th2 ratio up (Laso *et al.*, 1998) NK cell count up (Laso *et al.*, 1997) IgE up (Gonzalez-Quintala *et al.*, 1999; Dominguez-Santalla *et al.*, 2001) IgA up (Cook *et al.*, 1998)
Experimental	IL-12 down (acute) (Friedman *et al.*, 1998) IL-4, IL-10 up (acute) (Friedman *et al.*, 1998)	B cell count down (acute) (Lopez *et al.*, 1997) B cell count down (chronic) (Lopez *et al.*, 1997, Starkenburg *et al.*, 2001) Th1/Th2 ratio down (acute) (Na *et al.*, 1997) Th1/Th2 ratio down (chronic) (Starkenburg *et al.*, 2001) IgE up (chronic) (Starkenburg *et al.*, 2001)
+ Stimuli, acute, human (healthy volunteers)	Stimulus LPS: TNF-α, IL-1β down (Szabo *et al.*, 1996b) Stimulus LPS: IL-10 up (Szabo *et al.*, 1996b) Stimulus SEB: IL-1β down (Szabo *et al.*, 1996b) Stimulus SEB: IL-12 up (Girouard *et al.*, 1998)	
+ Stimuli, acute, experimental	Stimulus LPS: IL-10 up, IL-12 down (Mason *et al.*, 2000) Stimulus LPS: IL-6 down (Rivier, 1999)	
Alcohol and ALD		
Chronic, human	TNF-α up in AC (Schafer *et al.*, 1995) IL-6 up in AC (Genesca *et al.*, 1999) IL-8 up in AC (Homann *et al.*, 2000) TNF-α up in AH (Bird *et al.*, 1990; Felver *et al.*, 1990) IL-1 up in AH (McClain *et al.*, 1986) IL-6 up in AH (Hill *et al.*, 1992; Genesca *et al.*, 1999) IL-8 up in AH (Sheron *et al.*, 1993; Hill *et al.*, 1993) IL-6 up in FL (Schafer *et al.*, 1995)	Th1/Th2 ratio up in AC (Laso *et al.*, 1999) NK count and activity in AH up (Laso *et al.*, 1997) NK count and activity in ALD down (Cook *et al.*, 1997) B cell count down (Cook *et al.*, 1996) IgG, IgA up in ALD (Cook *et al.*, 1998) IgM up in AH (Cook *et al.*, 1998)
Alcohol and surgery		
Chronic, human	IL-6/IL-10 ratio down (Sander *et al.*, 2002)	Th1/Th2 ratio down (Tønnesen *et al.*, 1992)

Alcoholic liver disease (ALD); alcoholic cirrhosis (AC); alcoholic hepatitis (AH); fatty liver (FL); immunoglobulin A (IgA); immunoglobulin E (IgE); immunoglobulin G (IgG); immunoglobulin M (IgM); intercellular adhesion molecule (ICAM); interferon (IFN); interleukin (IL); lipopolysaccharide (LPS); natural killer (NK) cell; staphylococcal enterotoxin B (SEB); T helper cell (Th); tumour necrosis factor (TNF).

of the population aged 12 and older. Among that same population 55.7 million (24.9%) smoked cigarettes. Cigarette smokers are more likely to use other tobacco products, illicit drugs and alcohol than non-smokers. In 2000, 39.4% of smokers were binge drinkers and 13.6% were heavy alcohol users (Substance Abuse and Mental Health Services Administration, 2001). Cigarette smoking has long been reported as having negative health consequences. In the United States, cigarette smoking is responsible for over 400 000 deaths per year and the direct health care costs exceed $50 billion per year (US Department of Health and Human Services, 1994). The noxious effect of smoking can be due, in part, to its immunosuppressive activity (Sopori and Kozak, 1998; Hakki *et al.*, 2000). Recent studies suggest smoking as a risk factor for faster development of acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus (HIV)-1 seropositive individuals, higher susceptibility to *Pneumocystis carinii* infections in AIDS patients, and higher frequency of transmission from AIDS from smoking mothers to their offspring (Burns *et al.*, 1991; Nieman

et al., 1993; Burns *et al.*, 1994; Sopori and Kozak, 1998). Smokers have an increased susceptibility for respiratory tract diseases and cancer, which may reflect cigarette smoke-induced impairment of the immune system (Holt and Keast, 1977).

Cigarette smoke contains thousands of different compounds including nicotine, polycyclic aromatic hydrocarbons, tobacco glycoprotein and some metals (Sopori and Kozak, 1998). Chronic nicotine exposure leads to the inhibition of antibody responses, indicating that nicotine is a major immunosuppressive component in cigarette smoke (Geng *et al.*, 1996). Nicotine is also the major addictive component of tobacco. The mechanism of cigarette smoke-induced immunosuppression is not clear, and several potential mechanisms are being considered.

Immune Modulations

Mellon and Bayer (1999) suggest that nicotine mimics the effects of morphine on peripheral blood lymphocyte responses. These effects appear to decrease the overall magnitude of the response to mitogen treatment without clearly altering the sensitivity of the cells to this potent stimulus. They demonstrated that nicotinic agonists, such as epibatidine, produce their immunomodulatory effects on blood lymphocyte proliferation by acting predominantly at peripheral nicotinic receptors (Mellon and Bayer, 1999). The nicotinic receptor-mediated immunomodulation, like that produced by opioids, appears independent of the ability to activate the HPA axis (Mellon and Bayer, 1999).

CD4 T cells, NK and B cells, as well as the percentages of total lymphocytes, monocytes and granulocytes, were unaffected by acute nicotine in experimental settings. This suggests that the acute effect of nicotine on cell function in the blood, such as non-specific proliferation, is not due to changes in cell distribution (McAllister-Sistilli *et al.*, 1998).

Pro-/Anti-Inflammatory Immune Modulation

Matsunaga *et al.* (2001) showed that nicotine exposure to murine macrophages infected with bacteria enhanced the growth of *Legionella pneumophila*, and led to a downregulated production of IL-6, IL-12 and TNF-α. In this setting the IL-10 production induced by bacteria was not affected by nicotine (Matsunaga *et al.*, 2001). In another experimental study, pretreatment with nicotine inhibited LPS-induced IL-1β, IL-8 and prostaglandin E_2 (PGE_2) protein production (Sugano *et al.*, 1998).

It has been shown that LPS-stimulated human alveolar macrophages in heavy smokers release less IL-1 and PGE_2 (Brown *et al.*, 1989), as well as less TNF-α and IL-6 than in non-smokers (McCrea *et al.*, 1994).

Th1/Th2 Subsets

Geng *et al.* (1995) demonstrated that splenic lymphocytes from rats treated chronically with nicotine exhibited a substantial loss of the antibody-forming cell (AFC) responses to both T-dependent and T-independent antigens without significantly affecting the number or percentage of lymphocyte subpopulation. Similar results were shown in chronic smoke-exposed rats, which showed suppressed AFC response without altering the total number or subpopulation of lymphocytes (Kalra *et al.*, 2000). The inhibition of the AFC response was evident in nicotine-treated animals up to 14 days following the termination of nicotine exposure. These results indicate that nicotine-induced T cell anergy, a condition in which antigen-specific T cells fail to respond to an antigen and develop tolerance (Sopori and Kozak, 1998), lasts for a significant period after nicotine has been cleared from the circulation (Geng *et al.*, 1996). The nicotine-induced activation is thought to be responsible for making T cells refractory to antigen-induced biochemical changes. Furthermore, there are indications that nicotine only partially activates T cells (Geng *et al.*, 1996). This partial activation by nicotine may be a general mechanism for the induction of T cell tolerance leading to immunosuppression (Geng *et al.*, 1996).

Nicotine exposure decreases T cell CD28 expression, which results in T cell anergy (Zhang and Petro, 1996). Both CD4 and CD8 T cell subpopulations may be increased in smokers (Smart *et al.*, 1986). The altered Th1/Th2 ratios could explain how cigarette smoke affects the immune response. It has been demonstrated that the exposure of responding T cells to nicotine results in decreased plasma levels of IL-2 and IFN-γ and leads to an increased accumulation of IL-4 and IL-10 in mice (Zhang and Petro, 1996; Petro *et al.*, 1999). The leucocytes of smokers produce higher levels of IL-4, a cytokine which inhibits the Th1 cells required for the inflammatory response (Sopori and Kozak, 1998). In contrast, LeCam *et al.* (1996) did not find a nicotine effect on the *in vitro* release of IL-4 and IFN-γ. However, Ouyang *et al.* (2000) showed that even at concentrations as high as 200 $\mu g\,ml^{-1}$, nicotine inhibited the production of IL-2, IFN-γ, IL-1β and TNF-α. IL-2, IFN-γ, IL-1β and TNF-α are important mediators of antitumour immunity and suppression of these cytokines by components of cigarette smoke, especially nicotine, may increase the risk of infection, lung cancer and other malignancies (Ouyang *et al.*, 2000). In an experimental study nicotine caused an increase in IL-1α mRNA levels, but despite the increase in mRNA the IL-1α protein level in the rat adrenal gland was reduced (Andersson *et al.*, 1992).

The ability of nicotine to influence CD4 T cell cytokine production may not just have a significant impact on microbial disease progression (Zhang and Petro, 1996). Progression towards both human and mouse AIDS is associated with decreased production of Th1 cytokines such as IFN-γ and increased production of Th2 cytokines, like IL-4 and IL-10 (Clerici and Shearer, 1994).

B Cells

Nicotine stimulates IgE production (Sopori and Kozak, 1998). Gerrard *et al.* demonstrated that IgE levels in smoking humans are higher as compared to non-smokers (Gerrard *et al.*, 1980). Furthermore, the levels of IgM, IgA and IgG were found to be decreased in smokers (Gerrard *et al.*, 1980; Andersen *et al.*, 1982).

NK Cells

It has been shown that smokers have decreased NK activity (Tollerud *et al.*, 1989; Hughes *et al.*, 1985), which has also been confirmed in experimental settings (Nair *et al.*, 1990).

HPA Axis and ANS

Nicotine may affect immune and/or other physiological functions via activation of CRF and the eventual release of corticosterone and other neurohormones (Rosecrans and Karin, 1998). Nicotine and muscarinic receptors play an important role in corticotropin-releasing hormone (CRH) release, as they both mediate IL-2-stimulated CRH release (Karanth *et al.*, 1999). Nicotine together with cigarette smoke stimulates the release of noradrenaline (norepinephrine) and adrenaline (epinephrine) and ACTH (Cryer *et al.*, 1976; Seyler *et al.*, 1984). ACTH and catecholamines have been shown to inhibit the immune response (Fuchs and Sanders, 1994).

Both acute and chronic nicotine treatments regulate T cell proliferation via nicotinic acetylcholine receptors (nAChR). However, while the chronic effects of nicotine appear to be independent of the HPA axis, activation of this axis may play a critical role in the response of T cells to acute nicotine exposure (Singh

et al., 2000). Acute administration did not inhibit the proliferation of spleen cells to ConA, a T cell mitogen (Singh *et al.*, 2000). However, acute nicotine administration inhibited the ConA-stimulated proliferation of peripheral blood cells; but this effect was short lived and not observed at 24 h after nicotine administration (Singh *et al.*, 2000). It was also found that acute exposure increased the plasma corticosterone concentration. Adrenalectomized animals did not exhibit the inhibition of the ConA response after acute nicotine treatment. Because glucocorticoids primarily affect the activation of IL-2, and the IL-2-induced signalling cascade, acute nicotine may not significantly affect the early steps in the T cell receptor-mediated signalling in T cells (Almawi *et al.*, 1991; Iseki *et al.*, 1993; Paliogianni *et al.*, 1993; Singh *et al.*, 2000). Unlike acute treatment, chronic nicotine treatment does not increase the plasma corticosterone levels, and chronic, sustained exposure to nicotine in rats inhibited the response of spleen cells to ConA (Singh *et al.*, 2000; Geng *et al.*, 1995). These authors concluded that the effects of chronic nicotine on T cell function are independent of the HPA axis (Singh *et al.*, 2000).

CNS

Cytokines present in the CNS originate not only from the peripheral sources of the immune system but also through endogenous production by cells of the brain, including astrocytes and neurones whose production can be stimulated by peripheral cytokines (Carlson *et al.*, 1999). By itself, nicotine is neuroprotective against NMDA-mediated excitotoxicity. It is important to note that nicotine inhibits TNF-α-induced, but not IL-1- (both α and β) or IL-6-induced neuroprotection in cortical cell cultures (Carlson *et al.*, 1999). Nicotine stimulates neurones via activation of nAChRs and, specifically for neuroprotection, those nAChRs composed of $\alpha 7$ subunits (Lindstrom, 1996; Sargent, 1993). Activation of these ligand-gated ion channels induces a flux of calcium into the cell, leading to other intracellular events (Harkins and Fox, 1998; Tang *et al.*, 1998; Warashina, 1998; Darnay and Aggarwal, 1997). After the initial agonist action of nicotine the channel closes, rapidly terminating the effects of nicotine and its consequent pharmacological effects. A nicotine-induced nAChR desensitization associated with the development of tolerance and an upregulation of central nAChR was observed (Rosecrans and Karin, 1998; Marks *et al.*, 1983). How these intracellular events interfere with TNF-α-induced effects is unknown.

Summary (See Tables XVI-4.3 and XVI-4.4)

Taken together, there is a good evidence that nicotine peripherally and centrally suppresses pro- and stimulates anti-inflammatory responses. Except for IgE upregulation all cell-mediated responses seem to be downregulated.

BENZODIAZEPINES

Clinical Relevance

Benzodiazepines are one of the most prescribed and consumed medication groups in the world (Kapczinski *et al.*, 2001). For example, 10% of the inhabitants in a Dutch community of 13 500 people receive benzodiazepine prescriptions at least once a year (van Hulten *et al.*, 1998). Stress, depression and psychotic disturbances have been shown to provoke severe immunological disorders (Lechin *et al.*, 1994; Muller *et al.*, 1991; Stein *et al.*, 1991). The 1,5- and particularly the 1,4-benzodiazepines are the most widely prescribed anxiolytic drugs, and function by binding to the central benzodiazepine receptor, which is allosterically coupled to the γ-aminobutyric acid (GABA) A receptor (Fruscella *et al.*, 2001; Zavala, 1997). Indirectly, benzodiazepines can also regulate neurotransmitter release via the modulation of neurosteroid synthesis in the mitochondria (Freeman and Young, 2000). Peripheral benzodiazepine receptor binding sites have been found in all mammalian tissues, including glial cells and blood mononuclear cells, with the following cell distribution: monocytes > B cells and large granular lymphocytes > T cells (Ferrarese *et al.*, 1990, 1993; Rocca *et al.*, 1993).

Immune Modulation

Stimulation of peripheral benzodiazepine receptors may have an important impact on phagocyte activity, therefore influencing essential functions such as chemotaxis, the generation of oxidative bursts and cytokine production (Ruff *et al.*, 1985; Ferrarese *et al.*, 1993; Zavala, 1997). However, there are marked differences between the augmentation *in vitro* and inhibition *ex vivo* of cytokine production and oxidative burst potential (Zavala, 1997).

Benzodiazepine and LPS/Intestinal Permeability

To our knowledge nothing is known about benzodiazepine abuse or withdrawal and intestinal permeability or endotoxin liberation.

Pro-/Anti-Inflammatory Immune Modulation

Fruscella *et al.* (2001) demonstrated that benzodiazepine derivatives have an anti-inflammatory effect in mice. They reduce the exudate production of IL-6 and PGE_2 as well as vascular permeability, which is partially involved in the reduction of pouch leucocyte recruitment (Fruscella *et al.*, 2001). It has been shown that peripheral (Ro5-4864) and mixed-type benzodiazepine (diazepam) receptor agonists suppress phytohaemagglutinin- and ConA-induced proliferation of human peripheral blood mononuclear cells, and inhibit IL-2- and IL-3-like activity (Bessler *et al.*, 1992).

In mice, the production of bioactive TNF-α by splenocytes and peritoneal macrophages, and the LPS-induced production of IL-1 and IL-6 by spleen macrophages, is inhibited by injections of

Table XVI-4.3 Central immune effects of nicotine

Stimulus: Nicotine	Central	
	Cytokines	Cells
Acute, experimental	TNF-α in cortical cells down (Carlson *et al.*, 1999) IL-1α, IL-1β and IL-6 in cortical cells no change (Carlson *et al.*, 1999)	
Chronic, experimental		AFC response down (Sopori *et al.*, 1998)

Antibody plaque-forming cell (AFC); interleukin (IL); tumour necrosis factor (TNF).

Table XVI-4.4 Peripheral immune effects of nicotine

Stimulus: Nicotine	Peripheral	
	Cytokines	Cells
Acute, human	IL-1β, IL-2, IFN-γ, TNF-α down (Ouyang *et al.*, 2000)	CD4 T cells, total lymphocytes, monocytes, granulocytes, NK, B cells no change (McAllister-Sistilli *et al.*, 1998)
Chronic, human	IL-2 down (van Dijk *et al.*, 1998) IL-2, TNF-α no change (Madretsma *et al.*, 1996) IL-10 down (Madretsma *et al.*, 1996)	NK cell count down (Tollerud *et al.*, 1989) NK cell activity down (Hughes *et al.*, 1985) IgE up (Gerrard *et al.*, 1980) IgM down (Gerrard *et al.*, 1980) IgA, IgG down (Andersen *et al.*, 1982)
Acute, experimental	IL-2, IFN-γ down (Zhang and Petro, 1996; Petro *et al.*, 1999) IL-4, IL-10 up (Zhang and Petro, 1996; Petro *et al.*, 1999) IL-4, IFN no change (LeCam *et al.*, 1996) IL-1α mRNA up (Andersson *et al.*, 1992) IL-1α protein level down (Andersson *et al.*, 1992)	Th1/Th2 ratio no change (McAllister-Sistilli *et al.*, 1998) Th1/Th2 ratio down (Zhang and Petro, 1996) B cell count, NK cell count no changed (McAllister-Sistilli *et al.*, 1998) NK cell activity down (Nair *et al.*, 1990)
Chronic, experimental	IL-8 mRNA down (colonic mucosa) (Louvet *et al.*, 1999)	AFC response down (Geng *et al.*, 1995, Kalra *et al.*, 2000) Th1/Th2 ratio down (Zhang and Petro, 1996)
+ Stimuli, chronic, human heavy smokers	Stimulus LPS: IL-1, PGE_2 down (Brown *et al.*, 1989) Stimulus LPS: TNF-α, IL-6 down (McCrea *et al.*, 1994)	
+ Stimuli, acute, experimental	Stimulus LPS: IL-1β, IL-8, PGE_2 down (Sugano *et al.*, 1998) Stimulus *L. pneumophila*: IL-6, IL-12, TNF-α down (Matsunaga *et al.*, 2001) Stimulus *L. pneumophila*: IL-10 no change (Matsunaga *et al.*, 2001)	Stimulus ConA: proliferation of PBC down (Singh *et al.*, 2000)
+ Stimuli, chronic, experimental		Stimulus ConA: proliferation of PBC and spleen cells down (Singh *et al.*, 2000)

Antibody plaque-forming cell (AFC); cluster of differentiation (CD); concanavalin A (ConA); interferon (IFN); interleukin (IL); *Legionella pneumophila* (*L. pneumophila*); lipopolysaccharide (LPS); messenger ribonucleic acid (mRNA); natural killer (NK) cell; peripheral blood cell (PBC); prostaglandin E_2 (PGE_2); T helper cell (Th); tumour necrosis factor (TNF).

ligands selective for peripheral benzodiazepine receptors (Zavala *et al.*, 1997; Zavala and Lenfant, 1987; Fruscella *et al.*, 2001). A comparable study performed on human cells from subjects given a single injection of the mixed-type benzodiazepine receptor agonist midazolam (0.08 mg kg^{-1}) showed that LPS-mediated IL-1β, TNF-α and IL-6 production was decreased (Taupin, 1991). Taken together, the *in vivo* experiments show that peripheral benzodiazepine receptor ligands inhibited the synthesis of IL-1β, IL-6 and TNF-α in response to LPS (Zavala, 1997). However, in contrast to the *in vivo* data, the mixed/peripheral benzodiazepine receptor agonist increased rather than inhibited cytokine generation, producing bell-shaped concentration–response curves. Thus, at concentrations of 0.1–10 nm, Ro5-4864 and diazepam produced approximately a twofold increase in the production of bioactive and immunoreactive IL-1β, TNF and IL-8 induced by LPS. However, at lower or higher concentrations the effects of the drugs were diminished (Ruff *et al.*, 1985; Zavala, 1997; Fruscella *et al.*, 2001; Taupin *et al.*, 1991).

In addition, intra- and extracellular levels of benzodiazepines may differ. Midazolam has been shown to cause a significant decrease in the LPS-induced extracellular accumulation of IL-8 in human subjects, whereas the intracellular IL-8 levels increased with exposure to LPS and remained increased after treatment with midazolam. Furthermore, IL-8 mRNA levels also increased in the presence of midazolam, indicating that midazolam has a modulating effect on the transport or secretion of IL-8 from the cell, which would account for the differences in intra- and extracellular concentrations (Galley *et al.*, 1998).

The selective central benzodiazepine ligand clonazepam did not affect the cellular immune functions examined, suggesting an *in vitro* immunosuppressive activity of peripheral and mixed type, but not central type benzodiazepine ligands (Bessler *et al.*, 1992).

Using the high-affinity peripheral benzodiazepine ligand 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195), which was previously used to judge the activation of microglia *in vivo* by TNF-α as an increase in the density of peripheral benzodiazepine receptors (PBR) (Iida *et al.*, 2000; Stephenson *et al.*, 1995; Bourdiol *et al.*, 1991), the respiratory burst response was inhibited, the release of glutamate and IL-1β was reduced, and the secretion of products cytotoxic to neuronal cells was suppressed. Selectivity was suggested by the failure of PK11195 to influence TNF-α secretion or expression of human leucocyte antigen (HLA)-DR and CDw32. Therefore, PK11195 may be a useful inhibitor of selective macrophage functions, retarding both local and systemic inflammation. Since PK11195 enters the brain readily, it may be beneficial in treating central as well as peripheral inflammatory diseases (Klegeris *et al.*, 2000).

During HIV-1 encephalitis, the chemotaxis-inducing activity of terminal deoxynucleotidyltransferase (Tat) may enhance the viral life cycle through the recruitment of additional susceptible microglial cells to the foci of infection. Benzodiazepines readily penetrate the blood–brain barrier and are known to possess anti-inflammatory properties. Pretreatment of human microglial cells with peripheral (Ro5-4864) and mixed (diazepam), but not central (clonazepam), benzodiazepine receptor ligands was found to potently suppress HIV-1 Tat-induced chemotaxis. The inhibitory effect of diazepam was associated with the ability to block Tat-induced $[Ca^{2+})]_i$ mobilization. These data support the notion that PBR ligands could alter the neuropathogenesis of HIV-1 via effects

on microglia, and may provide new therapeutic options for AIDS dementia (Lokensgard *et al.*, 2001).

T and B Cells

In human benzodiazepine users a lower than normal CD4 (T helper/inducer) cell count was shown. Considering that CD8 (T-suppressor/cytotoxic) cells did not show abnormal values, the lower CD4/CD8 ratio registered in benzodiazepine users was due to lower CD4 values (Lechin *et al.*, 1994).

It was reported that PBR agonists and mixed-type ligands like diazepam stimulate the humoral immune response to sheep red blood cells in mice, a T cell-dependent antigen (Zavala *et al.*, 1984; Ferrarese *et al.*, 1993). Due to the observed immunomodulating activity the day after immunization, it was suggested that the immunomodulation observed *in vivo* could be mediated by the binding of these molecules to macrophages, which may result in a modulation of the physiological function of these cells in the immune system (Ferrarese *et al.*, 1993).

NK Cells

Pretreatment with the benzodiazepine diazepam completely blocked CRH-induced suppression of NK activity (Irwin *et al.*, 1993). Animals that received diazepam prior to CRH showed NK activity similar to the lytic activity observed in rats treated with diazepam alone (Irwin *et al.*, 1993). Benzodiazepine may antagonize sympathetic outflow, and thereby attenuate CRH-induced immune suppression, because benzodiazepine reduced plasma catecholamines (Vogel *et al.*, 1984) and elevated sympathetic activity following stress, which mediates the reduction of NK activity following CRH (Irwin *et al.*, 1990, 1993). The NK activity was significantly greater in benzodiazepine users than in controls (Lechin *et al.*, 1994).

Summary (See Tables XVI-4.5 and XVI-4.6)

Taken together, there is good evidence that peripheral and mixed benzodiazepine agonists influence the immune system. However, nothing is known at present about the effects of benzodiazepine abuse and withdrawal on immune functions, to the best of our knowledge.

OPIOIDS

Clinical Relevance

In 2000, approximately 130 000 people in the United States were current heroin users, which represents 0.1% of the population aged 12 and older (Substance Abuse and Mental Health Services Administration, 2001).

Although the idea that central opioids can modulate the immune response is a relatively recent one, the understanding that systemic opioids produced alterations in the immune system began more than 100 years ago. At this time, Cantacuzene (1898) described the effects of opium on the phagocytic function of guinea-pig immunocytes. More recent research has shown that people addicted to heroin have increased rates of infection (Hussey and Katz, 1950; Haverkos and Lange, 1990). Closer examination of addicts has revealed abnormalities in many immunological parameters, including decreased NK cytolytic activity, blood lymphocyte proliferation responses to mitogen, and alterations in more complex immune responses including antibody-dependent cell-mediated cytotoxicity and antibody production (Mellon and Bayer, 1998a; Brown *et al.*, 1974; Layon *et al.*, 1984; Nair *et al.*, 1986). Several studies in healthy non-addicted individuals exposed to opioids either

Table XVI-4.5 Central immune effects of benzodiazepines

Stimulus: Benzodiazepines	Central	
	Cytokines	Cells
Experimental	IL-1β down (Klegeris *et al.*, 2000) HIV-1-Tat-induced chemotaxis down (Lokensgard *et al.*, 2001)	

Human immunodeficiency virus type 1 (HIV-1); interleukin (IL); terminal deoxynucleotidyltransferase (Tat).

Table XVI-4.6 Peripheral immune effects of benzodiazepines

Stimulus: Benzodiazepines	Peripheral	
	Cytokines	Cells
Acute, human	IL-3-like activity, IL-2 down (Bessler *et al.*, 1992)	
Chronic, human		CD4 down, CD8 no change (Lechin *et al.*, 1994)
Experimental	IL-6, PGE_2 down (Fruscella *et al.*, 2001)	
+ Stimuli, human healthy volunteers	Stimulus LPS: IL-8 extracellular down (Galley *et al.*, 1998) Stimulus LPS: IL-8 mRNA intracellular up (Galley *et al.*, 1998) Stimulus LPS: IL-1, TNF-α up (Taupin *et al.*, 1991)	
+ Stimuli, acute, experimental	Stimulus LPS: IL-1β, IL-6, TNF-α down (Zavala *et al.*, 1997)	
Benzodiazepine and stress/trauma		
Acute, experimental		NK cell activity up (Irwin *et al.*, 1993)

Cluster of differentiation (CD); interleukin (IL); lipopolysaccharide (LPS); messenger ribonucleic acid (mRNA); natural killer (NK) cell; prostaglandine E_2 (PGE_2); tumour necrosis factor (TNF).

medicinally or occupationally have also demonstrated immunological abnormalities (Mellon *et al.*, 1998; Crone *et al.*, 1988; Biagini *et al.*, 1995). The immunosuppressive effects of the opiate could result in an increased susceptibility to infections in the post-operative period, a possible lack of defences in cancer patients and an increased susceptibility to HIV infections in drug abusers (Peterson *et al.*, 1990; Sacerdote *et al.*, 1997).

Animal studies have further reinforced the conclusions drawn from human subjects, showing that morphine can alter the resistance of an organism to infection. In rodents, multiple doses of morphine increased the susceptibility to *Klebsiella pneumoniae* and *Candida albicans* infections (Tubaro *et al.*, 1983), and increased lethality in *Toxoplasma gondii*-infected mice (Chao *et al.*, 1990). Even a single dose of morphine increased lethality in mice infected with Friend virus (Starec *et al.*, 1991). In swine, chronic morphine altered the course of the response to herpes virus infection (Risdahl *et al.*, 1993).

On the other hand, both human and rodent T lymphocytes, B lymphocytes and monocytes are able, upon appropriate stimulation, to produce and release β-endorphin (Buzzetti *et al.*, 1989; Blalock, 1989; Manfredi *et al.*, 1995; Sacerdote *et al.*, 1998). β-Endorphin is a potent local analgesic compound that is released by the activation of immune cell CRF receptors within inflamed tissue (Cabot, 2001; Schäfer *et al.*, 1996). It was shown that β-endorphin affects the tonic inhibition of the immune responses (Manfredi *et al.*, 1993; Panerai *et al.*, 1995).

Immune Modulation

Opioids can alter the function of all types of immunocytes (T cells, B cells, NK cells, lymphocytes, monocytes, macrophages and neutrophils) (Peterson *et al.*, 1993, 1998). Investigations have indicated that opioids can operate as cytokines (Bidlack, 2000), both within somatic tissues and in the CNS (Peterson *et al.*, 1998). All of the major properties of cytokines are shared by opioids, i.e. production by immune cells with paracrine, autocrine and endocrine sites of action, functional redundancy, pleiotropy and time- and dose-dependent effects (Peterson *et al.*, 1998). Because the CNS is populated predominantly by astroglia and microglia, which have properties of immune cells, it is possible that certain opioid effects on the CNS involve cytokine-like interactions with glial cells. Although there is mounting evidence to support the concept that opioids are members of the cytokine family, the relative contribution of chronic opioid use and abuse to immunoregulation remains unclear (Bianchi *et al.*, 1999; Roy *et al.*, 2001b).

The immunomodulatory activity of opioids is likely to be due to complex interplay between direct and indirect influences (McCarthy *et al.*, 2001). It is well established that the opioid system affects a number of parameters related to innate and acquired immunity (Radulovic *et al.*, 1995), which then indirectly affect the immune cells (Roy *et al.*, 2001b). When opioids have a direct effect on immune function, they must act through opioid receptors expressed on immune cells. These receptors are often the same as, or similar to, neuronal-type opioid receptors, i.e. κ-opioid, δ-opioid and μ-opioid receptors (Bidlack, 2000). But it was reported that the morphine binding sites are different from the classical neuronal μ-opioid receptor (Roy and Loh, 1996; Sharp *et al.*, 2001; Makman *et al.*, 1998; Roy *et al.*, 2001a). It has been shown that another morphine receptor is also present, namely the μ-3-opioid receptor, which is opiate alkaloid sensitive and opiate peptide insensitive (Makman *et al.*, 1998; Stefano and Scharrer, 1996). These μ-3 receptors have been identified in human peripheral blood monocytes and intervertebrate immunocytes (Stefano and Scharrer, 1996; Stefano *et al.*, 2000; Makman *et al.*, 1998; Roy *et al.*, 2001a; Law *et al.*, 2000). The influence of the opioids on the immune response *in vivo* is also likely to be a result of the participation of both the CNS and the HPA axis (McCarthy *et al.*, 2001). Through this indirect mechanism opioids bind to classical opioid receptors in the CNS, causing the release of catecholamines and/or steroids.

Phagocytic Cells and Pro-/Anti-Inflammatory Immune Modulation

In all but one study (Eisenstein *et al.*, 1998), morphine was found to suppress the phagocytosis and chemotaxis of phagocytic cells in animals and humans (animals: Casellas *et al.*, 1991; Szabo *et al.*, 1993; Tubaro *et al.*, 1993; Rojavin *et al.*, 1993; Pacifici *et al.*, 1994; humans: Perez-Castrillon *et al.*, 1992 — macrophages; Stefano *et al.*, 1993 — human PBMC; Makman *et al.*, 1995 — granulocytes). Pacifici *et al.* (1994) found a biphasic response in the phagocytic function of elicited murine polymorphonuclear leucocytes (PMN). They reported that 20 or 40 min after morphine administration there was an increase in phagocytosis and killing of the organism, whereas cells harvested 24 h later demonstrated a decrease in phagocytic activity. Roy *et al.* (1991) found that morphine pellets inhibited the ability of bone marrow macrophage precursors to develop into viable colonies in response to macrophage stimulating factor *in vitro*, and that the effect was inhibited by naltrexone. Also, the *in vitro* addition of morphine or β-endorphin to precursor cells had a similar result, indicating that morphine acted directly on the precursor cells.

Morphine blocked the activity of several chemotactic agents including TNF-α, IL-1, IL-8 and formyl-methionyl-leucyl-phenylalanine (fMLP) (Makman, 1995). In response to ConA or phorbol myristate, human peripheral blood mononuclear cells (PBMC) exposed to morphine had reduced production of superoxide and peroxide (Peterson, 1987a) due to the release of transforming growth factor (TGF)-β (Chao *et al.*, 1992). In a study by Peterson *et al.* (1989), methadone-maintained patients had similarly impaired generation of superoxide.

In PBMC, morphine has been shown to increase TGF-β production in cells stimulated by LPS or phytohaemagglutinin (PHA) (Chao *et al.*, 1992). However, morphine inhibited IFN-γ production in PBMC stimulated with ConA or varicella zoster virus. In this case, the morphine appeared to affect the macrophages directly, and be mediated by macrophage-reactive oxygen intermediates and PGE_2 (Peterson *et al.*, 1987b). Morphine also blocked the release of bioactive TNF-α from PBMC cultures treated with LPS or PHA (Chao *et al.*, 1993). The synthetic κ-opioid agonist U50, 488 H has also been shown to inhibit LPS-driven IL-1 and TNF-α release from the murine macrophage cell line P388D1 (Belkowski *et al.*, 1995), and from primary murine peritoneal macrophages (Alicea *et al.*, 1996). However, IL-6 levels were unaffected in the primary cultures (Alicea *et al.*, 1996). All of these effects on cytokines were shown to be opioid receptor mediated using appropriate antagonists.

Interestingly, almost all of the effects of morphine and of non-peptide opioids on phagocytes are inhibitory. Some opioid peptide effects are also inhibitory, such as with the phagocytosis of yeast (Szabo *et al.*, 1993). In contrast, it has been reported that opioid peptides that bind various types of opioid receptors are stimulatory in a variety of assays. For example, Ruff *et al.* (1985) reported that [D-Ala(2), D-Leu(5)]enkephalin (DADLE), β-endorphin and dynorphin (1–13) were stimulators of human mononuclear cell chemotaxis. This finding was confirmed and extended to show that DADLE, D-Ala(2), Mephe(4), Gly-ol(5)-enkephalin (DAGO), [D-Pen(2), D-Pen(5)]enkephalin (DPDPE), D-Ala2,Met5-enkephalinamide (DAMA), dynorphin A(1-17), D-Ala2-deltorphin I and methionine-enkephalin (met-enkephalin) were all chemotactic for human granulocytes (Makman *et al.*, 1995). Dynorphin A has also been reported to enhance tumoricidal activity of mouse macrophages (Foster and Moore, 1987; Hagi *et al.*, 1994) and to stimulate superoxide production of human PMN and

peritoneal macrophages (Sharp *et al.*, 1985). β-Endorphin was also active in stimulating superoxide activity (Sharp *et al.*, 1985).

The existence of two types of receptors (Carr *et al.*, 1991; Sedqi *et al.*, 1995; Stefano *et al.*, 1992; Makman *et al.*, 1995) would help to explain the generally divergent effects of the alkaloids and the opioid peptides on monocyte and granulocyte function. In general, the non-peptide opioids are functionally suppressive while the peptides upregulate. It is of interest that these differences do not seem to exist in the nervous system.

It has been shown that morphine treatment decreases IL-2 and IFN-γ and increases IL-4 and IL-5 as a function of concentration (Lauw *et al.*, 2000). In studies using high dosage (*in vivo*) or high concentrations (*in vitro*) that correspond to levels seen in drug-addicted populations (Roy *et al.*, 2001a), it was demonstrated that morphine inhibits dose-related ConA-induced IL-2 and IL-4 production that is not mediated through μ-, δ- or κ-opioid receptors (Jessop and Taplits, 1991; Carr *et al.*, 1993, 1994a; Eisenstein *et al.*, 1993; Flores *et al.*, 1995; Roy *et al.*, 2001a).

In contrast to human PBMC, morphine was shown to potentiate the production of TNF-α with murine (Chao *et al.*, 1994) and human microglial cells (Peterson *et al.*, 1994). The ability of morphine to potentiate TNF-α production by microglia appeared to explain the amplification of HIV-1 expression in a chronically infected promonocyte line (U1 cells) after the cells were co-cultured with LPS-stimulated human foetal brain cell cultures (Peterson *et al.*, 1994). Sundar *et al.* (1995) have shown that the endogenous opioid β-endorphin stimulated the expression of HIV-1 in acutely infected microglia via a naloxone-inhibitable mechanism involving IL-1, IL-6 and TNF-α. In studies with the κ-opioid receptor, the peptide dynorphin was shown to directly stimulate TNF-α and IL-6 expression in human brain cell cultures that were cultured with U1 cells. The direct stimulatory effects of dynorphin results in an enhanced upregulation of HIV-1 (Chao *et al.*, 1995) whereas the κ-opioid receptor ligands, when exposed to κ-agonists U50, 488 and U69, 593 in human microglia cells, suppressed HIV-1 expression if acutely infected (Peterson *et al.*, 1998). Thus, it appears that the effects of opioid agonists on HIV-1 expression in the brain may be influenced both by the type of opioid receptors on microglia and the nature of infection (acute vs. chronic).

T Lymphocyte Proliferative Responses

Hernandez *et al.* (1993) provided the first evidence that central opioid receptors modulate circulating T lymphocyte function. Using the polyclonal mitogen ConA, it was demonstrated that a systemic dose of morphine suppressed mitogen-stimulated whole blood lymphocyte proliferation, whereas *N*-methyl morphine had no effect. However, both morphine and *N*-methyl morphine administered into the third ventricle suppressed T lymphocyte proliferation. Therefore, T lymphocyte proliferative responses appear to be mediated predominantly, if not entirely, by opioid receptors located within the brain (Hamra and Yaksch, 1996; Hoffman *et al.*, 1995; Lysle *et al.*, 1996). These central opioid receptors appear to be located suprasinally, as intrathecal morphine does not alter peripheral lymphocyte function (Hamra and Yaksch, 1996). Extended periods of exposure to morphine may be required to suppress splenic lymphocyte activity (Hamra and Yaksch, 1996; Fecho *et al.*, 1996).

Taken together, the studies suggest that morphine inhibits mitogen-stimulated peripheral lymphocyte proliferation through central opioid receptors (Weber and Pert, 1989; Bayer *et al.*, 1990a, 1992; Fecho *et al.*, 1993a). Evaluating selective agonists ([(3)H]Tyr-D-AlaGly-MePhe-Gly-ol (DAMGO) μ-selective, DPDPE δ-selective and U50, 488 κ-selective), it was demonstrated that proliferative responses of peripheral lymphocytes are mediated by central μ-receptors (Mellon and Bayer, 1996, 1998a; Schneider and Lysle, 1996).

Cellular and Humoral Immune Responses

Veljic *et al.* (1990) first reported decreased humoral immune responses in rats chronically treated with met-enkephalin directly into the lateral ventricle. A 4-day treatment with this endogenous opioid produced a dose-dependent effect on the humoral immune response to sheep red blood cells, as measured by the number of plaque-forming cells (PFC) produced, as well as antibody production: A high dose of met-enkephalin decreased the number of PFC and antibody production, whereas lower doses enhanced the responses (Veljic *et al.*, 1990; Jankovic, 1991). A dose-dependent effect was also found for modified delayed-type hypersensitivity skin test (DTH) reactions (Jankovic *et al.*, 1991). The effects of low-dose met-enkephalin on both humoral and cellular immune responses were due to the tonic activation of δ-receptors, because the specific δ-receptor antagonist (ICI 174864) not only antagonized the met-enkephalin effects, but also suppressed the responses when administered alone (Radulovic *et al.*, 1994). Whether high-dose met-enkephalin effects are mediated centrally by μ- or κ-receptors or whether peripheral components are involved remains unclear (Radulovic *et al.*, 1995; Corbett *et al.*, 1993).

T Cells

McDonough *et al.* (1980) showed that heroin addicts have reduced numbers of cells that can rosette with sheep erythrocytes, and that treatment with naloxone restored binding to a normal frequency. Studies in addicts are complex, as many are polydrug abusers. Donahoe *et al.* (1986) found that simultaneous abusers of heroin and cocaine did not have depressed T cell rosettes, suggesting that the two drugs have cancelling effects. Heroin addicts have also been reported to have reduced CD4+ cells and elevated CD8+ cells (Donahoe *et al.*, 1987) in one study, but no change in T lymphocyte cell subsets in another study (Novick *et al.*, 1989). Whether the observed alterations in cell numbers are due to drug abuse or to other life-style alterations in abusers cannot be easily tested. Other parameters of T cell function in response to drug abuse have not been studied in humans.

Animal studies have provided a clearer delineation of the cause-and-effect relationship between opioid administration and alteration in T cell function. Morphine has been shown to alter the ratio of CD4+ and CD8+ T lymphocytes in both the spleen (Arora *et al.*, 1990) and thymus (Arora *et al.*, 1990; Freier and Fuchs, 1993) of mice implanted with a 75 mg morphine pellet, as well as the peripheral blood of monkeys which received daily injections of morphine for 2 years (Carr and France, 1993). On a functional level, morphine administration has been shown to inhibit the capacity for a DTH response (Pellis *et al.*, 1986; Bryant and Roudebush, 1990; Molitor *et al.*, 1992). Morphine has also been reported to inhibit the induction of cytotoxic T cell responses in mice following immunization with allogenic spleen cells (Carpenter and Carr, 1995; Carpenter *et al.*, 1994). Ho and Leung (1979) observed a depression in the proliferative response of lymphocytes to ConA in mice addicted to morphine, indicating a diminished T cell response to an antigen. Bryant *et al.* (1987) had a similar finding but, in contrast to the previous report, observed that the depression in T cell activation disappeared as mice became tolerant. Similar results were observed in monkeys given daily injections of morphine (Chuang *et al.*, 1993). In contrast to the immunosuppressive effects of morphine reviewed so far, there are two reports in the literature that demonstrate the of stimulation of T cell responses to mitogens by morphine (Bocchini *et al.*, 1983; Bidlack and Hemmick, 1990).

A number of investigators have examined mechanisms by which morphine induces suppression of T cell activities. Flores *et al.* (1994) showed that the depression of ConA responses in peripheral blood cells of rats was not dependent on glucocorticoids or on

pituitary factors, as neither adrenalectomy nor hypophysectomy abrogated the suppression. In addition, adrenalectomy, alone or in combination with drugs, suggested that the sympathetic system was involved in morphine suppression of the splenic responses to PHA (Fecho *et al.*, 1996). There are several studies which failed to find an effect when morphine was added to T cells *in vitro* and only a few investigations found direct morphine effects. This is a surprising situation, since the inhibition of T rosette formation has been confirmed by two groups using human cells (De Carolis *et al.*, 1984; Donahoe *et al.*, 1987, 1988) since it was first described in 1979 (Wybran *et al.*, 1979). The κ-opioid agonist U50, 488 H has been reported by Guan *et al.* (1994) to suppress the capacity of T cells to participate in an *in vitro* antibody response to sheep erythrocytes. A weak suppressive effect of heroin on IL-2 production by murine spleen cells was observed at some doses, but not others, and the effect of antagonists was not tested (Thomas, P.T. *et al.*, 1995).

B Cells

Following initial work by Lefkowitz and Chiang (1975), Bryant *et al.* (1988) found that implantation of a 75 mg morphine slow-release pellet reduced the mitogenic responses of splenic B cells to LPS, which has been confirmed using an injection dosing regimen (Bhargava *et al.*, 1994). Bussiere *et al.* (1992) reported that 75 mg morphine pellets also inhibited induction of antibody-forming cells to sheep red blood cells in a variety of mouse strains and showed that suppression was naltrexone inhibitable in some mouse strains, but not others (Pruett *et al.*, 1992). Further, Bussiere *et al.* (1992) showed that both mice with defects in response to LPS, and beige mice, which lack NK cells, had suppressed antibody responses when treated with morphine, while mice which lack κ-opioid receptors did not (Bussiere *et al.*, 1992). Formation of an antibody response to sheep red blood cells requires the interaction of macrophages, T cells and B cells. Thus, depression of the capacity to produce antibodies does not necessarily mean that the drug affects B cells. In fact, Bussiere *et al.* (1993) found that antibody responses could be restored by adding normal macrophages or IL-1, IL-6 or IFN-γ, cytokines which are produced by or which activate macrophages, suggesting that the morphine-induced suppression is due in part to a lack of macrophage activity. Weber *et al.* (1987) also found that morphine inhibited serum antibody responses to a T-dependent, but not to a T-independent antigen, suggesting that morphine did not affect B cell function.

Taub *et al.* (1991) has shown that morphine, as well as the κ-opioids U50, 88 H and U69, 593, inhibited antibody formation to sheep erythrocytes when added to mouse spleen cells *in vitro*. Eisenstein *et al.* (1995) confirmed that both morphine and U50, 488 H could inhibit *in vitro* antibody responses, showing that the drugs directly affect immune cells, and that suppression is not mediated by the HPA axis. In this study, there were marked differences in mouse strain susceptibility to the effects of morphine and the agonist, raising the possibility that *in vitro* effects of morphine may be affected by the strain used (Eisenstein *et al.*, 1995). A direct effect of morphine on murine splenic B cell proliferation to mitogen has also been reported (Thomas *et al.*, 1995a). Endogenous peptides have also been reported to be suppressive, which is interesting in the context of antibody formation (Johnson *et al.*, 1982a, b; Morgan, 1996).

NK Cells

A large number of studies examining the effects of opioids on immune function have focused on the perturbation of NK cell function. Provocative reports that supported a neuroimmune circuit involving NK cells were published starting in 1984 when Shavit *et al.* (1984b) first found that subcutaneous (s.c.) injections of morphine into rats suppressed splenic NK cell activity. Depression of NK activity has been demonstrated in heroin abusers (Novick *et al.*, 1989) and in polydrug abusers (Nair *et al.*, 1986b). In the latter study, depression of antibody-dependent cellular cytotoxicity (ADCC) was also observed (Nair *et al.*, 1986). In a definitive experiment, reduced NK cell activity, as well as reduced ADCC mediated by NK cells, has been shown using human volunteers that were given morphine intravenously (i.v.) for 24 h (Yeager *et al.*, 1995). Subcutaneous injection of morphine has also led to depressed NK cell function in rats (Bayer *et al.*, 1990a) and mice (Carr *et al.*, 1994a). Monkey NK cell function has also been reported to be suppressed when morphine was injected s.c., on a daily basis, for 2 years (Carr and France, 1993). It can be concluded that there is strong evidence that opioids given *in vivo* depress NK and ADCC activity.

A number of investigators have addressed the question of whether this depression is due to a direct effect on NK cells or is centrally mediated. Shavit *et al.* (1986) showed that delivery of morphine into the lateral ventricle of the brain suppressed NK cell activity to the same degree as a single s.c. injection. The observation that *N*-methylmorphine, which does not cross the blood–brain barrier, was ineffective when given peripherally to rats suggested that the depression of NK responses was mediated via opioid receptors in the brain (Shavit *et al.*, 1986). These studies were confirmed and extended by Weber and Pert (1989), who found that morphine depresses NK activity in the periaqueductal grey region of the rat brain. Evidence for involvement of sympathetic pathways (Carr *et al.*, 1993, 1994b) or the HPA axis (Freier and Fuchs, 1994) in *in vivo* morphine effects on NK cell function in the rat has been published. Carr *et al.* (1993, 1994b) have shown that pretreatment of mice with α-adrenergic receptor antagonists blocked the morphine-induced suppression in NK cell activity. In contrast, Fecho *et al.* (1993b) reported that β-adrenergic receptor antagonists could not block the suppressive effects of morphine on NK cell activity in the rat. A role for HPA axis involvement in morphine-induced suppression of NK cell activity was suggested by Freier and Fuchs (1994), and Band *et al.* (1992) reported that central administration of the κ-opioid agonist DAGO suppressed NK cell function without elevation of plasma corticosterone. These papers provide strong evidence that the effects of morphine on NK activity are centrally mediated.

There is little evidence that morphine can modulate NK function directly (Eisenstein *et al.*, 1998). Mathews *et al.* (1983) reported that β-endorphin added to human PBMCs enhanced NK cell activity, but that morphine was inactive. Variable stimulatory activity of β-endorphin and met-enkephalin was observed by other investigators (Puente *et al.*, 1992; Oleson and Johnson, 1988). Ochshorn-Adelson *et al.* (1994) reported that naloxone did not affect NK cell cytotoxicity when added to human PBMC. Similarly, other investigators have failed to find an effect of morphine *in vitro* on NK cell activity in rodents (Freier and Fuchs, 1994). It is interesting that, as with the phagocytic cells, the endogenous opioids seem to have an upregulating rather than a downregulating effect. Further, *in vitro* effects have been demonstrated with some of the peptides, but not with morphine. The discordance between peptide and alkaloid data indicates that if the receptor is the same, then downstream signalling events are different. The paucity of studies reporting an *in vitro* effect of morphine on NK cell activity and the substantial literature showing that effects on NK activity are centrally mediated support the conclusion that the alkaloid is not acting directly on the NK cell.

Neuroendocrine and Autonomic Involvement

Because the CNS communicates with the periphery via two major physiological systems, i.e. the neuroendocrine system and the

ANS, the identification of efferent mechanisms mediating central opioid modulation of the immune system has focused on these two systems.

Neuroendocrine Involvement

Although a central site of action for morphine-stimulated corticosterone release in the rat was suggested as early as 1969 (Lotti *et al.*, 1969), to date the exact neuronal structure(s) and receptor subtype(s) within specific neuronal tissues that mediate these responses have not been clearly delineated. In fact, μ, δ and κ-receptors appear to independently modulate the HPA axis (Iyengar *et al.*, 1987).

Chronic morphine administration in wild-type mice led to lymphoid organ atrophy and a diminished CD4+/CD8+ ratio in the thymus (Gavériaux-Ruff *et al.*, 1998). Roy *et al.* (2001b) used *in vivo* and *in vitro* studies to demonstrate that morphine induces thymic hypoplasia and results in a glucocorticoid-dependent lymphopaenia (Jessop and Taplits, 1991; Carr *et al.*, 1993, 1994a; Eisenstein *et al.*, 1993; Flores *et al.*, 1995; Roy *et al.*, 2001b). The immune modulatory effects of prolonged morphine exposure on both lymphocyte proliferative responses and NK cell activity appear to be the activation of the HPA axis, despite the fact that the effects of prolonged exposure are not entirely explained by glucocorticoids (Bryant *et al.*, 1987, 1988, 1990, 1991; Freier and Fuchs, 1994). In contrast, the effects of acute morphine showed that the lymphopaenia observed was dependent upon the HPA axis whereas the decreased proliferative response to mitogen was adrenal independent (Bayer *et al.*, 1990a, 1990b; Flores *et al.*, 1994, 1995; Bayer *et al.*, 1992).

Autonomic Nervous System

Several investigators have explored opioid modulation in the ANS (Appel *et al.*, 1986; Marson *et al.*, 1996). Direct administration of opioid into the CNS has been shown to produce elevations in plasma adrenaline, noradrenaline and dopamine, suggesting that catecholamines are released from both the adrenal medulla, as well as sympathetic nerve terminals (Gomes *et al.*, 1976; Vogel *et al.*, 1984; Kiritsy-Roy *et al.*, 1986). Therefore, catecholamines from either synaptic terminals or the adrenal medulla may mediate the effects of morphine on NK cell activity or lymphocyte proliferative responses. It is hypothesized that the activation of the sympathetic nervous system by morphine elicits the release of catecholamines into primary and secondary lymphoid organs (Hall *et al.*, 1998; Felten *et al.*, 1985), which results in the suppression of NK cells (Shavit *et al.*, 1986; Weber *et al.*, 1989) and lymphocyte functionality (Fecho *et al.*, 1993a; Felsner *et al.*, 1995). However, many of the drugs used at doses required to produce a significant effect have been shown to have their own effects on immune cell activity (Fecho *et al.*, 1996; Flores *et al.*, 1996) and therefore the specific role of the ANS in opioid-mediated effects remains unclear.

Tolerance

Animals made tolerant to an analgesic dose of morphine also appeared to develop tolerance to the effects of morphine on lymphocyte proliferation (Bayer *et al.*, 1994, 1996). This observation suggests that consistent use of opioid compounds may lead to a state of tolerance in the immune system. If tolerance develops to this immunological parameter, the potential significance of the altered lymphocytes in drug-naive animals must be questioned. However, animals made tolerant to morphine and exposed to stress demonstrated clear lymphocyte suppression (Bayer *et al.*, 1994), while previous studies showed that drug-naive animals did not show alterations in lymphocyte proliferation (Flores *et al.*, 1990). These observations suggest an increased susceptibility to stress in morphine-tolerant animals, a finding with significant implications regarding the immunological stability of individuals chronically exposed to opioids, either for pain relief or due to drug addiction.

Summary (See Tables XVI-4.7–XVI-4.10)

There is incontrovertible evidence that morphine inhibits the function of NK cells, B cells, T cells and phagocytic cells when it is given *in vivo*. Direct effects of the drug have also been demonstrated *in vitro* in phagocytic cells. It is less clear whether morphine has a direct effect on NK cells or B cells. There is strong evidence that morphine can directly modulate receptor expression on T cells, but consistent alteration of other functional parameters *in vitro* has been difficult to demonstrate.

Research to date indicates that the cellular responsiveness to opioids may depend on the state of cell activation, which can influence receptor expression. Alternatively, various immune cells may express more than one type of opioid receptor, and functional alterations may depend upon the relative activation of one type as compared with another. As endogenous peptides seem to have opposite functional effects from non-peptide agonists in a number of cases, and immune cells may even synthesize such peptides (Zurawski *et al.*, 1986; Kuis *et al.*, 1991), the interactions of exogenous and endogenous opioids may result in complex alterations in immune cell function. The presence of unrecognized endogenous opioid peptides that can antagonize the alkaloids or synthetic opioid peptides may account for the variable effects of morphine seen in different studies of T cell function (Eisenstein *et al.*, 1998).

Taken together, opiates down regulate pro-inflammatory cytokines, decrease IL-2 but increase IL-4, decrease phagocytosis and chemotaxis, decrease lymphocyte proliferation, decrease NK cell activity at high doses peripherally, whereas opiates increase TNF-α centrally. A decreased CD4/CD8 ratio was also seen in heroin addicts. However, opiates in higher doses decrease both IL-2 and IL-4, and diminish the depression in T cell activation to antigens, but with stress lymphocyte suppression was observed.

Table XVI-4.7 Central immune effects of opioids

Stimulus: Opioids	Central	
	Cytokines	Cells
Acute, human	Morphine: TNF-α up (human microglial cells) (Peterson *et al.*, 1994)	
Acute, experimental	Morphine: TNF-α up (murine microglial cells) (Chao *et al.*, 1994)	
	Morphine: μ receptor agonist: NFκB up (red cortical neurones) (Hou *et al.*, 1996)	

Nuclear factor κB (NFκB); tumour necrosis factor (TNF).

Table XVI-4.8 Peripheral immune effects of opioids

Stimulus: Opioids	Peripheral	
	Cytokines	Cells
Acute, human	Morphine: IL-2, IFN-γ down (Lauw *et al.*, 2000) Morphine: IL-4, IL-5 up (Lauw *et al.*, 2000) Morphine: IL-1,IL-8, TNF-α, fMLP down (Makman *et al.*, 1995)	Morphine: antibody-dependent cell-mediated toxicity down (Yeager *et al.*, 1992) Morphine: NK cell activity up (Yeager *et al.*, 1992) Morphine: NK cell activity down (Yeager *et al.*, 1995) Morphine: phagocytosis, chemotaxis down. (Perez-Castilloun *et al.*, 1992; Stefano *et al.*, 1993; Makman *et al.*, 1995)
Chronic, human	Morphine: IL-2, IL-4 down (Roy *et al.*, 2001a) Heroin: IL-2 down (weak effect) (Thomas, P.T. *et al.*, 1995)	Heroin: CD4+ cells down, CD8+ cells up (Donahoe *et al.*, 1987) Heroin: T cell subsets no change (Novick *et al.*, 1989) Heroin: NK cell activity down (Roy *et al.*, 2001; Novick *et al.*, 1989; Nair *et al.*, 1986b)

Cluster of differentiation (CD); concanavalin A (ConA); formyl-methionyl-leucyl-phenylalanine (fMLP); interferon (IFN); interleukin (IL); natural killer (NK) cell; tumour necrosis factor (TNF).

Table XVI-4.9 Peripheral immune effects of opioids

Stimulus: Opioids	Peripheral	
	Cytokines	Cells
Acute, experimental		Morphine and all opioid peptides: phagocytosis, chemotaxis down (Casellas *et al.*, 1991; Szabo *et al.*, 1993) Morphine: NK cell activity down (Weber and Pert, 1989) Morphine: splenic NK cell activity down (Shavit *et al.*, 1986; Hoffman *et al.*, 1995; Lysle *et al.*, 1996)
Chronic, experimental		Morphine: CD4+/CD8+ ratio up (chronic) (Arora *et al.*, 1990) Morphine: CD4+/CD8+ ratio down (chronic) (spleen, thymus) (Freier and Fuchs, 1990; Gavériaux-Ruff *et al.*, 1998) Morphine: NK cell activity down (Gavériaux-Ruff *et al.*, 1998) High-dose met-Enk: humoral response down (plaque-forming cells, antibody production) (Jankovic *et al.*, 1991; Radulovic *et al.*, 1994) High-dose met-Enk: DTH response down (Jankovic *et al.*, 1992; Radulovic *et al.*, 1994) Low-dose met-Enk: humoral response up (plaque-forming cells, antibody production) (Jankovic *et al.*, 1992; Radulovic *et al.*, 1994) Low-dose met-Enk: DTH response up (Jankovic *et al.*, 1992; Radulovic *et al.*, 1994) Morphine: lymphocyte proliferation no change (Bayer *et al.*, 1994)

Cluster of differentiation (CD); concanavalin A (ConA); delayed-type hypersensitivity reaction (DTH); formyl-methionyl-leucyl-phenylalanine (fMLP); interferon (IFN); interleukin (IL); lipopolysaccharide (LPS); methionine-enkephalin (met-Enk); natural killer (NK) cell; nuclear factor κB (NFκB); peripheral blood mononuclear cells (PBMC); phorbol myristate acetate (PMA); phytohaemagglutinin (PHA); transforming growth factor (TGF); tumour necrosis factor (TNF).

COCAINE

Clinical Relevance

In 2000, an estimated 1.2 million Americans were current cocaine users. This represents 0.5% of the population aged 12 and older. The estimated number of crack users in 2000 was 265 000 (Substance Abuse and Mental Health Services Administration, 2001).

Cocaine abuse is associated with a variety of clinical disorders. These disorders involve cardiovascular complications, including cardiac arrhythmias and sudden death (Ferreira *et al.*, 2001), neurovascular complications such as cerebral infarction, intraparenchymal and subarachnoid haemorrhage and cerebral vasculitis (Conway and Tamargo, 2001). Ischaemic as well as haemorrhagic neurovascular sequelae have been associated with the alkaloid form of cocaine ('crack'), whereas cocaine hydrochloride has generally been associated only with intracranial haemorrhage (Conway and Tamargo, 2001; Levine *et al.*, 1991). Cocaine use has been linked to hypertension and acute and chronic renal failure (Norris *et al.*, 2001).

In the last 10 years the increased susceptibility to infections among cocaine users has caught the attention of many researchers and clinicians. For example, the incidence of HIV seroprevalence is significantly higher in cocaine addicts (Anthony *et al.*, 1991; Chaisson *et al.*, 1989; Pellegrino and Bayer, 1998; Pellegrino *et al.*, 2001), and cocaine users have a higher incidence of hepatitis C infection than other intravenous drug abusers (Thomas, D.L. *et al.*, 1995; Ward *et al.*, 2000). It has also been suggested, however, that the increased susceptibility to HIV and hepatitis C cannot be explained by intravenous drug abuse alone (Pellegrino *et al.*, 2001).

Pharmacologically, cocaine binds to monoamine reuptake pumps, preventing removal of serotonin, dopamine and noradrenaline from

Table XVI-4.10 Peripheral immune effects of opioids

Stimulus: Opioids	Peripheral	
	Cytokines	Cells
+ Stimuli, acute, human (healthy volunteers)	Morphine: stimulus ConA/PMA: O_2- down, H_2O_2 down (PBMC) (Peterson *et al.*, 1987) Morphine: stimulus LPS/PHA: TNF-α down (PBMC) (*in vitro*) (Chao *et al.*, 1993) Morphine: stimulus LPS/PHA: TGF-β up (PBMC) (*in vitro*) (Chao *et al.*, 1993) Morphine: stimulus ConA/varicella zoster: IFN-γ down (PBMC) (Peterson *et al.*, 1987)	
+ Stimuli, chronic human	Methadone: stimulus PMA: O_2- down (Peterson *et al.*, 1989)	Opiate exposed: stimulus pokeweed mitogen: lymphocyte proliferation down (Biagini *et al.*, 1995)
+ Stimuli, acute, experimental	κ-Opioid-agonists: stimulus LPS: IL-1, TNF-α down (Belkowski *et al.*, 1995; Alicea *et al.*, 1996) κ-Opioid-agonists: stimulus LPS: IL-6 no change (Alicea *et al.*, 1996) Morphine: stimulus stress: lymphocyte suppression (tolerant mice) (Bayer *et al.*, 1994) Morphine: stimulus ConA/LPS: T cell response down (addicted mice) (Bryant *et al.*, 1987) Morphine: stimulus ConA/LPS: T cell response no change (tolerant mice) (Bryant *et al.*, 1987)	Morphine: stimulus ConA: T lymphocyte proliferation down (Hamra *et al.*, 1996; Hoffman *et al.*, 1995; Lysle *et al.*, 1996) Morphine: stimulus variety of mitogens: splenic lymphocyte proliferation down (Fecho *et al.*, 1995; Hamra *et al.*, 1996)
+ Stimuli, chronic, experimental		Stimulus stress + morphine: lymphocyte suppression (Bayer *et al.*, 1996)

Concanavalin A (ConA); formyl-methionyl-leucyl-phenylalanine (fMLP); interferon (IFN); interleukin (IL); lipopolysaccharide (LPS); natural killer (NK) cell; nuclear factor κB (NFκB); peripheral blood mononuclear cells (PBMC); phorbol myristate acetate (PMA); phytohaemagglutinin (PHA); transforming growth factor (TGF); tumour necrosis factor (TNF).

the synapse (Szabo *et al.*, 1995). Cocaine also acts as a muscarinic cholinergic antagonist and as a local anaesthetic (Szabo *et al.*, 1995; Pellegrino *et al.*, 2001). The multiplicity of pharmacological actions may contribute to be varied immune response to cocaine, particularly when doses are varied (Pellegrino *et al.*, 2001).

Immune Modulation

Pro-/Anti-Inflammatory Immune Modulation

In addition to measures of cellular and humoral immunity, the effects of chronic cocaine treatment on cytokine production have also been examined. It was hypothesized that an altered cytokine expression during cell activation can result in immune changes (Pellegrino and Bayer, 1998). Only a few studies have examined the possible effects of cocaine on cytokine production by immune cells. The pattern of cytokine secretion from LPS-stimulated mouse splenocytes was altered by chronic cocaine exposure. Decreased secretion of IL-4 and IL-10, but increased levels of TNF-α, IL-6 and IL-2, were observed 24 h after mitogen stimulation. Contrary to the increase at 24 h, at 72 h there was a decrease in TNF-α (Chen and Watson, 1991; Wang *et al.*, 1994; Pellegrino and Bayer, 1998). Cocaine administered to mice during *in vivo* antigen-stimulated differentiation of effector T cells suggests that the immunomodulatory effects of cocaine may be due to the upregulation of IL-2 and IFN-γ production by CD8+ cells with a type 0 cytokine profile (Di Francesco *et al.*, 1999).

Cell-Mediated Immune Functions

One possibility is that cocaine decreases cell-mediated immune function in cocaine addicts. In studies measuring cell-mediated immunity alterations in immune cell numbers and distribution, mitogen-induced lymphocyte proliferation and NK cytolytic activity have been observed (Pellegrino and Bayer, 1998).

A significant dose-dependent decrease in the number of thymocytes and white blood cells was detected 96 h after acute cocaine administration in mice (Ou *et al.*, 1989). Acute intravenous cocaine exposure resulted in a decrease in mitogen-induced T lymphocyte proliferation in rats in a dose- and time-dependent manner (Bayer *et al.*, 1995). Cocaine and its major metabolites increased B cell proliferation in human B-lymphoblastoid cells at concentrations equivalent to serum levels produced during cocaine abuse in humans (Phillips *et al.*, 1995). The effects varied with gender (Xu *et al.*, 1997): acute *in vivo* cocaine exposure inhibited the proliferation of T lymphocytes in response to ConA in both thymocytes and splenocytes. However, the attenuated IL-2 production was only seen in thymocytes. These effects on T cells were greater in male mice than in female mice. The function of macrophages was also impaired with acute cocaine exposure. However, the impact was greater in female than in male mice.

Chronic treatment of cocaine resulted in a decrease in thymus weight and a decrease in CD8+ cells, and is, therefore, possibly related to the inhibition of thymocyte proliferation (Choi *et al.*, 1998; Lopez *et al.*, 1992). But after 30 days of cocaine administration the decrease in cell number was no longer present, so it was suggested that a possible tolerance to the effect developed (Di Francesco *et al.*, 1994; Pellegrino and Bayer, 1998). When rats were chronically exposed to cocaine, the decrease in mitogen-induced T lymphocyte proliferation was no longer observed (Bayer *et al.*, 1996).

Human studies have shown only small changes in lymphocyte function and number in some cocaine addicts (Ruiz *et al.*, 1998). But the same group found decreases in CD4+ T cells and increases in NK cell number among different group of addicts (Ruiz *et al.*, 1994).

Although tolerance may develop to some immune parameters after chronic cocaine exposure, this does not necessarily imply

Table XVI-4.11 Central immune effects of cocaine

Stimulus: Cocaine	Central	
	Cytokines	Cells
Acute, human	Endothelial adhesion molecule up (Gan *et al.*, 1999) ICAM-1, VCAM-1, ELAM-1 up (Gan *et al.*, 1999)	
Chronic, experimental	NFκB induction p105 up (nucleus accumbens, cytoplasmatic) (Ang *et al.*, 2001)	
+ Stimuli, acute, experimental	Stimulus LPS: IL-6, TNF-α up (BMVEC/monocyte co-culture) (Gan *et al.*, 1999)	

Brain microvascular endothelial cell (BMVEC); endothelial leucocyte adhesion molecule (ELAM); intercellular adhesion molecule (ICAM); interleukin (IL); lipopolysaccharide (LPS); nuclear factor κB (NFκB); tumour necrosis factor (TNF); vascular cell adhesion molecule (VCAM).

that the immune function of the animal is normal. Potentially, compensatory mechanisms could develop, which under stress or infection may result in inadequate immunocompetence (Pellegrino and Bayer, 1998). Chronic ingestion of coca alkaloids has been shown to result in a decrease in the DTH response in mice (Watson *et al.*, 1983). Cocaine at low doses was found to be associated with an accelerated tumour growth in mice, suggesting a potential decrease in NK cell or cytotoxic T cell function (Ou *et al.*, 1989).

Doses of cocaine which were not accompanied by increases of corticosterone did not induce the suppression of blood lymphocyte proliferation to mitogens, which suggests a possible role for increased steroid levels in the immunosuppressive effects of cocaine (Pellegrino and Bayer, 1998). Cocaine administration has been found to result in the activation of the HPA axis, which leads to an elevation in plasma corticosterone levels in rodents and cortisol levels in humans (Baumann *et al.*, 1995; Bayer *et al.*, 1995; Saphier *et al.*, 1993). At least, the T cell-dependent antibody response is evidently mediated by corticosterone (Stanulis *et al.*, 1997). However, in a previous study an HPA axis-independent mechanism was favoured (Pellegrino *et al.*, 2001). In the study by Pellegrino *et al.* (2001) it was found that cocaine was acting through peripheral receptors to decrease lymphocyte proliferation. This hypothesis is supported by the fact that systemic cocaine methiodide, which should not cross the blood–brain barrier, decreased lymphocyte proliferation in a similar way, and that cocaine and cocaine methiodide were unable to alter lymphocyte activity when administered directly into the CNS. However, peripheral effects should not be equated with direct effects on lymphocytes. In fact, the high doses required to decrease immune cell function *in vitro* make it unlikely that cocaine was acting directly on lymphocytes to decrease proliferation (Berkeley *et al.*, 1994; Javaid *et al.*, 1978; Luo *et al.*, 1992; Martinez and Watson, 1990). Therefore, cocaine-induced decreases in lymphocyte function may be produced indirectly through alteration of other neurotransmitters or the neuroendocrine system. Another possibility is that cocaine is decreasing lymphocyte function through alterations in peripheral monoamine levels. This is supported by the observation that the monoamine reuptake inhibitor RTI-55 resulted in a similar decrease in lymphocyte activity. However, the effects of systemically administered RTI-55 could be the result of elevations in central monoamine levels, especially since a similar decrease in lymphocyte proliferation was observed following central administration of high doses of RTI-55. The inhibitory effect might also be mediated via serotonin reuptake inhibition (Pellegrino *et al.*, 2001). The ability of the local anaesthetic lidocaine to decrease lymphocyte proliferation in a similar manner as cocaine suggests that this pharmacological property may also be involved in cocaine-induced lymphocyte suppression (Pellegrino *et al.*, 2001).

Whether signal transduction, such as the induction of NFκB or mitogen-activated protein (MAP) kinases, is affected by cocaine remains to be determined (Ang *et al.*, 2001; Pierce *et al.*, 1999). NFκB p105 is a precursor that is cleaved into NFκB p50 and IκB-γ p70, while NF-κB p65 is a distinct gene. Ang *et al.* (2001) found that chronic cocaine administration increased the levels of the precursor NFκB p105, the inhibitory subunit IκB-γ p70, and the active transcription factor NFκB p65. It is unclear from these results what the net activity of the NFκB system is. In cultured neurones, cocaine and amphetamine increase the neurotoxic effects of the HIV proteins Tat and gp 120 (Nath *et al.*, 2000) and NF-κB activates the HIV promoter (Rattner *et al.*, 1993). Whether this immunological disorder is associated with HIV-1-associated dementia remains unclear (Goodkin *et al.*, 1998). It has been shown that cocaine enhances brain endothelial adhesion molecules and monocyte migration across the cerebral vessel wall (Gan *et al.*, 1999; Fiala *et al.*, 1998) and opens the blood–brain barrier to HIV-1 invasion (Zhang *et al.*, 1998).

Prenatal cocaine exposure did enhanced B cell responsiveness (Bohn *et al.*, 1997). Chronic administration to female rats during pregnancy significantly elevated IgG levels in the absence of lymphocyte proliferation (Masten *et al.*, 1996). However, PBMC stimulated with phytohaemagglutinin and phorbol-12-myristate PBMC isolated from foetal cord blood in cocaine-using mothers showed significantly decreased IL-1 and IL-2 concentrations (Karlix *et al.*, 1998).

Summary (See Tables XVI-4.11 and XVI-4.12)

Although these studies suggest cocaine may suppress overall immunity (decreased pro- and enhanced anti-inflammatory cytokines, decreased lymphocyte proliferation, decreased DTH response), alterations in dose duration and frequency of cocaine administration, as well as differences in the animal models used in these studies, make it difficult to reach a definitive conclusion. The differences between the human and animal studies could be accounted for by multi-drug use, differences in dose, route of administration, timing, duration, species and assay protocols (Pellegrino and Bayer, 1998).

SYNTHETIC DRUGS

LSD

Clinical Relevance

The hallucinogenic agent lysergic acid diethylamide (LSD) is used recreationally by large numbers of individuals in developed countries (Ghuran and Nolan, 2000). In 2000, approximately 1 million Americans were current users of hallucinogens. This number represents 0.4% of the population 12 and older (Substance Abuse and Mental Health Services Administration, 2001). The

Table XVI-4.12 Peripheral immune effects of cocaine

Stimulus: Cocaine	Peripheral	
	Cytokines	Cells
Acute, human		B cell proliferation up (Phillips *et al.*, 1995)
Chronic, human		CD4+ cells down (Ruiz *et al.*, 1994) NK cell count up (Ruiz *et al.*, 1994)
Experimental		Thymocyte proliferation down (chronic) (Choi *et al.*, 1998) Cytotoxic T cells down (acute) (Ou *et al.*, 1989) CD8 count down (chronic) (Lopez *et al.*, 1992) T-dependent antibody response up (via corticosterone release) (Stanulis *et al.*, 1997)
+ Stimuli, acute, human	Stimulus IL-2: IL-8, IFN-γ down (*in vitro*, human PBL) (Mao *et al.*, 1996)	
+ Stimuli, acute, experimental	Stimulus ConA: IFN-γ down (Chen and Watson, 1991) Stimulus ConA: IL-2 down (thymocytes, male > female) (Xu *et al.*, 1997) Stimulus influenza virus: IL-2, IFN-γ up (CD8+ T cells) (Di Francesco *et al.*, 1999)	Stimulus ConA: lymphocyte proliferation down (Bayer *et al.*, 1995, 1996) Stimulus ConA: T lymphocyte proliferation down (Pellegrino *et al.*, 2001) Stimulus ConA: lymphocyte proliferation down (thymo-, splenocytes, male > female) (Xu *et al.*, 1997) Stimulus ConA: T lymphocyte proliferation no change (Pellegrino *et al.*, 2001)
+ Stimuli, chronic experimental	Stimulus ConA: IL-2, IL-5, IL-6 and TNF-α up (splenocytes) (Wang *et al.*, 1994) Stimulus ConA: IFN-γ no change (splenocytes) (Wang *et al.*, 1994) Stimulus ConA: IL-4, IL-10 down (splenocytes) (Wang *et al.*, 1994) Stimulus LPS: IL-6 up (macrophages/peritoneal) (Wang *et al.*, 1994) Stimulus LPS: IL-1α no change (macrophages/peritoneal) (Wang *et al.*, 1994) Stimulus LPS: TNF-α down (macrophages/peritoneal) (Wang *et al.*, 1994)	Stimulus DNFB: DTH response down (Watson *et al.*, 1983) Stimulus ConA: lymphocyte proliferation no change (Bayer *et al.*, 1996)

Cluster of differentiation (CD); concanavalin A (ConA); dinitrofluorobenzene (DNFB); delayed-type hypersensitivity reaction (DTH); interferon (IFN); interleukin (IL); lipopolysaccharide (LPS); natural killer (NK) cell; peripheral blood lymphocytes (PBL); tumour necrosis factor (TNF).

drug is an indole derivative and chemically resembles serotonin. It is usually ingested orally. The adrenergic effects are usually mild and do not produce the profound sympathetic storms that can occur after taking cocaine, amphetamine or 'ecstasy' (Ghuran and Nolan, 2000). LSD is well known for its powerful mind-altering properties, causing feelings of euphoria, anxiety, paranoia, and visual and auditory hallucinations which can occur days, months or even up to 5 years after the last use (Abraham and Aldridge, 1993; Ghuran and Nolan, 2000). Clinical symptoms that correspond to general sympathetic arousal include dilated pupils, tachycardia, hypertension and hyporeflexia (Abraham and Aldridge, 1993; Ghuran and Nolan, 2000).

Immune Modulation

LSD alters immune function. *In vitro* it was demonstrated that LSD suppresses the production of the cytokines IL-2, IL-4 and IL-6. Additionally, LSD is able to suppress the B cell proliferation and the induction of cytotoxic T lymphocytes (House *et al.*, 1995a). LSD affects the NK cell activity in a dose-related manner. *In vitro* exposure to LSD had differential effects on NK cell activity, showing significant enhancement of both basal and IL-2-augmented NK cell function at concentrations that correspond to average levels of human exposure, and showing suppression of the NK response at higher doses (House *et al.*, 1994). *In vivo* immunological effects of LSD are not yet clear and remain to be investigated.

Summary (See Table XVI-4.13)

Taken together, LSD in high doses suppresses all immune functions (cytokines, T and B cells, NK cells).

3,4-Methylenedioxymethamphetamine

Clinical Relevance

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is a ring-substituted phenylisopropylamine that is structurally related to both amphetamines and hallucinogens (Connor *et al.*, 1998). MDMA was first synthesized in 1912 but never marketed. It became popular in the 1970s and 1980s because it induces feelings of euphoria, friendliness, closeness to others and empathy (Mas *et al.*, 1999). Known under the street name 'ecstasy', which is derived from the characteristic euphoria that the drug produces, MDMA is one of the most popular recreational drugs in the Western world (Croft *et al.*, 2001). In the United States, an estimated 6.4 million persons have tried 'ecstasy' at least once in their lifetime (Substance Abuse and Mental Health Services Administration, 2001).

Adverse effects associated with the recreational use of MDMA include loss of appetite, jaw clenching, nausea, headache, trismus, bruxism, sweating, muscle aches, fatigue and insomnia. MDMA abuse can lead to the life-threatening serotonin syndrome (Sternbach *et al.*, 1991; Mas *et al.*, 1999; Dowling *et al.*, 1987; Henry *et al.*, 1992; Huether *et al.*, 1997; Croft *et al.*, 2001; Ricaurte *et al.*,

Table XVI-4.13 Peripheral immune effects of LSD. No central immune modulatory effects were found

Stimulus: LSD	Peripheral	
	Cytokines	Cells
Acute, experimental	IL-2, IL-4, IL-6 down (House *et al.*, 1995a)	Cytotoxic T cell down (House *et al.*, 1995a) NK cell activity in low doses up (House *et al.*, 1995a) NK cell activity in high doses down (House *et al.*, 1995a) B cell proliferation down (House *et al.*, 1995a)

Interleukin (IL); natural killer (NK) cell.

1988). MDMA ingestion can lead to an increased risk for immune system-related diseases (Pacifici *et al.*, 1999).

Immune Modulation

The main neurochemical action of MDMA is that it is a potent releaser of 5-hydroxytryptamine (5-HT, serotonin) within the CNS (Hegadoren *et al.*, 1999; Connor *et al.*, 2001). To evaluate the role of 5-HT release in MDMA-induced immunosuppression Connor *et al.* (2001) examined the *in vivo* effect of pretreatment with the selective paroxetine and the tryptophan hydroxylase inhibitor *p*-chlorophenylamine, on MDMA-induced suppression of IL-1β and TNF-α secretion following LPS challenge in rats. The treatment regimen of *p*-chlorophenylamine caused in excess of a 90% depletion of 5-HT concentration in both the frontal cortex and hypothalamus. In addition, MDMA administration produced a reduction in both cortical and hypothalamic 5-HT concentration, and further enhanced the *p*-chlorophenylamine-induced 5-HT depletion in both brain regions (Connor *et al.*, 2001). The suppressive effects of MDMA on LPS-induced IL-1β and TNF-α were equivalent in both saline and *p*-chlorophenylamine-treated groups (Connor *et al.*, 2001). Thus the authors suggest that the immunosuppressive effects of MDMA occur by a mechanism independent of 5-HT release (Connor *et al.*, 2001). Connor *et al.* (1998) pointed out that although *in vitro* data can be valuable in assessing the effect of MDMA on immune cells, these data also have limitations. Peripheral metabolism of MDMA may yield active metabolites which may act directly or indirectly on the immune system. Furthermore, MDMA exposure resulted in a release of CRF from the median eminence in the hypothalamus and HPA axis, as well as sympathetic nervous system (SNS) activation (Connor *et al.*, 1998). Both increased HPA axis and SNS activity can alter leucocyte distribution and function (Dhabhar *et al.*, 1995; Irwin, 1993). Another limitation is that *in vitro* studies are limited because they do not account for the effect of MDMA metabolites on immune function (Connor *et al.*, 1998).

MDMA and Mitogen

In rats, MDMA impairs the ability to respond to an *in vivo* challenge with bacterial LPS. An MDMA-associated reduction in IL-1β and TNF-α production after LPS challenge was recently shown (Connor *et al.*, 2000b, 2001). It was demonstrated that LPS-induced secretion of TNF-α was much more sensitive to the suppressive effects of MDMA than LPS-induced secretion of IL-1β (Connor *et al.*, 2000b). Connor *et al.* (2000b) demonstrated that *in vitro* exposure to MDMA at concentrations equivalent to serum levels following *in vivo* administration did not alter LPS-stimulated IL-1β or TNF-α production in diluted whole blood cultures. This suggests that the suppressive effect of MDMA on IL-1β and TNF-α following an *in vivo* LPS challenge might not be attributed to a direct effect on immune cell function.

Acute MDMA administration in rats has shown a reduction in the ConA-induced lymphocyte proliferation, and a reduction in the total leucocyte count (Connor *et al.*, 1998, 2000a). The effect of MDMA on ConA-stimulated Th1/Th2 cytokine production was associated with an increase in IL-2 production and a decrease in IL-10 and IFN-γ production. LPS treatment in MDMA-exposed rats resulted in a decrease in TNF-α production, whereas LPS-stimulated IL-1β secretion was not significantly altered (Connor *et al.*, 2000a).

Pro-/Anti-Inflammatory Immune Modulation

The immunological effects of MDMA administration include a reduction in the number of circulating lymphocytes, suppression of T lymphocyte proliferation and changes in Th1 and Th2 cytokine production (Connor *et al.*, 2000a, 2001). Cytofluorimetric analysis in men revealed that the observed reductions in lymphocyte subset counts were mainly due to T cells and not to B cells. It was also shown that MDMA treatment was associated with decrease in the production of Th1-type cytokines IL-2 and IFN-γ and an increase in the production of Th2-type cytokines IL-4 and IL-10. These results suggest that MDMA might be inducing an unbalanced differentiation of lymphocytes in peripheral blood based on the observed decrease in CD4 T lymphocytes. This partially explains the immunodysfunction caused by MDMA (Pacifi *et al.*, 2001).

In an *in vitro* study MDMA exposure resulted in the enhancement of IL-2 at low doses but resulted in suppression at high doses. The IL-4 production was not affected by MDMA treatment (House *et al.*, 1995b).

Cells

MDMA produced a suppression of cytotoxic T lymphocyte induction *in vitro* (House *et al.*, 1995b). Exposure to MDMA in rats demonstrated a reduced count of total leucocytes, lymphocytes, monocytes and neutrophils (Connor *et al.*, 2000a).

MDMA exposure had no effect on B cell proliferation at any concentration (House *et al.*, 1995b). In human MDMA experiments there were no differences found in the amount of cytotoxic/suppressor lymphocytes (CD8) and of B lymphocytes, and the total leucocyte count remained unchanged (Pacifici *et al.*, 2001).

Th1/Th2 Subsets

MDMA treatment on human PBMC was associated with a decrease in the production of the Th1-type cytokines IL-2 and IFN-γ (Pacifici *et al.*, 2001). When MDMA-treated lymphocytes in rats were ConA-simulated it has been shown that there is an increase in IL-2 levels, but IFN-γ levels were unchanged (Connor *et al.*, 2000a).

MDMA has been shown to result in the modulation of NK cell activity and macrophage function (House *et al.*, 1995b). Acute MDMA treatment resulted in a time-dependent immune dysfunction associated with MDMA plasma concentrations (Pacifici *et al.*, 2000). A decrease in CD4 T cells and in the CD4 T helper/CD8 T suppressor cell ratio was observed, whereas the percentage of NK cells was significantly increased. The total leucocyte count remained unchanged. After MDMA administration a decrease in CD4 T cell counts and simultaneous increase in NK cell activity

were confirmed, and a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation was also observed (Pacifici *et al.*, 1999, 2001). The combination of MDMA with ethanol produced the greatest suppressive effect on CD4 T cell count and phytohaemagglutinin-stimulated lymphoproliferation (Pacifici *et al.*, 1999, 2001).

Summary (See Tables XVI-4.14 and XVI-4.15):

In conclusion, the recreational use of MDMA alters the immunological status in humans. The correlation between MDMA pharmacokinetics and the profile of MDMA-induced immune dysfunction suggests that the alteration may be mediated by the CNS. Based on previous findings, MDMA may act directly on immune cells or indirectly by promoting the release of serotonin from platelet stores in the periphery. To the best of our knowledge there are only a few clinical studies which examine the immunological parameters in MDMA abusers. However, there was no change in the pro-inflammatory, cell-mediated immune reaction and it is not yet clear how MDMA doses taken by social users affect immune function.

Amphetamines

Clinical Relevance

Among the US population aged 12 and older in 2000, an estimated 800 000 persons used stimulants such as amphetamines (Substance Abuse and Mental Health Services Administration, 2001). Amphetamines are CNS stimulants that produce 'pro-conflict' and 'anxiogenic-like' effects, enhance behavioural responsiveness to stressful stimuli, and increase corticosterone secretion (Freire-Garabal *et al.*, 1991). Anxiety as well as other emotional or psychosocial stresses were reported to produce a series of well-known adverse effects on host-defence mechanisms, particularly cell-mediated immune responses (Freire-Garabal *et al.*, 1991). Amphetamines cause a marked reduction in the resistance to bacteria, viruses and tumours (Nunez-Iglesias *et al.*, 1996). Chronic treatment with amphetamine in mice strongly inhibits natural and specific cellular immune responses in a dose-related manner (Nunez-Iglesias *et al.*, 1996).

Immune Modulation

In another experimental study amphetamine treatment resulted in the suppression of IL-2, but not IL-4 production by T lymphocytes

Table XVI-4.14 Central immune effects of 'ecstasy'

Stimulus: Ecstasy	Central	
	Cytokines	Cells
+ Stimuli, acute, experimental	Stimulus LPS: IL-6, TNF-α down (paroxetine pretreatment) (cortical and hypothalamic cells) (Connor *et al.*, 2001)	

Interleukin (IL); lipopolysaccharide (LPS); tumour necrosis factor (TNF).

Table XVI-4.15 Peripheral immune effects of 'ecstasy'

Stimulus: Ecstasy	Peripheral	
	Cytokines	Cells
Acute, human healthy volunteers	IL-2, IL-6, TNF-α, IFN-γ down (Pacifici *et al.*, 2001) IL-4, IL-10, TGF-β1 up (Pacifici *et al.*, 2001)	Th1/Th2 ratio down (Pacifici *et al.*, 2001) Total leucocyte count no change (Pacifici *et al.*, 2001) T helper cell (CD4) count down (Pacifici *et al.*, 2001) Cytotoxic T cell (CD8) count no change (Pacifici *et al.*, 2001) T helper cells, CD4/CD8 ratio down (Pacifici *et al.*, 2001) NK cell count up (Pacifici *et al.*, 2001) B cell count no change (Pacifici *et al.*, 2001)
Acute, experimental	TNF-α no change (House *et al.*, 1995b) IL-2 up (low dose) (House *et al.*, 1995b) IL-2 down (high dose) (House *et al.*, 1995b) IL-4 no change (House *et al.*, 1995b) IL-2 up (Connor *et al.*, 2000a) IFN-γ no change (Connor *et al.*, 2000a)	Cytotoxic T cell induction down (House *et al.*, 1995b) B cell proliferation no change (House *et al.*, 1995b) Total leucocyte count down (Connor *et al.*, 2000a) Lymphocyte count down (Connor *et al.*, 2000a) Neutrophil count no change (Connor *et al.*, 2000a) Monocyte count no change (Connor *et al.*, 2000a) NK cell activity up (House *et al.*, 1995b)
+ Stimuli, acute, experimental	Stimulus ConA: IL-2 up (Connor *et al.*, 2000a) Stimulus ConA: IL-10, IFN-γ down (Connor *et al.*, 2000a) Stimulus LPS: TNF-α down (Connor *et al.*, 2000a) Stimulus LPS: IL-1β no change (Connor *et al.*, 2000a) Stimulus LPS: IL-1β, TNF-α down (*in vivo*) (Connor *et al.*, 2000b, 2001) Stimulus LPS: IL-1β, TNF-α no change (*in vitro*) (Connor *et al.*, 2000b)	Stimulus ConA: lymphocyte proliferation down (Connor *et al.*, 2000a) Stimulus KLH: IgG down (Connor *et al.*, 2001) Stimulus PHA: lymphocyte proliferation down (Pacifici *et al.*, 1999) Stimulus ethanol: CD4 cell count down (Pacifici *et al.*, 2001) Stimulus ethanol: NK count up (Pacifici *et al.*, 2001)

Cluster of differentiation (CD); concanavalin A (ConA); interferon (IFN); interleukin (IL); keyhole limpet haemocyanin (KLH); lipopolysaccharide (LPS); natural killer (NK) cell; T helper cell (Th); transforming growth factor (TGF); tumour necrosis factor (TNF).

Table XVI-4.16 Central immune effects of amphetamines

Stimulus: Amphetamines	Central	
	Cytokines	Cells
Acute, experimental	bFGF up in astrocytes (amphetamine) (Flores *et al.*, 2000) NFκB activity up in striatum (methamphetamine) (Asanuma and Cadet, 1998)	

Basic fibroblast growth factor (bFGF); nuclear factor κB (NFκB).

Table XVI-4.17 Peripheral immune effects of amphetamines

Stimulus: Amphetamines	Peripheral	
	Cytokines	Cells
Acute, human		NK cell activity up (*dl*-amphetamine) (Gagnon *et al.*, 1992)
Acute, experimental	IL-2 down (amphetamine) (House *et al.*, 1994) IL-4 no change (amphetamine) (House *et al.*, 1994)	Cytotoxic T cell down (amphetamine) (Nunéz-Iglesias *et al.*, 1996) NK cell activity down (amphetamine) (Nunéz-Iglesias *et al.*, 1996; House *et al.*, 1994) NK cell activity up (methamphetamine) (House *et al.*, 1994) B cell proliferation down (amphetamine) (House *et al.*, 1994)
Chronic, experimental		T cell count down (amphetamine) (Freire-Garabal *et al.*, 1991) NK cell activity up (amphetamine) (Swerdlow *et al.*, 1991)
+ Stimuli, chronic, experimental		Stimulus ConA: spleen cell response down (Freire-Garabal *et al.*, 1991) Stimulus PHA: T cell proliferation down (Gagnon *et al.*, 1992) Stimulus Stress: T cell count down (amphetamine) (Basso *et al.*, 1999) Stimulus Stress: DTH reaction down (amphetamine) (Basso *et al.*, 1999)

Delayed-type hypersensitivity reaction (DTH); interleukin (IL); natural killer (NK) cell; phytohaemagglutinin (PHA).

(House *et al.*, 1994). Nunez-Iglesias *et al.* (1996) showed a reduction in the T cell-mediated generation of cytotoxic T lymphocytes both in mixed lymphocyte cultures and *in vivo* assays with amphetamine. This indicates that amphetamine depresses the specific immune responses. After chronic amphetamine treatment in mice, the number of cells in the thymus fell to approximately 40% of baseline. Changes in spleen were similar. The decrease in peripheral blood lymphocytes was greater than the decrease in thymus cells. Amphetamine exposure also showed a reduced in the blastogenic response to ConA (Freire-Garabal *et al.*, 1991). The *in vitro* effects of 'designer' amphetamines, including *d*-, 1-, 4-proproxy- and *dl*-amphetamine, on human peripheral blood mononuclear leucocytes resulted in a wide range of suppressive actions. Treatment with each of these amphetamines led to reduced proliferation in response to T cell mitogen PHA (Gagnon *et al.*, 1992). Since T cells are essential for the development of immune responses to *Listeria* in mice, Freire-Garabal *et al.* (1991) postulated that the capacity of mice to develop immunity to an intracellular pathogen would be impaired at the time of, or immediately following, amphetamine treatment. The number of bacteria in the spleens and livers of mice injected with amphetamine was greater than that of placebo mice. Column-purified spleen cells from amphetamine-immunized mice had a reduced capacity to confer immunity as compared to placebo-immunized mice, thus confirming that T cell immunity was defective (Freire-Garabal *et al.*, 1991). Stress, i.e. foot shock, resulted in an immunosuppressive effect in rats previously subjected to chronic amphetamine treatment. These rats had a reduced percentage of T lymphocytes and a decreased DTH reaction (Basso *et al.*, 1999).

The function of NK cells was slightly suppressed by amphetamine exposure, but was enhanced by methamphetamine exposure (House *et al.*, 1994). An inhibition of NK cell activity by chronic amphetamine treatment has been shown in a dose-related manner (Nunez-Iglesias *et al.*, 1996). In contrast, *in vitro* studies have shown that only very low concentrations of *dl*-amphetamine (10^{-10} M) could increase the human NK cell activity (Gagnon *et al.*, 1992). Chronic amphetamine treatment in rats causes a significant increase in NK cell activity (Swerdlow *et al.*, 1991).

The murine B cell proliferation was suppressed in high-dose exposure to amphetamine (100 μM) (House *et al.*, 1994).

Amphetamines can either directly target cells or act indirectly by affecting neuroendocrine pathways. Amphetamines have numerous effects on neuronal and endocrine systems (Nunez-Iglesias *et al.*, 1996). Other experimental studies show that chronic amphetamine exposure has a stimulatory effect on ACTH secretion which is proportional to the decrease in T cell numbers and blastogenic response (Freire-Garabal *et al.*, 1991). Adrenalectomized mice showed reduced, but still significant, immunosuppression in response to amphetamine administration (Nunez-Iglesias *et al.*, 1996). It was suggested that other neuropeptides and neurotransmitters could be involved in the immunological response to amphetamine (Nunez-Iglesias *et al.*, 1996).

Lasting structural changes in the neurones of the nucleus accumbens and prefrontal cortex have been observed after repeated exposure to amphetamines (Robinson and Kolb, 1997, 1999b). Amphetamine injections induced increased expression of the neurotrophic and neuroprotective basic fibroblast growth factor (bFGF) in astrocytes, in the ventral tegmental area and substantia nigra pars compacta (Flores *et al.*, 2000). There are increases in both extracellular dopamine and glutamate in the ventral tegmental area after amphetamine injection (Wolf and Xue, 1999). Increased extracellular glutamate activates astrocytic bFGF (Pechan *et al.*, 1993), which acts directly on neurones or indirectly through astrocytes (Gómez-Pinilla *et al.*, 1995).

A cytokine that might contribute to methamphetamine-induced severe neurological deficits is IL-6. IL-6 is considered to play an important role in the development, differentiation, regeneration and degeneration of the CNS (Gruol and Nelson, 1997). It was shown that methamphetamine-induced damage to dopamine and serotonin terminals, apoptotic cell death and reactive gliosis are attenuated in IL-6 knock-out mice. Although the role of IL-6 in methamphetamine-induced damage is not yet clear, the protection afforded by the IL-6 null genotype suggests that the cytokine might be an important component of the toxic cascade induced by the drug (Ladenheim *et al.*, 2000). In addition to influencing neural development, IL-6, which can penetrate the blood–brain barrier (Banks *et al.*, 1994), affects the neuroendocrine and neurochemical effects of amphetamines (Zalcman *et al.*, 1999). IL-6 increased the sensitivity to the locomotor-stimulating effects of amphetamines (Zalcman *et al.*, 1999).

Summary (See Tables XVI-4.16 and XVI-4.17)

Taken together, amphetamines decreased in particular cell-mediated immune responses.

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XVI-5

The Psychophysiology of Substance-Related Disorders

Alex Gamma and Matthias E. Liechti

INTRODUCTION

The psychophysiological study of substance abuse and related disorders is based on three major rationales. The first rationale is the identification of the biological underpinnings and functional consequences of substance abuse and dependence and their development; the second rationale is the search for biological 'markers' that are present before the onset of substance use and that identify young individuals who are at risk for developing substance abuse or related disorders during adolescence or adulthood. This rationale also opens an interface between psychophysiological and molecular genetic research. As seen in chapter XVI-9, a substantial part of the risk for substance abuse is genetically transmitted. By finding a marker that is linked to these high-risk genes, psychophysiology can assist molecular genetics in the identification of high-risk gene carriers who are not detected by psychiatric diagnosis (be it that they remain unaffected throughout their lifetime or that they are children not yet affected by the disorder). The third rationale is to use the knowledge gained by these efforts to improve intervention and prevention strategies. A better understanding of the neurobiological mechanisms of substance abuse behaviour will render treatment interventions more effective and specific. Furthermore, given a reliable marker for increased susceptibility for substance abuse and related psychopathology, prevention efforts could be geared selectively to high-risk children years before they reach the age of possible onset of the disorder.

Psychophysiological research into substance-related disorders has largely taken place during the past 30 years. In general, these three decades have substantially advanced the field, although in our view there has been more palpable progress for the work based on the second rationale than that based on the first. Studies using event-related brain potentials have been successful in finding a promising marker for the risk of developing substance abuse and a spectrum of associated disturbed behaviours. The identification of the biological underpinnings and functional significance of substance-related psychophysiological alterations, however, still faces important difficulties. Some of these problems have to do with limitations in the inferences from and functional interpretations of psychophysiological data (see final section). Another set of problems that is largely unavoidable in this field of research is rooted in the fact that most studies are cross-sectional and compare two or more different groups of subjects that differ in some dimension of interest. Typically, a group of substance users is experimentally compared with a group of non-drug-using subjects, and differences in test results between the groups are attributed to the variable of interest subjects were grouped by; i.e. in this case, substance abuse. But in order for this attribution to be valid, the two study groups would have to be identical in every respect but the fact that one uses substances and the other does not; otherwise some other difference between the groups (e.g. age, sex, psychopathology) could be responsible for the observed experimental differences. Although many researchers try to control this problem by matching the study groups for as many of these confounding variables as possible, a complete matching of the groups will never be possible. This is a serious limitation for the interpretation of research data in this field, since substance abuse is characterized by substantial comorbidity with internalizing disorders on the one hand, and externalizing disorders like antisocial personality on the other. In addition, there are major differences in lifestyle between drug users and non-users that might affect psychophysiological variables. Finally, polydrug use is a frequent phenomenon, so that the biological and behavioural effects of a given drug are difficult to study in isolation. Thus, it is an important task of research to disentangle the associations of substance abuse with comorbid psychopathology, variations in personality traits, use of other drugs and substance-related disturbed behaviours, and to isolate the contributions of these various factors to the observed experimental differences.

In acknowledging the essentially comorbid nature of substance abuse, we will, after reviewing the literature on single substances, deal with the possible common causes and psychophysiological and behavioural dispositions underlying the propensity to use various kinds of mind-altering substances. We start our review with alcohol, which, to date, has garnered the most attention within psychophysiological drug research. The study of alcoholism is an exemplary case that will bring up many of the issues and problems that also pertain to the other substances. We therefore suggest that our readers read this section also if their main interest is with another substance of abuse.

The methods most frequently used by psychophysiological research include the electroencephalogram (EEG), the recordings of event-related and evoked potentials (ERP/EP) as well as measurements of autonomic, including cardiovascular and electrodermal, activity. These will be introduced in the following sections whenever needed.

ALCOHOL

Studies on alcoholism or alcoholism risk have so far mainly focused on male subjects. The reasons for this bias may be that alcoholism is far more frequent in men than in women, and that evidence for the heritability of alcoholism has been strong in men, but equivocal in women. Recent studies, however, have found genetic influences on alcoholism to be comparable in women and men. The future may thus see an increased interest in research on female subjects. In the present review, studies focusing on female subjects will be specifically mentioned.

Sensory Evoked Potentials

Over two decades ago, reports on electrophysiological abnormalities in adults increasingly surfaced in the literature. Measurements of

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brain stem auditory evoked responses (BAERs) showed a prolonged neural transmission time and delayed peak latencies in alcoholics (Begleiter *et al.*, 1981; Cadaveira *et al.*, 1991). BAERs occur in response to a train of auditory ultra-short-duration clicks. They are characterized by seven peaks that mark the path of the neural impulse from the auditory nerve via the cochlear nucleus and inferior colliculus to higher cortical areas. Neural brain stem transmission time is usually measured as the latency between peaks 1 and 5 of the evoked response. A slowing of brain stem transmission was observed primarily in chronic alcoholics who already showed signs of neurological dysfunction, and substantiated evidence for demyelination of nerve tracts as a consequence of excessive drinking.

Visual evoked potentials (VEPs) can be elicited by a checkerboard-reversal paradigm, in which the colours of a black-and-white checkerboard are inverted about every second. VEPs are characterized by several peaks, including the P100, a positive-voltage deflection occurring about 100 ms after stimulus onset. The P100 has been found to be prolonged in some (Kriss *et al.*, 1982; Meinck *et al.*, 1990; Cadaveira *et al.*, 1991) but not all (Emmerson *et al.*, 1987; Bauer and Easton, 1996), studies in alcoholics. It has been suggested that the presence of a P100 delay depends on the severity of the patients' neurological alterations, since P100 latency is sensitive to neurological disorders such as multiple sclerosis, glaucoma, optical neuritis or occipital tumours. Animal studies indicate that heightened VEP amplitudes may reflect transient cortical hyperexcitability during withdrawal. However, in humans, increased VEP amplitudes persist beyond withdrawal in some (Coger *et al.*, 1976; Emmerson *et al.*, 1987), but not all (Chan *et al.*, 1986) studies. Another measure of CNS hyperexcitability is the amplitude–intensity gradient (A/I slope). It has been found that alcoholics are 'augmenters', who tend to react to increasing stimulus intensity with increasing physiological responses. 'Reducers', on the other hand, tend to reduce their responses to increasing stimulus intensities. Thus, alcoholics show increasing VEP responses (P100 amplitudes) to increasing stimulus intensities (positive A/I slopes), while controls do not. Interestingly, alcohol ingestion reduces augmentation in alcoholics. It has therefore been suggested that alcoholics may use alcohol to dampen their oversensitivity to sensory stimuli. However, enhanced A/I slopes have been found in alcoholics only during early, but not after prolonged abstinence (Coger *et al.*, 1976; Emmerson *et al.*, 1987). Thus, while A/I slopes appear to normalize after withdrawal, P100 amplitudes and latencies may not. Possibly, A/I gradients are a measure of transient CNS hyperexcitability during withdrawal, whereas P100 characteristics may reflect structural changes of visual pathways.

The P300 Component of the Event-Related Potential

The event-related potential (ERP) is a time-locked electrophysiological response to a stimulus and shows a characteristic temporal profile of positive and negative peaks occurring up to about 1000 ms after stimulus onset. These peaks (called 'components') are named after the direction of their deflection from baseline and their latency (e.g. 'N100' denotes a negative-going wave with a peak latency of about 100 ms after stimulus onset). In general, early, 'sensory' components reflect the physical parameters of the stimulus, while later 'cognitive' components reflect the subjective significance of the stimulus and the attention allocated to it. The P300 (also simply called 'P3') is a positive-going cognitive ERP component with a modal latency of 300 ms. The P3 is elicited by stimuli that are novel or that are particularly attended to, independent of the sensory modality in which they occur. A typical method of evoking a P3 is the auditory oddball paradigm: a sequence of frequent tones of a given frequency is presented to a subject. Randomly interspersed are rare tones of a different frequency. The subject is instructed to attend to the rare, target tones (e.g. by counting them or by pressing a button whenever they occur) and to ignore the frequent, non-target tones. The ERP in response to the target tones, but not to the non-target tones, will then show a P3 wave. A P3 can also be elicited by novel non-target tones, i.e. tones that are rare but not attended to. It has been shown that the P3 in response to rare targets (called P3b) is maximal over the parietal cortex, while the P3 as an orienting response to novel non-targets (called P3a) shows a central–frontal distribution. (In the remainder of this chapter, 'P3' will refer to the P3b, unless otherwise stated.) The P3 is thought to reflect the allocation of attentional resources when a new stimulus is processed. It occurs whenever the model of the stimulus environment in the working memory is updated. P3 amplitude increases with the amount of attention demanded by the stimulus and its informational value. P3 latency, on the other hand, is thought to reflect the speed and timing of attentional processing of the stimulus. It increases with increasing level of task difficulty, when degraded stimuli are used and with increasing age. On some cognitive tasks, increased P3 amplitude has been linked to better memory performance, while increases in P3 latency were associated with increased reaction time (Pfefferbaum *et al.*, 1991).

Early studies in chronic male alcoholics indicated ERP deficits in the form of prolonged P3 latency and reduced P3 amplitude. Initially, these findings were thought to reflect a direct consequence of chronic alcohol abuse. However, subsequent attempts to replicate these findings yielded mixed results. While a majority of investigations confirmed the P3 amplitude deficits (Figure XVI-5.1) (Pfefferbaum *et al.*, 1991; Cohen *et al.*, 1995; Holguin *et al.*, 1999a; Hada *et al.*, 2000; Malone *et al.*, 2001; Prabhu *et al.*, 2001), there was a not much smaller number of negative reports (Hermanutz *et al.*, 1981; Steinhauer *et al.*, 1987; Lille *et al.*, 1987; Cadaveira *et al.*, 1991; Hill *et al.*, 1995b, 1999a). P3 latency slowing was even more resistant to replication: most later studies did not find deviations of P3 latency in alcoholics as reported in the early work. These discrepancies indicated that alcohol use alone could not completely account for the observed alterations in P3 characteristics. There had to be some other factor(s) that substantially contributed to the variance in these findings. A number of alternative sources of variance have been evaluated: differences in study sample source, ERP task, severity of alcoholism, length of abstinence, comorbid psychiatric or personality disorders, or family history of alcoholism. We will now examine each one of these candidates for their possible contribution to the conflicting findings.

Sample Source

Alcoholic study subjects have been recruited from three main sources: hospital and VA treatment programmes, community treatment programmes and from the community at large. Typically, there is an increasing degree of cognitive impairment and of the severity of alcoholism from hospitalized patients to community-recruited alcoholics. Since the P3 reflects cognitive processes, it is reasonable to assume that P3 measures may also differentiate between alcoholics from these different sources. A look at the relevant literature, however, shows that neither positive nor negative P3 findings are distributed across these three sources in a systematic way. Particularly, P3 findings obtained in community alcoholics do not differ from those found in their hospitalized counterparts. Glenn *et al.* (1996) explicitly addressed the hypothesis that alcoholics from VA treatment programmes may have stronger P3 deficits than alcoholics from community treatment programmes, reflecting their poorer neuropsychological performance. Contrary to their hypothesis, they found no differences in P3 measures across groups. The conclusion is that differences in the source of subject recruitment do not appear to explain the variability of P3 findings in alcoholics.

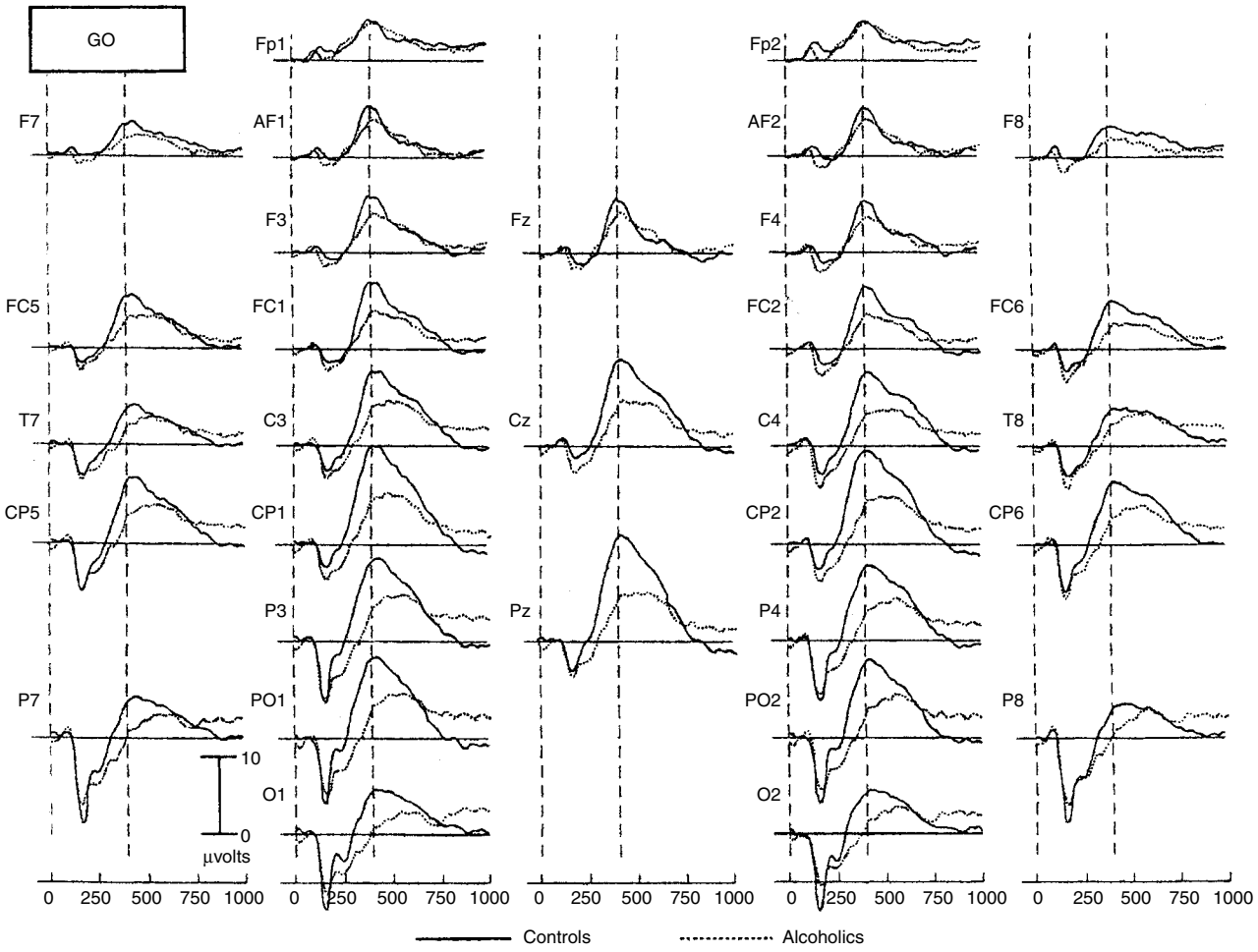

Figure XVI-5.1 Visual P3 amplitudes in controls (solid line) and alcoholics abstinent for 1 month (dotted line) at 31 scalp electrode locations. Note that the size of P3 differences increases from anterior to posterior. Reprinted with permission from Cohen, H.L., Porjesz, B., Begleiter, H. and Wang, W., 1997. Neurophysiological correlates of response production and inhibition in alcoholics. *Alcoholism: Clinical and Experimental Research* **21**, 1398–1406. Copyright 1997 by the Research Society on Alcoholism

ERP Task

Tasks employed to produce ERPs can vary along several axes, two important ones being stimulus modality and level of difficulty. The majority of studies reporting decrements in P3 amplitude used tasks involving visual stimulation, while auditory tasks were less effective in producing P3 alterations in alcoholics. It thus appears that stimulus modality has some bearing on the P3 effects in alcoholics. A higher likelihood for P3 amplitude reductions to occur with visual tasks is also seen in studies of non-alcoholic children or adolescents who are at increased risk for later alcohol abuse owing to a positive family history of alcoholism (see below). It is possible that deficits in P3 amplitude related to current alcoholism or risk for alcoholism are largely restricted to the brain substrate for visual as opposed to auditory P3 generation. An alternative explanation is that the visual tasks used in these studies were more powerful in revealing P3 amplitude decrements in virtue of their being more difficult, i.e. cognitively demanding. In fact, it has been suggested that visual tasks are generally more difficult than auditory tasks because of visual stimuli being less alerting than auditory stimuli, thereby demanding more 'effortful processing'. Apart from this possible general effect of stimulus modality on task difficulty, however, the tasks used to reveal P3 deficits in alcoholics do not seem to be in any consistent way more difficult than those that failed to produce P3 deficits. Pfefferbaum *et al.* (1991) and Realmuto *et al.* (1993) even observed P3 amplitude reductions in an automatic ERP paradigm involving no task and therefore very little processing resources.

Severity of Alcoholism

Assuming that the severity of previous alcohol exposure is one cause of P3 deficits, then the variability in P3 findings in adult alcoholics might stem from the fact that studies reporting negative results included subjects whose previous exposure to alcohol on average was not severe enough to produce significant changes in the P3. This hypothesis, particularly regarding deficits in P3 amplitude, finds little support in the literature. A number of studies failed to find associations between several measures of severity of drinking and visual (Pfefferbaum *et al.*, 1987, 1991; Malone *et al.*, 2001) or auditory (Hill *et al.*, 1995b) P3 amplitude and latency. Olbrich *et al.* (2000) extended these findings to topographical P3 measures, finding no correlation between the gravity centre of visual and auditory P3 scalp distribution and severity of alcoholism. Furthermore, a lack of association between P3 amplitude and drinking history was reported in non-alcoholic young men (Baribeau *et al.*, 1987).

In contrast, Polich and Bloom (1987) reported an association between quantity of alcohol consumed per drinking occasion and reduced amplitude and increased latency of the P3 in non-alcoholic college students. However, in a subsequent study (Polich and Bloom, 1988), they failed to replicate these results. Steinhauer *et al.* (1987) found delayed P3 latency in the more difficult of two auditory ERP paradigms only in alcoholic subjects and their alcoholic siblings, but not in their non-alcoholic siblings or controls. They inferred that P3 latency prolongation could be primarily related to drinking history, and less to a positive family history of alcoholism. It should be noted, however, that this conclusion was not based on a statistical assessment of the direct relationship between drinking history and P3 latency. In sum, it appears that severity of alcoholism as indexed by various measures of alcohol exposure had little influence on P3 characteristics in these studies. This does not necessarily mean that excessive drinking has no effect on P3, but only that there does not seem to be a linear relationship between the two. Porjesz and Begleiter (1981) point out that even among alcoholics with similar drinking histories there are great individual differences in susceptibility to alcohol-related brain deficits, and thus, possibly, in P3 characteristics. In fact, this individual variability may be the main reason for the general lack of correlations of severity of alcoholism with P3 measures.

Length of Abstinence

Brain pathology in most alcoholics has been shown to recover at least partially with increasing time of abstinence. The question therefore arises whether abstinence may also normalize the observed deficits in the P3 component. The literature offers little support for this view. Alterations in P3 amplitude and latency can invariably be observed in alcoholics with different durations of abstinence ranging up to several years (Whipple *et al.*, 1991; Glenn *et al.*, 1994). Several studies (Pfefferbaum *et al.*, 1991; Realmuto *et al.*, 1993; Hill *et al.*, 1995b) found no significant correlations of duration of abstinence with any ERP component measure in alcoholics abstinent up to 3 months. This apparent immunity of P3 deficits to the length of abstinence has attracted several explanations: either the effects of alcohol on the neural substrate of P3 generation are irreversible, or the persistent P3 deficits are not primarily the consequence of alcohol use but of some other, co- or premorbid, factor. The first conjecture seems at odds with findings of brain recovery in abstinent alcoholics, but cannot be definitely ruled out. The second suggestion has gained credibility in the light of studies demonstrating reduced P3 in alcoholics with comorbid internalizing disorders like depression and externalizing disorders like antisocial personality disorder, as well as a positive family history of alcoholism. These issues will be discussed below.

Comorbid Psychopathology

P3 alterations are not specific to alcoholism. Decreases in P3 amplitude have, for example, also been found in clinical psychiatric disorders like schizophrenia or depression and in externalizing and personality disorders. There is substantial comorbidity between psychiatric disorders and alcoholism or other substance dependence. It is therefore possible that the positive P3 findings in alcoholics might be due to the presence of comorbid psychiatric disorders rather than to alcohol abuse itself. Two gender-specific forms of this hypothesis have received a fair amount of support in recent research. In women, there is a link between internalizing disorders, particularly depression, alcoholism and P3 deficits. Parsons *et al.* (1990) investigated male and female alcoholics and failed to find an effect of alcoholism on P3 in women without comorbid psychiatric diagnosis, whereas the effect in men was present despite the lack of a psychiatric diagnosis. Hill *et al.* (1999a) compared depressed with non-depressed female alcoholics and controls, and found auditory P3 amplitude reductions in the depressed group relative to the non-depressed alcoholics and the controls. There was no difference between the non-depressed alcoholics and the controls. The authors concluded that the observed P3 deficits in alcoholic women were most likely due to comorbid depression. Using a visual P3 paradigm, Prabhu *et al.* (2001) found no effect of depression on P3 decrements in alcoholic women. However, these researchers relied on a not further specified self-report measure of depression, which makes their findings difficult to interpret.

In men more than in women, alcohol and drug abuse are frequently comorbid with and predicted by a spectrum of externalizing disorders, which, in turn, has been found to be associated with P3 amplitude reductions. These externalizing disorders include antisocial personality disorder (ASPD) in adults, as well as conduct disorder (CD), oppositional defiant disorder (ODD) and attention deficit with hyperactivity disorder (ADHD) in children. A common basis underlying these disorders is the lack of behavioural inhibition and control, as expressed by personality traits such as low constraint and impulsivity. These and related personality traits are known to be significant risk factors for the development of externalizing psychopathology and substance abuse. With regard to alcoholism in particular, low harm avoidance and high novelty-seeking in children have been identified as personality traits predictive of later alcoholism. Several studies indicate that externalizing psychopathology as an expression of these critical personality traits is characterized by reduced P3 amplitudes. Bauer *et al.* reported significantly reduced frontal P3 amplitudes in a sample of non-alcoholic (Bauer *et al.*, 1994) and cocaine-abusing (Bauer, 1997) men with ASPD, independent of any family history of alcoholism. Similarly, Bauer and Hesselbrock (1999) found P3 amplitude reductions in adolescents with CD that were unrelated to a family history of substance dependence. Some studies have found that in addition to being related to ASPD and childhood externalizing disorder, small P3 amplitudes were also associated with increased substance (including alcohol) dependence (Carlson *et al.*, 1999; Costa *et al.*, 2000) and a family history of alcoholism (O'Connor *et al.*, 1994). Several longitudinal studies support these results. Stabenau (1990) found that ASPD and a positive family history of alcoholism (FHA) made independent, additive contributions to the development of alcoholism 5 years later. The risk for alcoholism was increased threefold by an ASPD diagnosis and 1.5-fold by an FHA. Windle (1990) reported that antisocial delinquent behaviour in early adolescence was significantly predictive of alcohol use and dependence in late adolescence, particularly in men. A possible contribution of family history status was not analysed in that study. In a male sample, Mannuzza *et al.* (1993) found that childhood ADHD predicted ADHD symptoms, ASPD and drug abuse at age 26, with significant comorbidity between ASPD and drug disorders. There are a few contrasting reports. Herning *et al.* (1989) studied delinquent male adolescents and found that the presence of drug use, not delinquency, differentiated between low and high P3 amplitude, although delinquency itself was associated with alterations in other ERP components and with delayed BAERs. A very recent investigation by Malone *et al.* (2001) revealed P3 amplitude decrements in 'pure' alcoholics without comorbid psychiatric diagnoses. In pure ASPD subjects, the reduction in P3 amplitude did not reach statistical significance, but drug-using subjects with concomitant ASPD had particularly low amplitudes. The authors concluded that the presence of alcoholism alone may be sufficient to affect P3 amplitude, but that drug use and ASPD can amplify this effect.

Summed up, comorbid depression may largely account for the P3 deviations found in alcoholic women. In male alcoholics, the presence of comorbid CD or ASPD explains some of the variance in P3 findings, but alcohol and drug use appear to make additional, independent contributions.

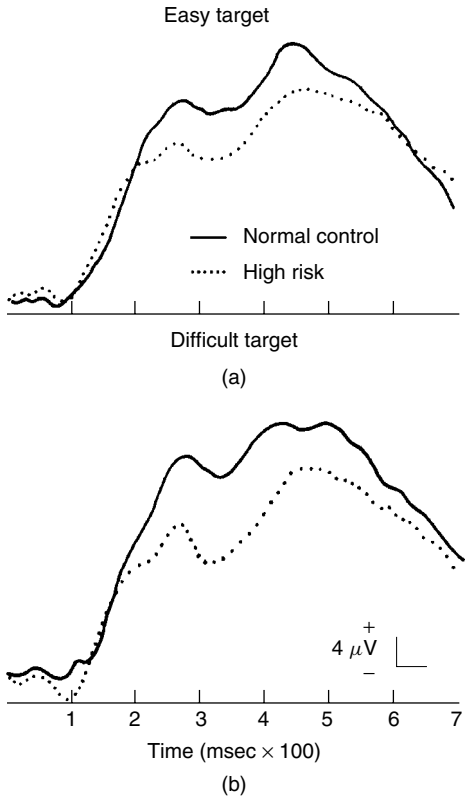

Figure XVI-5.2 Visual P3 amplitudes in control children and children who are at high risk for alcoholism due to a positive family history of alcoholism. High-risk children have significantly smaller amplitudes in both easy and difficult task conditions. Reprinted with permission from Begleiter, H., Porjesz, B., Bihari, B. and Kissin, B., 1984. Event-related brain potentials in boys at risk for alcoholism. *Science* **225**, 1493–1496. Copyright 1984 by the American Association for the Advancement of Science

Family History of Alcoholism

Two decades ago, two seminal papers first reported decrements in P3 amplitude in non-alcoholic boys (Begleiter *et al.*, 1984) and young men (Elmasian *et al.*, 1982) who were at risk for developing alcoholism due to a positive FHA (Figure XVI-5.2). A commonly applied criterion for a positive FHA is the presence of one or several alcohol-dependent or -abusing first-degree relatives (parents, siblings). Because of the strong heritability of alcoholism, family history positive (FHP) individuals are at high risk for developing alcoholism. The findings by Begleiter and colleagues and Elmasian and colleagues opened up the intriguing possibility that reduced P3 amplitudes in high-risk children could be a psychophysiological marker for an inherited susceptibility to alcoholism. The research efforts that followed these initial findings largely confirmed the presence of P3a and P3b decrements in FHP children (Begleiter *et al.*, 1987; Hill and Steinhauer, 1993a, Brigham *et al.*, 1995; Hill *et al.*, 1995a, 1995c, 1999b), adolescents (van der Stelt *et al.*, 1997; Steinhauer and Hill, 1993) and young adults (O'Connor *et al.*, 1987; Ramachandran *et al.*, 1996; Cohen *et al.*, 1997; Ramsey and Finn, 1997; Holguin *et al.*, 1999b; Hada *et al.*, 2001). In a meta-analysis of this literature, Polich *et al.* (1994) came to the conclusion that positive P3 findings in FHP individuals were most consistently found in studies using difficult visual ERP tasks. Thus, as in studies of adult alcoholics, the visual stimulus modality is more effective in producing P3 differences in FHP subjects, but task difficulty appears to be more relevant to P3 in FHP than in alcoholic subjects. Of course, these two types of studies cannot be completely separated. In fact, one important question is to what extent and how family history status may have interfered with the results attributed to the effects of alcohol abuse in alcoholism studies. Several studies investigated alcoholism and FHA status in the same subject samples, all between 30 and 40 years of age. Pfefferbaum *et al.* (1991) tested male alcoholics in an automatic and two easy auditory and visual ERP paradigms and found significant auditory, and near-significant visual P3 decrements in FHP subjects. They also reported a correlation between increasing family density of alcoholic relatives and P3 amplitude. Similarly, Cohen *et al.* (1995) found a significant auditory P3 amplitude reduction over the occipital lobe in high-density compared to low-density alcoholics. Using a mixed audiovisual oddball paradigm, Parsons *et al.* (1990) found no FHA effect in either a male or female alcoholic sample. However, a subgroup of their male sample had earlier shown a significant FHP effect on P3 amplitude in the same audiovisual paradigm (Patterson *et al.*, 1987). Neither alcohol nor FHA effects on auditory P3 measures were found by Hill *et al.* (1995b) in male alcoholics from high-density families, using a counting task and a choice-reaction task. The same paradigm was used by Steinhauer *et al.* (1987), who reported an FHA effect on P3 latency, but not amplitude. Hill and Steinhauer (1993) employed auditory and visual ERP paradigms of increasing difficulty in an exclusively female sample, and found P3 decrements across all levels of task difficulty in alcoholic FHP subjects, but not in their non-alcoholic FHP sisters, indicating an effect of alcoholism, but not FHA. A similar lack of an FHA effect in female alcoholics was reported by Prabhu *et al.* (2001). Finally, results from the longitudinal, multi-centred Collaborative Study on the Genetics of Alcoholism (COGA) in mixed-gender samples demonstrated P3 decrements in alcoholics vs. non-alcoholics, but also in unaffected relatives of alcoholics vs. unaffected relatives of controls, indicating both an FHA and an alcohol effect (Porjesz *et al.*, 1998; Hesselbrock *et al.*, 2001).

Although these reports, particularly the opposite findings of Patterson and colleagues and Parsons and colleagues in overlapping subject samples, are difficult to reconcile, it appears that FHA effects are more consistently found in male than in female subjects, while task difficulty and modality do not seem to influence P3 findings in FHP alcoholics. Possible clues to understanding this putative gender-specific effect might come from a hypothesis that has been most strongly advocated and supported by Hill and colleagues. These authors argue that the P3 decrements seen in FHP children reflect a developmental delay. The idea is that FHP children are lagging behind family history negative (FHN) children with respect to the maturation of brain processes underlying P3 generation. Thus, at a given age, FHP children will evince smaller P3 amplitudes than FHN control children. Assuming that these differences do not resolve by adulthood in boys, we could attribute the P3 differences in alcoholic FHP men found in some studies to a developmental delay persisting into adulthood.

However, this assumption is not consistently supported by the relevant work. In a large longitudinal study (Hill and Steinhauer, 1993a; Steinhauer and Hill, 1993; Hill *et al.*, 1999a), Hill and co-workers followed a group of FHN and FHP children up to 7 years to document the developmental course of auditory and visual P3 amplitude from childhood (age 8) to late adolescence (age 18). These researchers found that auditory P3 amplitude increases with age, whereas visual P3 amplitude decreases with age. With regard to family history status, the earlier studies by this group indicated that the auditory P3 amplitude in FHP boys starts at the same level as in FHN boys, but increases much more slowly with age, producing increasing differences with increasing age. Visual P3 amplitude in FHP boys, however, started at a lower level than in FHN control boys, and increased with age to catch up with control levels by about age 18. In girls, the developmental trajectories for

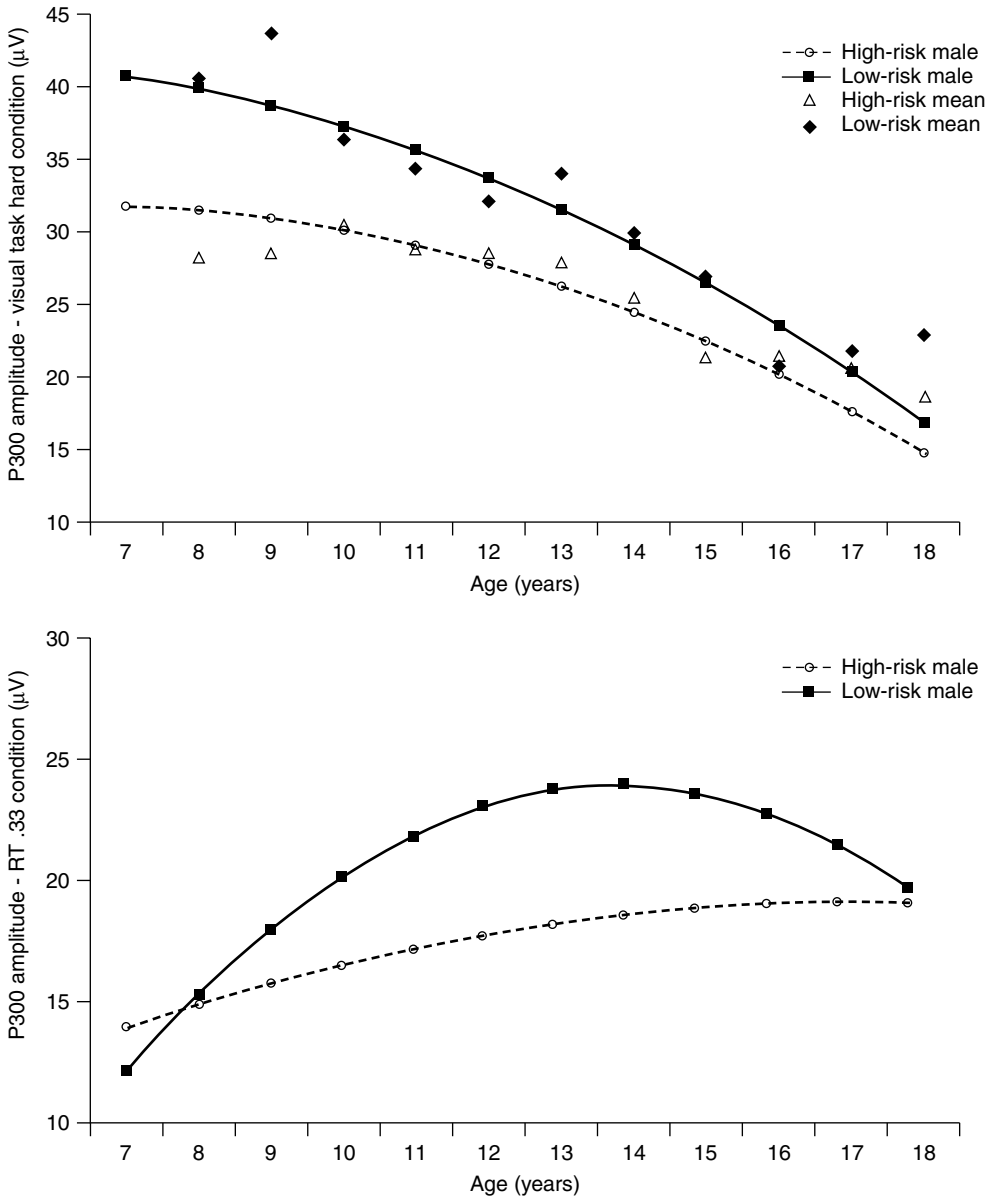

Figure XVI-5.3 Developmental trajectories of visual (above) and auditory (below) P3 amplitudes in 8- to 18-year-old male children at low and high risk for developing alcoholism. Note the convergence of visual and auditory P3 trajectories at age 18 and the inverted U-shaped curve for auditory P3 in low-risk children. Reprinted by permission of Elsevier Science from 'Developmental delay in P300 production in children at high risk for developing alcohol-related disorders' by Hill, S.Y., Shen, S., Locke, J., Steinhauer, S.R., Konicky, C., Lowers, L. and Connolly, J., *Biological Psychiatry* **46**, 970–981. Copyright 1999 by the Society of Biological Psychiatry

the auditory and visual P3 amplitude did not differ significantly by family history status. At this point, we find our assumption of persisting P3 decrements in boys but not girls confirmed, but only for the auditory modality. However, the 1999 study, using a much larger number of subjects and quadratic rather than linear modelling, found that the auditory P3 trajectories in boys did not continuously diverge with increasing age, but met again at age 18. This difference was obtained because the quadratic modelling algorithm fitted an inverted U-shaped curve to the amplitude data from the FHN boys. As a result, the trajectory of auditory P3 amplitudes in FHN boys started and ended at the same level as those in FHP boys, but in between it was sharply convex, resulting in the largest differences between FHN and FHP boys occurring at age 12–14 (Figure XVI-5.3). Thus, the latest findings indicate that developmental delays in the size of the P3 in both female and male FHP children resolve by adulthood, leaving little room for arguing that P3 decrements in male FHP alcoholics are residual childhood effects.

Summary

We can now summarize our extensive discussion of the possible sources of variance in the P3 findings in adult alcoholics. When observed, P3 decrements probably reflect to some extent an independent effect of chronic alcohol abuse that is not linearly related to the severity of drinking, indicating a more complex

relationship. P3 decrements occur independently of the duration of abstinence, suggesting that they are irreversible or pre-existing. In some, but not all, studies a positive FHA was found to make an additive contribution to the P3 deficits in alcoholics. This effect may be specific to men, but whether it represents a residual childhood effect or not is unclear. Externalizing disorders such as ADHD, CD and adult ASPD appear to make another independent contribution to the P3 decrements in some studies, particularly in alcoholic men. In alcoholic women, adult P3 reductions may be partly attributable to comorbid depression. P3 differences are more reliably detected in the visual than in the auditory modality, but are largely unaffected by task difficulty. There is a similar lack of effect of the source of subject recruitment. Delays in P3 latency may reflect structural brain changes related to chronic alcohol use, since P3 delays are typically found in organic brain disease.

Is the P3 a Marker for Alcoholism Risk?

The findings of P3 decrements in FHP children or adolescents are rather robust, which has led some (e.g. Iacono, 1998; Hesselbrock *et al.*, 2001) to propose that a reduced or small P3 response could be a psychophysiological 'endophenotype', i.e. a risk marker for the later development of alcoholism. The concept of an endophenotype defines it as an endogenous characteristic of a person that is a product of the predisposing genotype (Iacono, 1998). According to Iacono, there are several requirements for something to qualify for an endophenotype. Let us briefly examine how well a small or reduced P3 amplitude satisfies these criteria.

Potential endophenotype is evident in unaffected relatives.

This requirement is met for children and adolescents, where P3 decrements can be found in unaffected, i.e. non-alcoholic, individuals with a positive FHA. It is also met for unaffected male, but possibly not female, adults.

It is evident in affected relatives.

As discussed above, about half of the studies in FHP alcoholic men have observed P3 decrements. Two studies in FHP alcoholic women have not. This requirement is therefore only partly satisfied.

It is specific to the disease in question.

This requirement is not satisfied. As mentioned earlier, P3 deficits are not specific to alcoholism. Both in adults as well as in children and adolescents, they are also associated with other substance abuse, internalizing disorders such as depression and schizophrenia, or externalizing disorders such as ADHD, ODD, CD and ASPD.

It has predictive power.

The evidence we have so far indicates that this requirement is met. Two longitudinal studies in teenagers reported that reduced P3 amplitude predicted alcoholism or general substance abuse 4 (Berman *et al.*, 1993) and 8 (Hill *et al.*, 1995a) years later, respectively.

It indicates risk for the disease.

This requirement is met: the prevalence of alcohol and substance dependence is significantly higher in adolescents with a small rather than a large P3, irrespective of family history status (Carlson *et al.*, 1999). Furthermore, the presence of a small P3 is linked to a positive FHA, which in turn has been shown to increase the risk for alcoholism by about five times or more (Cloninger *et al.*, 1981).

It is heritable (follows from the definition).

This requirement is satisfied: various characteristics of the ERP, including the P3 amplitude, are heritable (e.g. O'Connor *et al.*, 1994a; Katsanis *et al.*, 1997; Almasy *et al.*, 2001).

As we see, a small P3 response basically satisfies four out of six criteria required for an endophenotype. Fortunately, the failure to completely satisfy the other two requirements need not worry us too much — it merely calls for a modification of the scope of our marker. Even if the second requirement were never met and the P3 findings in adult FHP alcoholics turned out to be unrelated to a heightened risk for alcoholism, a small P3 amplitude would still be a valuable risk marker in children and adolescents up to about age 18 (as seen in the work on developmental delay discussed above). This means that, although it would not identify adult high-risk individuals (both affected and unaffected), it would identify high-risk non-adults before they reach the age of onset of substance abuse, which seems to us to be much more important with regard to early prevention and intervention. The other issue is specificity. Here we should first say that the presence of our marker in diseases other than alcoholism does not necessarily mean that the marker indexes a *risk* for these other diseases. For example, while reduced P3 amplitude can be found in schizophrenia, it does not seem to *predict* or indicate an increased risk for this disorder in unaffected non-adults. However, our marker also appears to index risk for externalizing (and possibly internalizing) psychopathology and the abuse of substances other than alcohol. But this should not lead us to discard the marker, since it is unreasonable to expect a complete absence of the endophenotype in disorders that are comorbid with the target disorder and show considerable symptom overlap. In fact, it is even possible, although not necessarily the case, that the presence of the marker in a cluster of disorders indicates a shared aetiology, which may finally lead to a redefinition of these disorders at the clinical level.

In conclusion, our reading of the extant literature supports the following modified definition: *The presence of a small or reduced P3 amplitude is an endophenotype in children and adolescents marking the risk for alcoholism and other substance disorders as well as a spectrum of externalizing (and possibly internalizing) disorders.*

Other Components of the ERP

Although the P3 component has attracted most research, earlier ERP components, including the N100, P100, N200 and P200, have also been studied in alcoholics. The few studies that have focused on female subjects failed to find differences in pre-P3 components (Parsons *et al.*, 1990; Hill and Steinhauer, 1993). The results obtained in male subjects are mixed: for every positive finding, there are usually equally many, if not substantially more, negative findings. The most robust results are decreases in N100 amplitude and increases in N200 latency, while alterations in P100 or P200 characteristics are seldom reported.

The N100 is a negative wave with a typical post-stimulus latency of 120 ms. The N100 is larger after stimuli that occur in an 'attended channel', regardless of whether they are task relevant (i.e. targets) or task irrelevant (i.e. non-targets). The attended channel can be stimulus modality or some other stimulus dimension. Besides having deceased N100 amplitudes, alcoholics also failed to enhance N100 amplitude in response to stimuli in the attended vs. non-attended channel (Lille *et al.*, 1987; Parsons *et al.*, 1990). Lille *et al.* (1987) even found an inverted N1 effect, with alcoholics responding more strongly to stimuli in the non-attended channel. These findings can be interpreted as a failure of selective attention. Alcoholics seem unable to discriminate electrophysiologically between signals from the attended and non-attended channel.

The N200 is a negative wave occurring at around 200 ms after stimulus delivery. It is an automatic reaction to deviant stimuli within a homogeneous, repetitive stimulus sequence, reminiscent of the orienting response. It is largely independent of task relevance or stimulus significance. The N200 or its subcomponent, the N2a,

is therefore also called 'mismatch negativity' (MMN), because it is thought to be produced by a comparison process between the current deviant sensory input and a neural representation or 'template' of the 'standard input' (Realmuto *et al.*, 1993). A mismatch between the current and the standard input will elicit an MMN response. The latency of this component increases with increasing difficulty of discrimination between the standard and the deviant stimuli, suggesting that MMN latency is an index of stimulus evaluation time. Increases in N200 latency reported in alcoholics may reflect delays in stimulus evaluation (Porjesz *et al.*, 1987; Parsons *et al.*, 1990; Cadaveira *et al.*, 1991). Porjesz *et al.* (1987) additionally observed that alcoholics as opposed to controls did not show increasing N200 latencies with increasing difficulty of stimulus discrimination. Given a concomitant delay in P3 latency, these authors concluded that their alcoholic subjects have difficulties evaluating stimuli either for task relevance or for ease of discrimination.

A few studies further reported abnormalities in the amplitude of the MMN (Realmuto *et al.*, 1993; Ahveninen *et al.*, 2000), although in different directions. Realmuto and colleagues interpreted the MMN decrements found in their sample of alcoholic men as possibly reflecting a deficient neuronal template, which may fail to form in the first place, rapidly decay, or may be inaccessible. Ahveninen and colleagues, however, reported increased MMN amplitudes in alcoholic men with similar characteristics. They further found increased MMN responses to be significantly correlated with increased distractibility by the task-irrelevant deviant stimuli and with poorer hit rates. The authors took these data to indicate impaired neural inhibition of involuntary attention shifting. Despite MMN deviations in opposite directions, both reports agree that their findings represent a deficit in mismatch processing. The source of the differences between the two reports is unclear, but may be related to differences in task (automated processing vs. forced-choice-reaction time task) or in component detection. In view of the many negative reports, the findings concerning MMN in particular, and pre-P3 components in general, should be interpreted cautiously. The reasons for the mixed picture of results are currently unknown and await future research.

Topographical ERP Changes

So far, we have focused on the size and latency of various ERP components and ignored their topographical dimension. This approach reflects the preponderance of reports in the literature that used very few electrode sites (typically the midline leads Fz, Cz, Pz, and sometimes two adjacent bilateral leads) and offers little in the way of topographical analysis. Only a few studies have made recordings from the full or even an extended 10/20 system array. What most studies with reduced electrode sets agree on is that deviations of (primarily visual) P3 amplitude in alcoholics are largest over parietal areas (Pz) (Patterson *et al.*, 1987; Porjesz *et al.*, 1987; Parsons *et al.*, 1990; Whipple *et al.*, 1991; Glenn *et al.*, 1996), although some reports located the largest deviations at central (Cz) (Olbrich *et al.*, 2000) or right central (C4) (Emmerson *et al.*, 1987) recording sites.

Studies using a full electrode array have yielded conflicting results. Some studies showed that visual P3 decrements in alcoholics follow a gradient from anterior, where they are smallest, to posterior, where they are largest (Figure XVI-5.1) (Pfefferbaum *et al.*, 1991; Cohen *et al.*, 1995, 1997). More recent work has found the opposite: differences in visual P3 amplitude in alcoholics were more pronounced at anterior than posterior sites (Costa *et al.*, 2000; Prabhu *et al.*, 2001). Prabhu and colleagues also used a new EEG source localization method called low-resolution brain electromagnetic tomography (LORETA; Pascual-Marqui *et al.*, 1994; Pascual-Marqui, 1999) and confirmed prefrontal P3 differences located in the right dorsolateral prefrontal cortex and ventromedial frontocentral cortex. Not only the P3b, but also the visual and auditory P3a components have been topographically analysed and found to be globally different between male alcoholics and controls (Holguin *et al.*, 1999a; Hada *et al.*, 2000). In adult FHP individuals, topographical P3a differences were found globally (Holguin *et al.*, 1999b), frontally (Hada *et al.*, 2001), and posteriorly (van der Stelt *et al.*, 1997). The reason for the discrepancies in P3b results in alcoholics and in P3a results in FHP individuals is currently unclear.

Olbrich *et al.* (2000) analysed the electric gravity centres (centroids) of the negative and positive half-field of the N100 and P3 distribution, and found a shift of the positive P3 centroid to the right and of the negative P3 centroid posteriorly and to the left. The authors remark that a similar right shift of the positive P3 centroid along with decreased N100 amplitudes (as also found by Olbrich *et al.*) can be observed in schizophrenic patients, suggesting a common cerebral dysfunction in alcoholism and schizophrenia manifested by deficits in attentional filtering.

EEG

The EEG in Alcoholics

The use of the EEG in the study of acute and chronic alcohol effects goes back over half a century. Early studies using clinical EEG evaluation were primarily concerned with the search for salient abnormalities such as irregular spike and wave complexes characteristic of withdrawal seizures. With the advent of increasingly powerful tools for computerized analysis, the focus of research shifted to the identification of more subtle quantitative EEG (qEEG) changes. Although much the same confounding factors as discussed in the ERP section also afflict the study of EEG in alcoholics, there have been some fairly consistent findings. The most common changes in baseline EEG are elevated beta (>12 Hz) (Spehr and Stemmler, 1985; Kaplan *et al.*, 1985; Frank *et al.*, 1986; Krauss and Niedermeyer, 1991; Bauer, 1994a; Costa and Bauer, 1997; Günther *et al.*, 1997) and decreased alpha (8–12 Hz) activity (Coger *et al.*, 1976; Spehr and Stemmler, 1985; Kaplan *et al.*, 1985). Increases (Kaplan *et al.*, 1985; Pollock *et al.*, 1992) or decreases (Propping *et al.*, 1981) in delta or theta activity are also occasionally reported.

Despite its being frequently observed in alcoholics, elevated beta is not specific to alcoholism and probably is not even directly related to alcohol use. In fact, it is likely that multiple independent factors can produce increases in beta activity. For example, elevated beta activity has been found to depend on the degree of cognitive impairment (Coger *et al.*, 1978) and the level of medication administered during withdrawal (Kaplan *et al.*, 1985) in alcoholics, on the presence of depression (Pollock and Schneier, 1990), a positive FHA (Gabrielli *et al.*, 1982; Pollock *et al.*, 1995) or ASPD (Bauer and Hesselbrock, 1993). This multifactorial dependence may also explain why some studies found beta activity to normalize with increasing abstinence (Kaplan *et al.*, 1985; Pollock *et al.*, 1992), while others found beta changes to persist for up to half a year (Costa and Bauer, 1997). Recent work has shown that beta activity can be independent of drinking history, and may index the risk for relapse in alcohol and other substance abusers. Bauer (1994a) found that abstinent alcohol-dependent patients who later relapsed were characterized by enhanced high EEG beta activity while those who would remain abstinent did not differ from controls. Bauer (2001d) extended these findings, demonstrating that fast beta activity significantly predicted later relapse (with 75% accuracy) in a group of alcohol- and drug-abusing subjects. The beta elevation in relapsing subjects was related to increased levels of childhood CD and paternal alcoholism, but not to drug use. In a similar manner, other studies have observed beta elevations in alcohol- and cocaine-dependent (Costa and Bauer, 1997) as well as in 'ecstasy'-using subjects

(Gamma *et al.*, 2000) and these changes were not correlated to the degree of drug exposure. Thus, elevated beta may be a premorbid factor common to the abuse of several substances.

Low alpha activity in alcoholics may also have different sources. Most studies indicate that decreases in alpha activity recover with continued abstinence (Zilm *et al.*, 1981; Pollock *et al.*, 1992), suggesting that alpha is a state-dependent measure, possibly related to transient withdrawal-related CNS hyperexcitability. However, low alpha voltage may also be a heritable trait in a subgroup of alcoholics with comorbid anxiety disorder (Enoch *et al.*, 1999).

Despite the many possible sources for EEG frequency changes, some investigations suggest a general relationship between the degree of EEG changes and the severity of alcoholism or alcohol-related symptoms. Most studies that report EEG deviations have been conducted in hospitalized patients, who typically exhibit the most severe forms of alcoholism in the population. An investigation in young, abstinent, medication-free, non-hospitalized community alcoholics revealed no EEG changes (Emmerson *et al.*, 1987). Similarly, a subgroup of alcoholics with little neurological and cognitive impairment showed a normal EEG compared to both a neurologically and cognitively impaired subgroup (Zilm *et al.*, 1980). Early-onset alcoholics within a larger group of alcoholics were found to have the most EEG abnormalities (Johannesson *et al.*, 1982). As a caveat, it should be said that Emmerson *et al.* (1987) limited frequency analysis to the range between 4–14 Hz, thus excluding most beta activity. Also, the findings in early-onset alcoholics may not be a consequence of severe alcoholism but could mark the presence of a predisposing trait.

The EEG in High-Risk Individuals

A branch of alcoholism research has been concerned with possible EEG differences between non-alcoholic FHP and FHN children and adolescents in order to evaluate potential EEG markers for alcoholism risk, but also to identify the possible contribution of family history status to the findings in adult alcoholics. These studies have either been conducted under baseline conditions or have involved an ethanol challenge. The results were mixed, particularly those concerning baseline EEG findings. A majority of studies reported alpha differences in the baseline or placebo-induced EEG of (predominantly male) FHP children, adolescents and young adults. But while some observed lower levels of slow (<9 Hz) and fast (>9 Hz) alpha activity (Pollock *et al.*, 1983) others found higher levels of fast alpha and no difference in slow alpha activity (Ehlers and Schuckit, 1991; Bauer and Hesselbrock, 1993). Still others reported no alpha differences at all (Kaplan *et al.*, 1988; Cohen *et al.*, 1991). Higher levels of beta have been found in FHP children (Gabrielli *et al.*, 1982), young FHP adults (Bauer and Hesselbrock, 1993), and in a sample of older (65 years) FHP subjects (Pollock *et al.*, 1995), but only in male individuals. Several studies, however, failed to confirm this finding in young FHP men (Kaplan *et al.*, 1988; Ehlers and Schuckit, 1990; Cohen *et al.*, 1991).

As with the studies in alcoholics, the variability in beta findings may have various origins. Bauer and Hesselbrock (1993) found higher beta levels in FHP subjects only in the presence of concomitant ASPD. Some of the older FHP subjects investigated by Pollock and colleagues had relatives with depressive, bipolar or substance use disorders other than alcoholism, but without ASPD. The parents of the FHP children in the report by Gabrielli *et al.* were not only alcoholic, but also included schizophrenic, psychopathic and character-disordered individuals. Thus, it is possible that rather than a positive FHA being the primary determinant of higher beta activity in these studies, other factors such as comorbid ASPD or an inherited risk for personality, mood or psychotic disorder were responsible. In fact, it has been proposed that high beta activity may have a similar function in EEG studies as small P3 amplitudes have in ERP studies: that of an unspecific marker for a spectrum of psychopathology including substance abuse and psychiatric disorders.

The variability in alpha results may reflect a — perhaps genetically mediated — electrophysiological heterogeneity within FHP individuals. Pollock *et al.* (1988) found that two subgroups of young FHP men, formed on the basis of differences in their EEG alpha responses to ethanol, also differed with respect to indices of self- and observer-rated alcohol intoxication. The first group (called HR-A) showed larger ethanol-induced decreases of mean alpha frequency than controls and less self-reported intoxication. The second group (HR-B), which had normal ethanol-induced decreases of mean alpha frequency, showed even fewer signs of self- and observer-rated intoxication, compared to both the HR-A and control group. The authors relate these two different profiles to two different theories of the aetiology of alcoholism. One (Tarter *et al.*, 1984) posits that pre-alcoholics are characterized by excessive physiological lability and are prone to use alcohol because it stabilizes their physiological functions. The other (Goodwin, 1981) postulates that pre-alcoholics have a high 'innate' tolerance to alcohol and are therefore less physiologically sensitive to alcohol. Both theories predict that pre-alcoholics are less subjectively sensitive to alcohol. Obviously, the response profile of subgroup HR-A with their high EEG and low subjective response would be more consistent with Tarter and colleagues' theory, while the low EEG and subjective responses of subgroup HR-B are more compatible with the perspective of Goodwin. There is evidence from other studies that bears on these theories. Abnormally low subjective as well as cardiovascular and electrodermal sensitivity to alcohol indeed seems to be a general characteristic of high-risk, FHP individuals, as evidenced by lower ratings of self-reported intoxication (Pollock, 1992), and reduced heart rate, blood volume amplitude and skin conductance (Finn and Pihl, 1987; Pihl *et al.*, 1988; Finn *et al.*, 1990) after alcohol intake. Schuckit (1994) even found that a low subjective response to alcohol in male adolescents entailed a fourfold risk of developing alcoholism (but no other substance abuse) 12 years later, indicating that subjective sensitivity to alcohol may have predictive power.

In addition, EEG alpha responses to alcohol may be predictive of later alcoholism. A preliminary but intriguing finding by Volavka *et al.* (1996) indicates that the two FHP subtypes identified by Pollock and colleagues may be at differential risk for developing alcoholism. These researchers measured ethanol-induced mean alpha changes in 19-year-old sons of alcoholic fathers and conducted psychiatric follow-up assessments 10 years later. They found that those FHP subjects with the smallest amount of ethanol-induced alpha slowing (corresponding to the HR-B group) developed alcohol dependence 10 years later, whereas those with larger amounts of alpha slowing (the HR-A subjects) were either 'mere' alcohol abusers or without alcohol-related problems.

Ethanol-induced changes of EEG alpha and subjective ratings of intoxication are not unrelated. In a study by Bauer and Hesselbrock (1993) the presence of ethanol-induced alpha slowing (i.e. a relative decrease in fast vs. slow alpha activity) was associated with reduced feelings of intoxication. Interestingly, these researchers also found that alpha acceleration (i.e. a relative *increase* in fast vs. slow alpha activity), as observed in FHP individuals after placebo, was associated with *increased* feelings of intoxication. A related observation was made by Kaplan *et al.* (1988), who found alpha acceleration in FHP individuals after *ethanol*, not placebo, but also associated with higher ratings of intoxication.

By way of summarizing the psychophysiological findings in FHP individuals, we would like to offer the following hypotheses: (1) FHP subjects show a large variability in ethanol-induced alpha frequency changes, which may reflect heritable EEG patterns associated with different subtypes of high-risk subjects that may be predisposed for different types of alcoholism, possibly involving different aetiological pathways; (2) low levels of subjective, and possibly autonomic, sensitivity to alcohol may also predict or

predispose to later alcoholism; (3) ethanol-induced alpha slowing is associated with decreased intoxication, while alpha acceleration is related to increased intoxication in FHP individuals; (4) high levels of beta activity may serve as an unspecific marker for a spectrum of psychopathology including substance abuse and psychiatric disorders.

Although at present these hypotheses do not have a strong empirical basis, they nevertheless outline an area of research whose exploration may be very fruitful for understanding how psychophysiological processes influence, and are influenced by, heritable and environmental risk factors for the development of alcoholism.

COCAINE

ERP/EP

P300

There are relatively few electrophysiological studies in chronic cocaine abusers. This paucity may be due to the fact that cocaine dependence, as opposed to alcoholism, has only recently become a socially relevant problem. Like alcoholics, adult cocaine abusers or addicts have been found to show decrements in P3 amplitude (Herning *et al.*, 1990; Branchey *et al.*, 1993; Kouri *et al.*, 1996; Herning and King, 1996; Biggins *et al.*, 1997; Bauer, 2001b) and delays in P3 latency (Amass *et al.*, 1989; Biggins *et al.*, 1997; Bauer, 2001b). While P3 decrements are quite consistently observed during withdrawal or early abstinence, it is unclear whether P3 amplitudes recover with longer periods of abstinence. Two studies by Bauer (1997, 2001b) reported correlations between increasing time of abstinence from 1 to 5 months and increasing P3a and P3b amplitudes (Figure XVI-5.4). Bauer also found that P3a decrements were seen in cocaine-dependent patients only up to 2 months post withdrawal and then disappeared. Biggins *et al.* (1997), on the other hand, did not find evidence for recovery of P3a amplitudes in patients dependent on cocaine or dually dependent on cocaine and alcohol. However, these patients had been drug free for 2–6 weeks, which is less than the 2 months of abstinence that marked the transition from reduced to normal P3a amplitude in Bauer's 1997

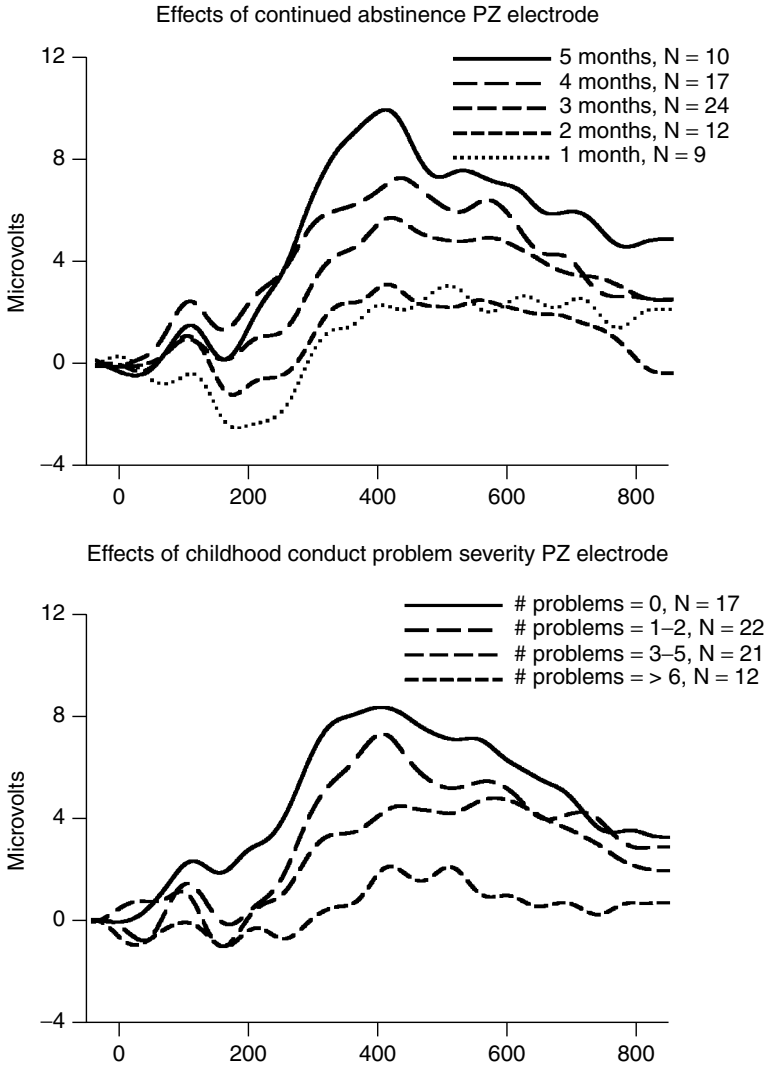

Figure XVI-5.4 Visual P3 amplitude in cocaine-, cocaine–alcohol- and opioid-dependent subjects recovers with increasing time of drug abstinence (above), but decreases with increasing number of childhood conduct disorder symptoms (below). Reprinted by permission of Elsevier Science from 'CNS recovery from cocaine, cocaine and alcohol, or opioid dependence: a P300 study' by Bauer, L.O., *Clinical Neurophysiology* **112**, 1508–1515. Copyright 2001 by the Society of Biological Psychiatry (See Colour Plate XVI-5.4)

study. Thus, shorter periods of abstinence may have obscured a possible relationship between abstinence and P3a recovery in the study by Biggins and colleagues. An earlier study by Branchey *et al.* (1993) appears to be more difficult to reconcile with the notion of P3 recovery. Although these researchers did not assess correlations of P3 size with duration of abstinence, they found reduced P3 responses in cocaine-, opioid- and mixed cocaine-opioid-dependent patients drug free for over half a year, suggesting that P3 does not recover even with prolonged abstinence.

There is, however, a theoretical framework which has gained considerable empirical support particularly by the work of Bauer and co-workers, that could reconcile findings of P3 recovery with the results of Branchey and colleagues. Several investigations by Bauer and co-workers have focused on the influence of an ASPD diagnosis or ASP features such as aggression, impulsivity or psychopathy on ERP characteristics in substance abusers. As we have already seen in our discussion of alcoholism, antisocial personality features are associated with P3 decrements over and above those produced by drug use or family history. In some cases, these features may even solely account for P3 findings previously attributed to the latter sources. The relationship between ASP symptoms and substance abuse does also hold for cocaine abuse. The lifetime prevalence of ASPD in cocaine-dependent patients is about 50%, and conduct disorder (CD), the childhood form of ASPD, is a known risk factor for cocaine abuse. Comorbid ASPD has been found to be linked to P3 decrements not only in healthy subjects (Bauer *et al.*, 1994; O'Connor *et al.*, 1994b) and alcoholics (Costa *et al.*, 2000) but also in cocaine-dependent patients (Bauer, 1997). The latter study included ASPD-negative controls as well as two cocaine-dependent groups, with and without comorbid ASPD. The ASPD-positive cocaine abusers were found to have lower P3a amplitudes than the two other groups, between which there was no difference. Furthermore, P3 size negatively correlated with the number of childhood, but not adult, CD/ASP symptoms (Figure XVI-5.4) (Bauer, 1997, 2001b). Other studies found P3 decrements in relation to aggressiveness, impulsivity or violent behaviour (Branchey *et al.*, 1988, 1993).

These results provide strong evidence that antisocial personality characteristics that predate substance abuse are one important determinant of P3 size. On the other hand, P3 in cocaine addicts also appears to be influenced by chronic drug use, as it is found to recover once the pharmacological stimulation provided by the drug is cut off. Given these two determinants, Bauer (2001b) speculated that pre-existing P3 decrements in individuals with childhood CD may limit the degree to which P3 can recover with prolonged abstinence from cocaine. This means that P3 amplitudes in abstinent cocaine abusers with childhood CD may recover only to the level present before cocaine use, which is still below the level of healthy individuals without childhood CD. Thus, the P3 decrement found by Branchey and colleagues after 6 months of abstinence could reflect the residual effect of childhood CD after P3 recovery from chronic drug use was largely complete. Consistent with this view, these authors in fact found the lowest P3 amplitudes in subjects with high impulsivity or with a history of incarceration. In sum, two effects might operate independently to lower P3 size: the first one is a pharmacological effect of cocaine that recovers within a few months of drug abstinence; after that, any residual decrement in P3 size solely reflects the effect of pre-existing antisocial personality features. An interesting piece of evidence in favour of this view comes from the Bauer (1997) study. He found that the P3a response that recovered with prolonged abstinence was topographically distinct from the P3a that differentiated ASPD-positive from ASPD-negative subjects. The former P3a was more frontally located than the latter, suggesting that there are different neural subsystems that mediate the effects of abstinence and of ASP features. Interestingly, the frontal location of the ASPD-related P3a is consistent with mounting evidence for a frontal dysfunction in aggression, violence and other antisocial behaviours. In sum, substance use and the presence of childhood antisocial personality features make independent, additive contributions to the P3 decrements seen in cocaine- and alcohol-dependent individuals. The ASPD-related contribution may be more frontally located, consistent with evidence for frontal dysfunction in subjects with ASP features.

Other factors such as depression, anxiety, severity of drug use and family history of alcoholism may also influence P3 in cocaine users. Unfortunately, the lack of studies does not offer much of a handle on assessing these factors. The few studies that addressed these issues found no effects of depressive mood, anxiety, duration of cocaine use or a positive FHA (Bauer, 1997, 2001b) on P3 measures. Biggins *et al.* (1997) found auditory and visual P3a amplitudes in cocaine users to be inversely related to the amount or duration of alcohol use, respectively.

Other ERP Components

Very few studies have examined ERP components other than the P3. Two recent studies focused on the P50, an early sensory component occurring about 50 ms after brief auditory stimuli (Fein *et al.*, 1996; Boutros *et al.*, 2000). The P50 is elicited in the absence of a cognitive task and is independent of attentional manipulations. Two paradigms are frequently used to evoke the P50: a trains paradigm involving trains of identical auditory clicks, with P50 becoming smaller as the interstimulus interval decreases; and pairs of auditory clicks, with short intervals between clicks of a pair (e.g. 500 ms) and longer intervals between pairs of clicks (e.g. 7–8 s). In the paired-click paradigm, the P50 response to the second click relative to the first click is suppressed in normal subjects. This 'gating' is thought to reflect a pre-attentive mechanism underlying the filtering out of irrelevant sensory information.

Both studies found P50 gating in the paired-click paradigm to be impaired in cocaine-abusing or -dependent subjects, although this effect just missed statistical significance in the study by Boutros and colleagues. The studies differ in that Fein *et al.* also observed large decreases in P50 responses to the first click, while Boutros *et al.* did not. However, the latter researchers found reduced P50 amplitudes with longer interstimulus intervals in the trains paradigm (this paradigm was not used by Fein *et al.*). The differences between these studies are not surprising given substantial differences in subject selection. Subjects in Fein *et al.* were abstinent from cocaine for 2 weeks, were heavy cannabis and alcohol users, and included cases with neurological or psychiatric disorders secondary to chronic substance abuse. Boutros and colleagues included subjects who had used cocaine in the last 48 hours before testing, but did not use alcohol or other substances and were free of neurological and psychiatric disorders. Despite these differences, both studies indicate that pre-attentive processing as indexed by P50 characteristics are impaired in chronic cocaine users, possibly reflecting impaired sensory gating. Interestingly, the lack of P50 abnormalities in a control group of active alcoholics included by Fein and colleagues indicates that P50 deficits may be specific to cocaine abusers. These authors therefore propose the P50 as a candidate electrophysiological measure that may differentiate between the effects of chronic alcohol and cocaine use.

Findings involving other ERP components are isolated. Biggins *et al.* (1997) reported significant increases in auditory N200 latency and no changes in auditory P200 amplitude or latency. This finding is in agreement with similar results in alcoholic subjects. Bauer (2001a) found the contingent negative variation (CNV), a negative wave in preparation of a motor response, to be subject to the same dependencies as the P3: its size at frontal electrodes differentiated between cocaine abusers with and without APS features, it recovered with increasing periods of abstinence, and

it was relatively unaffected by depressed or anxious mood and the duration of cocaine use. Another study by Bauer and Easton (1996) demonstrated a slight delay of P100 latency in a checkerboard-reversal paradigm, similar to that found in some studies in alcoholics. In a related study, Bauer (1994b) found decreased EEG responsiveness (i.e. decreasing alpha power with increasing light intensity) to square-wave modulated light, but increased responses to sine-wave modulated light, in chronic cocaine abusers. Taken together, it appears that chronic cocaine abuse attenuates the responsiveness of the visual system to square-wave, but not sine-wave modulated input. Since cocaine is known to be a potent vasoconstrictor, and numerous cases of cerebrovascular ischaemic and haemorrhagic complications are associated with its use, these EEG/ERP alterations may originate from detrimental effects on the optic nerve or occipital vasculature. There is also evidence for chronic cocaine effects on olfactory perception. Olfactory-evoked potentials (OEP) in cocaine-dependent patients have been found to be reduced, possibly reflecting damage to the peripheral or even central olfactory apparatus (Bauer and Mott, 1996).

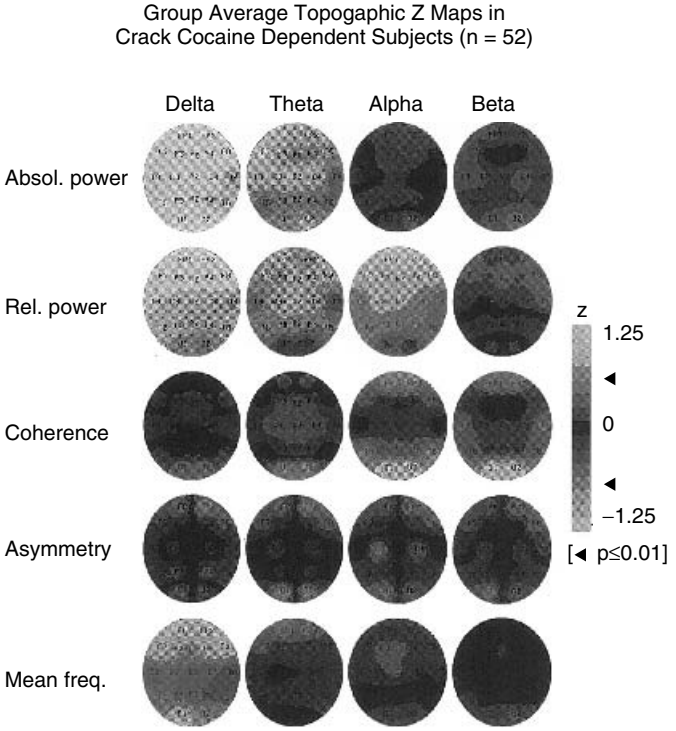

Figure XVI-5.5 Colour-coded topographic brain maps showing differences in absolute and relative power, coherence, hemispheric asymmetry and mean frequency between control subjects and recently detoxified cocaine abusers. Cocaine abusers have decreased absolute and relative slow wave and increased relative alpha power. Reprinted from Prichep, L.S., Alper, K.R., Kowalik, S., Merkin, H., Tom, M., Roy John, E. and Rosenthal, S., 1996. Quantitative electroencephalographic characteristics of crack cocaine dependence. *Biological Psychiatry* **40**, 986–993, copyright 1996, with permission from Elsevier Science (See Colour Plate XVI-5.5)

EEG

The recency of cocaine abuse as a socially important phenomenon is also reflected in the fact that the first EEG study in cocaine-dependent subjects appeared as late as 1990 (Alper *et al.*, 1990). This study reported increases in alpha activity and decreases in delta and theta activity. Later work only partly replicated these findings. Several studies found decreases in delta and/or theta (Roemer *et al.*, 1995; Prichep *et al.*, 1996, 1999; Herning *et al.*, 1997; King *et al.*, 2000) and increases in alpha activity (Figures XVI-5.5 and XVI-5.6) (Roemer *et al.*, 1995; Prichep *et al.*, 1996, 1999), sometimes paired with elevated beta activity (Roemer *et al.*, 1995; Herning *et al.*, 1997; Prichep *et al.*, 1999; King *et al.*, 2000). Other studies reported beta increases in the absence of alterations in slow wave and alpha activity (Costa and Bauer, 1997), while still others found no EEG changes at all (Bauer and Kranzler, 1994; Bauer, 1994b; Hersh *et al.*, 1995). Finally, one study (Pascual-Leone *et al.*, 1991) found beta elevations together with a pattern of slow wave and alpha activity opposite to the one typically reported, characterized by increased delta and theta and decreased alpha activity. In all bands but delta, changes predominantly occurred in relative compared to absolute EEG activity.

This heterogeneity of findings stems from important differences between the studies, mainly involving criteria for subject selection and experimental procedures. Many researchers have studied samples of cocaine-dependent subjects contaminated by the presence of other substance dependence or abuse or by comorbid DSM Axis I psychiatric diagnoses, all of which are known to have an impact on the EEG. Although these studies certainly reflect the clinical reality, they make it difficult to ascribe positive EEG findings to the chronic use of cocaine alone. For example, it is striking that increases in alpha and decreases in delta activity, although sometimes described as typical for cocaine addicts, are found almost exclusively in studies including subjects with a diagnosis of major depression (Alper *et al.*, 1990; Roemer *et al.*, 1995; Prichep *et al.*, 1996, 1999). It is tempting to speculate that comorbid depression may account for these findings, since increased alpha and decreased delta have been found in clinically depressed patients. Consistent with this view, Prichep *et al.* (1999) found higher depression scores and a higher incidence of concurrent depression in cocaine-dependent patients characterized by excess alpha and reduced delta activity. Roemer *et al.* (1995), however, failed to find any EEG deviations in a subsample of their subjects diagnosed with depression. In addition, some studies (Pascual-Leone *et al.*, 1991; Roemer *et al.*, 1995; Prichep *et al.*, 1996) found changes in delta and alpha frequency to be related to measures of cocaine exposure, although these relations appear somewhat inconsistent. A relation of changes in delta and alpha, but also theta activity, to chronic cocaine use and not depression is further suggested by studies showing that changes in this frequency range do not persist beyond early abstinence (about 1 month). Although not longitudinal in nature, these studies found either no changes (Bauer, 1994b, 1994c), or increased activity exclusively in the beta band (Costa and Bauer, 1997) after several months of abstinence. This may indicate recovery of chronic drug-induced slow wave and alpha abnormalities.

Regarding the increases in beta activity, the situation is very similar to that in alcoholism, and many of the same considerations apply. Although there is some limited evidence that beta activity is related to cocaine exposure (Herning *et al.*, 1997) and partially recovers during early abstinence (Noldy *et al.*, 1990; Noldy and Carlen, 1997), other studies found beta elevations to be unrelated to previous drug use and to persist beyond early abstinence (Costa and Bauer, 1997). Possibly, altered beta activity may reflect both short-term drug effects and long-term premorbid features, similarly as was hypothesized for P3 alterations. These premorbid features could be related to the presence of ASPD (Bauer and Hesselbrock, 1993) or the risk for relapse (Bauer, 1994a; Prichep *et al.*, 1999; Bauer, 2001d). Evidence for a relation of beta to a positive FHA in non-drug-using populations is inconsistent (see section on alcohol). In cocaine users, this relation has been ignored by most studies so far. One exception is Costa and Bauer (1997), who found no effect of a positive FHA on beta activity. Furthermore, beta alterations may be gender specific: King *et al.* (2000) reported that male cocaine abusers abstinent for 6 days have increased

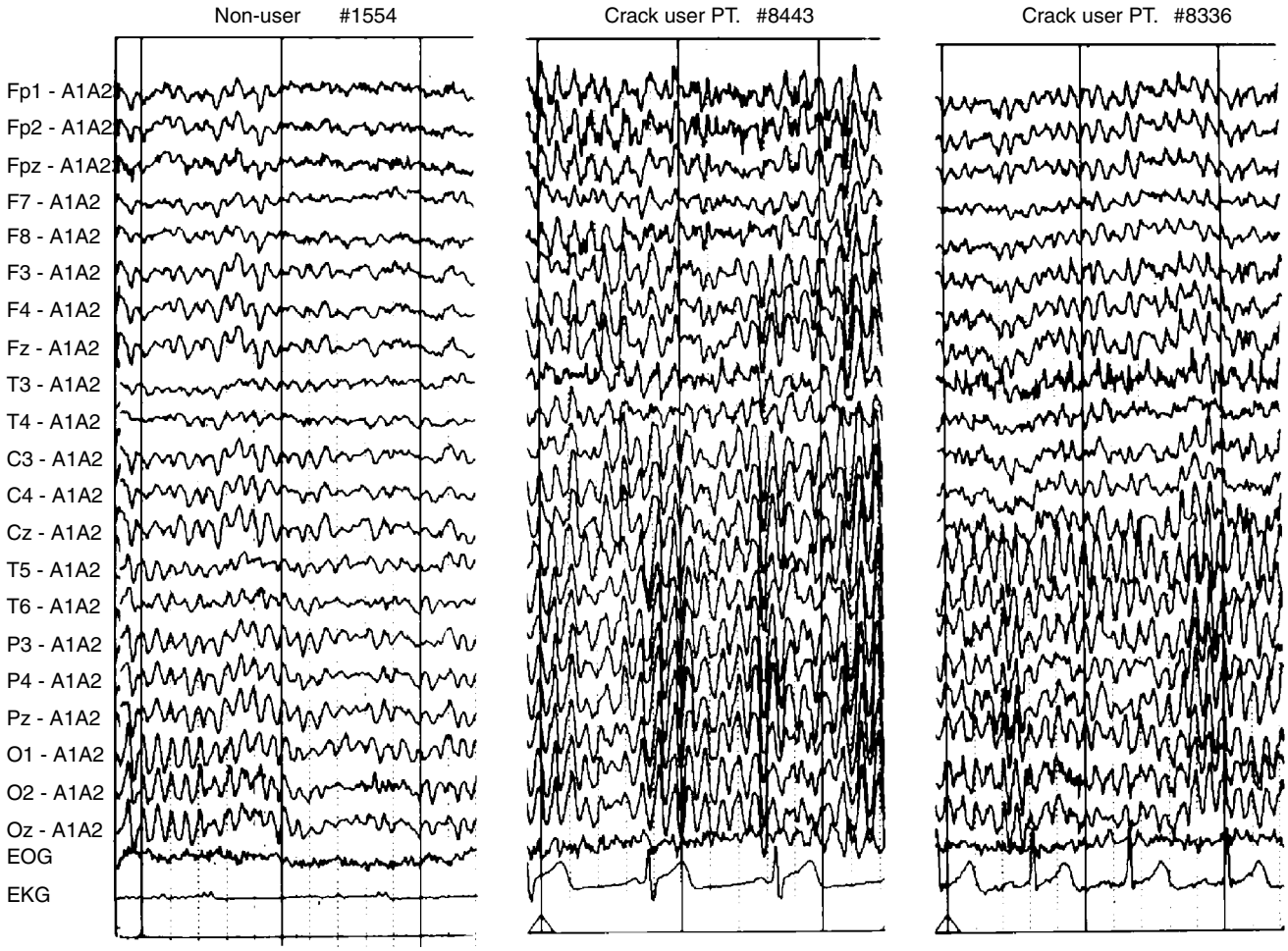

Figure XVI-5.6 Representative 2.5 s EEG epochs from a normal subject and two crack cocaine-dependent individuals. Note the excessive high-amplitude alpha activity (8–10 Hz) in the cocaine-dependent subjects. Reprinted from Alper, K.R., Chabot, R.J., Kim, A.H., Prichep, L.S. and Roy John, E., 1990. Quantitative EEG correlates of crack cocaine dependence. *Psychiatry Research: Neuroimaging* **35**, 95–105, copyright 1990, with permission from Elsevier Science

levels of beta activity compared to matched female cocaine abusers and healthy controls. This increase was not related to psychiatric status, since males had lower psychiatric distress, anxiety and depression than females. A similar failure to relate symptoms of depression and anxiety to beta activity has been reported by Costa and Bauer (1997) and Herning *et al.* (1997). Thus, although a diagnosis of major depressive disorder is commonly associated with increased beta activity (Pollock and Schneier, 1990), it does not appear to account for the presence of beta changes in male and their absence in female cocaine abusers. King and colleagues speculated that, since female compared to male cocaine abusers show a similar absence of changes in regional cerebral blood flow (rCBF), beta increases in male users might stem from a heightened susceptibility to cocaine-induced cerebrovascular complications. Cocaine is a potent vasoconstrictor, and may reduce rCBF through an overstimulation of dopaminergic innervation of cortical vasculature. Reductions of rCBF, in turn, may be linked to heightened beta activity. It has been suggested that women might be relatively protected from these complications by the presence of oestrogens.

A similar neurobiological hypothesis is available for explaining reductions in delta activity, to the extent they are cocaine induced. Some authors (e.g. Prichep *et al.*, 1999) have linked delta decreases to a current theory of the pathophysiology of cocaine dependence. This theory states that chronic cocaine use induces a desensitization of dopaminergic neurons in the ventral tegmental area, which, through inhibitory projections to the forebrain, may lead to a disruption of burst-firing of pyramidal cells in deeper cortical layers. Burst-firing has been implicated in the generation of delta rhythms and its disruption would therefore result in diminished delta wave activity. Functionally, normal delta has been suggested to mediate inner concentration by suppressing extraneous inputs in order to allocate maximal attention. In this view, deficits in delta may have similar consequences as is hypothesized for deficient P50 suppression: a failure of a pre-attentive gating mechanism filtering out irrelevant sensory information.

Finally, methodological differences between studies may account for some of the heterogeneity of EEG findings in cocaine-dependent subjects. Bauer (1994b, 1994c) failed to detect any, including beta, changes in the EEG of cocaine abusers abstinent for 3 months. This result is in contrast to a later study by the same group (Costa and Bauer, 1997), which found beta power to be increased in cocaine addicts after similar abstinence times. In the later study, the researchers attribute their previous negative beta finding to an overly conservative bandpass filter which cut off parts of the relevant beta spectrum.

In conclusion, changes in EEG frequency spectrum in cocaine-abusing or -dependent subjects are probably to some degree effects of cocaine exposure that tend to normalize during abstinence, but may, particularly in the case of increased beta activity, also reflect pre-existing factors possibly related to gender, the risk for relapse or the presence of ASP features. Alpha and delta changes are observed in studies including depressed cocaine addicts, suggesting a contribution of depressive disorder. On the neural level, delta and beta abnormalities may result from hyperactive dopaminergic projections to cortical vasculature and pyramidal cells. Increased beta activity is common to alcoholism and cocaine dependence, but changes in slow wave and alpha rhythms tend to be opposite in alcoholism.

Cocaine/Alcohol Co-Dependence

Both cocaine and alcohol dependence are associated with abnormalities in ERP and EEG measures. The question arises whether co-dependence on both substances produces additive effects greater than those produced by either drug alone. A specific pharmacological mechanism for such an additive effect could be the formation of cocaethylene, a cocaine metabolite that is produced in the liver exclusively in the presence of alcohol. Cocaethylene is more toxic than cocaine and might potentiate possible harmful consequences that alcohol or cocaine use has by itself. It has not been possible so far to examine levels of this metabolite in relation to electrophysiological characteristics of individuals co-dependent on both substances. Some researchers, however, have compared cocaine- and alcohol-dependent subjects with dually dependent subjects with respect to ERP and EEG measures. They found that alcohol/cocaine co-dependent, but not pure alcohol- or cocaine-dependent, subjects had normal EEGs (Costa and Bauer, 1997). Two other studies (Biggins *et al.*, 1997; Bauer 2001b) found P3 decrements similar in magnitude in both cocaine-dependent and cocaine/alcohol-co-dependent patients. It therefore appears that co-dependence on alcohol and cocaine does not affect ERP and quantitative EEG measures to a greater extent than cocaine dependence alone. Some have argued that this may be so because chronic cocaine effects on the ERP are larger (Bauer, 1996) and obscure the additional smaller contribution of alcohol use. There is, however, isolated evidence from clinical EEG evaluation for a unique effect of alcohol/cocaine co-dependence. Roemer *et al.* (1995) reported a pattern of abnormal alpha bursts with paroxysmal characteristics they call C-complexes. This pattern was observed in co-dependent, but not in pure cocaine- or alcohol-dependent, patients. The authors consider the possibility that C-complexes are a consequence of cocaethylene formation. Future studies should clarify whether this finding is replicable and can be quantified. Possibly, also animal electrophysiological studies involving the administration of cocaethylene could shed light on this issue.

OPIOIDS

ERP/EP

Although opioids are among the most addictive drugs, there has been very little electrophysiological research into their chronic effects. Opioid addicts are often concurrently dependent on cocaine, therefore the effects of pure opioid abuse are difficult to investigate. There is one study that compared nearly pure opioid-dependent male and female patients with cocaine-dependent, cocaine–alcohol-co-dependent and control subjects (Bauer, 2001b). All drug-using subjects, who were abstinent for 1–5 months, were found to have significantly lower P3 responses in a visual ERP paradigm. Opioid-dependent patients exhibited the lowest P3 of all drug users, but they were essentially not different from them in terms of demographic, psychological or substance use characteristics. P3 amplitude significantly decreased with increasing number of childhood symptoms of conduct disorder and with increasing duration of abstinence, but was not affected by a positive FHA, depression, anxiety, duration of drug use or the number of drug-related problems. These findings agree with a smaller study in male opioid and opioid–cocaine-dependent patients abstinent for over half a year (Branchey *et al.*, 1993). These researchers found reduced auditory P3 amplitudes in all drug-using subjects, with lowest amplitudes found in subjects with a history of incarceration. P3 amplitudes significantly decreased with increasing impulsiveness. Taken together, these two studies suggest that P3 decrements in opioid addicts are related to pre-existing antisocial personality characteristics present in childhood and/or adulthood, but also to opioid exposure as evidenced by their dependence on the length of abstinence.

A somewhat different interpretation of P3 decrements is suggested by results from Kouri *et al.* (1996). These researchers found auditory P3 decrements in male opioid–cocaine co-dependent patients *after*, but not *before*, detoxification. This may indicate that P3 reductions are a sign of drug withdrawal rather than previous drug exposure or pre-existing antisocial symptoms. If this were true, however, then these abnormalities would be expected to normalize with prolonged abstinence, which does not seem to be the case.

One study (Bauer, 1998) used pattern-shift VEPs to compare a group of opioid-dependent patients, abstinent for 2–4 months, with two groups of recent opioid users maintained on methadone, one positive and one negative for HIV, and a control group. Compared to the controls and the abstinent users, methadone-maintained opioid patients showed significantly delayed N75 and P100 components. N75 latency significantly correlated with the duration of opioid abuse, but not with duration of cocaine, alcohol or other drug use, length of abstinence or methadone levels. Possibly, altered N75 shows some specificity to opioid abuse, while P100 alterations have also been found in alcohol and cocaine abusers.

EEG

The EEG in chronic opioid addicts is largely unexplored territory. An early study by Volavka *et al.* (1970) reported irregularities in the clinical EEG of 50% of 64 heroin-dependent patients abstinent for at least 1 week. Recently, Costa and Bauer (1997) have resumed this line of research. They recorded the resting EEG in groups of alcohol-, cocaine- and opioid-dependent patients and control subjects. The opioid group included cases with concurrent cocaine or alcohol dependence. All patients were abstinent from their drug of choice for 1–6 months. As we have discussed in the previous sections, both the alcohol- and cocaine-dependent group showed increased EEG beta power. The authors concluded that enhanced beta activity, being common to both drugs, may not be a consequence of drug use, but a premorbid factor. However, the lack of beta abnormalities in the opioid-dependent patients seemed to challenge this interpretation, since these were the subjects with the highest prevalence of premorbid features such as CD, ASPD and other risk factors. To address this concern, Bauer (2001c) reanalysed this data set, hypothesizing that premorbid beta enhancement is indeed present in opioid patients, but masked by the antagonizing effect of chronic opioid exposure. He compared two subgroups of the opioid group, one with less and one with more than the median number of years of opioid abuse (6.5 years). Compared to the controls, those patients with less than 6.5 years of opioid use showed significantly more beta power, while those with longer histories of opioid use showed significantly less beta power and an additional increase in alpha power, similar to that previously found

in active opioid abusers (Shufman *et al.*, 1996). The dramatic EEG slowing associated with a longer history of opioid use suggests a drug-induced progressive beta reduction. On the other hand, the beta enhancement present in patients with shorter drug histories still leaves room for premorbid influences, possibly related to antisocial personality traits.

CANNABIS

ERP/EP

ERP findings in chronic cannabis users appear to be inconsistent. Most frequently, reduced amplitudes and prolonged P3 latencies were found in chronic cannabis users (reviewed in Patrick *et al.*, 1995). Struve and Straumanis (1990) measured auditory ERPs in psychiatric inpatients with a history of chronic cannabis use compared to non-cannabis-using psychiatric patients and normal controls. P3 responses were significantly reduced and delayed in the cannabis group compared to both non-user groups. Because psychiatric comorbidity and medication effects were not controlled for in this study, the investigators repeated the study with daily cannabis users and non-user controls that were both psychiatrically screened to be healthy volunteers (Patrick *et al.*, 1995). Auditory and visual P3 responses were elicited by a relatively simple oddball paradigm. The researchers found no differences in test performance, but the cannabis users displayed reduced auditory and near-significantly reduced visual P3 amplitudes. However, when age differences between the cannabis users and controls were statistically removed, significant P3 differences were removed as well. These results illustrate the need for well-matched control groups. They further indicate that reduced P3 in chronic cannabis users may only be found in those with comorbid psychopathology.

However, it is of note that there were no differences in test performance between the cannabis users and non-users in that study, although such differences are commonly found when difficult tasks are used. Solowij *et al.* (1991) recorded ERPs from long-term pure cannabis users during a complex multidimensional auditory selective attention task, where users performed clearly worse than controls. Subjects had to respond to stimuli that were long-duration tones of a particular pitch and location, with all three factors being varied. P3 amplitudes were found to be decreased in cannabis users as opposed to controls. Thus, a P3 task with difficult target–non-target discrimination is needed to detect any differences in P3 amplitudes between cannabis users and non-cannabis-using subjects. Another interesting finding observed in the user group of the Solowij *et al.* study was enhanced early processing negativity to short-duration stimuli which matched the target on location only. According to the investigators, this would be indicative of users engaging in unnecessary pitch processing and thus having difficulty in setting up an accurate focus of attention and in filtering out irrelevant information. This finding may reflect a dysfunction in the allocation of attentional resources and stimulus evaluation strategies in cannabis users, similarly as it is hypothesized for alcoholics.

In a further study Solowij *et al.* (1995) confirmed and extended their findings of cognitive impairments and related ERP changes in a group of cannabis users who had used cannabis for a mean time of 6.7 years at a mean frequency of three times per week. It appeared that frequency and duration of cannabis use differentially affected cognitive functions as assessed by this ERP paradigm. Frontal processing negativity to irrelevant stimuli was increased, indicating a decreased ability to focus attention and filter out irrelevant information, and this effect was progressive with the number of years of use, but was unrelated to frequency of use. In contrast, the speed of information processing, as measured by the latency of P3, was delayed significantly with increasing frequency of use, but was unaffected by duration of use. Solowij *et al.* note that with regard to the real world these ERP findings may reflect increased distractibility and hence impairment in any situation where concentration and focused attention are essential. In addition, long-term effects of cannabis may only become readily apparent after about 5 years of exposure.

Patrick *et al.* (1999) compared auditory P50 measures in chronic, psychiatrically normal daily cannabis users and control subjects. P50 suppression was reduced in the cannabis users. These sensory gating deficits were not significantly associated with the duration or frequency of cannabis use in this study, but were correlated with the frequency of cannabis use per week in another small group of cannabis users assessed by the same researchers (Patrick and Struve, 2000).

In sum, although there is evidence that chronic cannabis use might be associated with electrophysiological changes including reduced and delayed P3 responses and deficits in sensorimotor gating, these data are inconclusive given the small samples and the many possible confounding factors. Particularly, most cannabis users assessed in studies so far have also used cannabis on the days prior to the test session and cannabis intoxication may therefore be an important confounding factor given the long half-life of THC plasma levels. Nevertheless, the electrophysiological changes described above might reflect subtle impairments of memory, attention and processing of complex information in chronic cannabis users. It remains unclear, however, to what extent these deficits relate to a state of chronic intoxication with cannabis that would be reversible after prolonged abstinence. Since cannabis is the most widely used recreational drug, the effect of chronic cannabis administration should be more thoroughly studied.

EEG

A number of clinical EEG studies in the 1970s (reviewed in Struve and Straumanis, 1990) reported mild qualitative EEG abnormalities in casual to chronic cannabis users. Many of these studies, however, failed to include normal control subjects or to report population base rates of such abnormalities. Moreover, the effects of polydrug abuse or concomitant psychiatric or medical pathology were not considered. Finally, clinical, qualitative EEG assessment is based on subjective judgement and the decision what to count as abnormal is therefore prone to rater bias. This effect is particularly concerning when, as was mostly the case, intra- or interrater reliabilities are not reported or the rater is not blind to experimental condition or subject status. The prevalence of EEG abnormalities in most of these early studies ranged from 0 to 17%, which is in fact not higher than that in the normal population. Those studies that did include control groups confirmed that the prevalence of abnormal EEG findings was similar for cannabis users and normal subjects. One early study reported striking rates of EEG abnormalities as high as 90%. However, this study was severely criticized for methodological flaws by several authors. Early quantitative EEG studies of chronic cannabis effects have also often not compared cannabis users with control groups, but instead focused on the effects of daily cannabis consumption by very small numbers of normal subjects over several weeks under experimental observation. Two such studies found evidence for time-dependent increases in alpha and theta, and decreases in beta activity. One early study that included cannabis users and an age- and sex-matched control group reported increased delta activity and coherence, increased theta activity and, contrary to the other studies, decreased alpha. In a more recent qEEG study, Struve and colleagues found elevations of absolute and relative power and interhemispheric coherence of alpha activity over the frontal cortex in cannabis using inpatients, but also in healthy daily cannabis users, compared to non-users (Struve *et al.*, 1989). Although differences in psychiatric diagnosis,

medication and the use of other drugs were not controlled for, these results were later confirmed in a replication study in an independent sample. In addition, a more recent investigation by the same researchers (Struve *et al.*, 1998), including cannabis users free of serious psychopathology and without significant use of other drugs, found that users with previous daily exposures to cannabis for 15–24 years had more absolute theta power over the frontal cortex than non-users or subjects who had used cannabis for a shorter period (Struve *et al.*, 1998). This theta increase apparently developed with increasing duration of cannabis use. Trudeau *et al.* (1999) analysed EEG data from subjects with mixed cannabis and stimulant use disorder. In the stimulant-only group, relative power differences were dramatic and included diffuse frontal and right parietal excess alpha and deficit theta, as well as diffuse deficit beta. Cannabis and stimulant dependence together produced more EEG changes than either drug alone. In the absence of stimulant use, however, the EEG effects of cannabis were relatively small. There was only a moderate deficit in frontal relative delta power which was not seen in the stimulant-only group. Thus, the Struve *et al.* findings of alpha hyperfrontality were not replicated. However, subjects in the Trudeau *et al.* study were abstinent for at least 1 month, whereas Struve and colleagues tested active cannabis abusers. Possibly, alpha hyperactivity is a withdrawal-related EEG characteristic that disappears with prolonged abstinence.

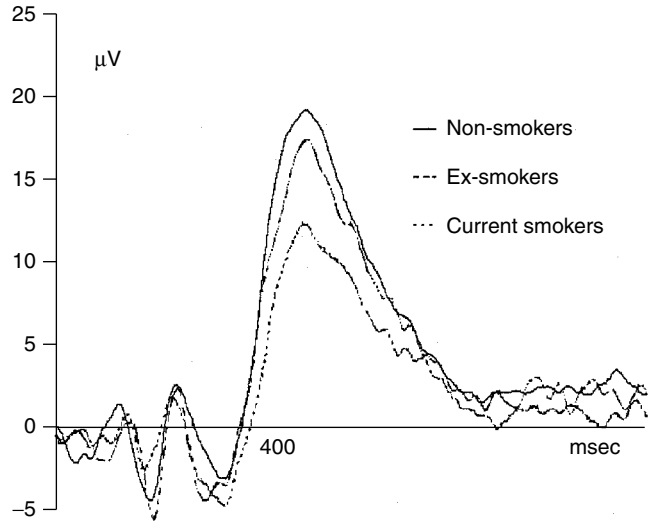

Figure XVI-5.7 Visual P3 amplitudes at the parietal lead in non-smokers, ex-smokers and current smokers. Current smokers show significant P3 decrements compared to both controls and ex-smokers. Reprinted with permission from Anokhin, A.P., Vedeniapin, A.B., Sirevaag, E.J., Bauer, L.O., O'Connor, S.J., Kuperman, S., Porjesz, B., Reich, T., Begleiter, H., Polich, J. and Rohrbaugh, J.W., 2000. The P300 brain potential is reduced in smokers. *Psychopharmacology* **149**, 409–413. Copyright 2000, Springer-Verlag

NICOTINE

ERP

Most studies involving electrophysiological techniques focused on acute effects of nicotine rather than on chronic changes due to nicotine dependence. The literature on possible ERP alterations in smokers compared to non-smokers is therefore very scant. Among the very few studies, Anokhin *et al.* (2000) assessed P3 of the ERP in 17- to 74-year-old current daily tobacco smokers, ex-smokers and never-smokers in a large sample drawn from the COGA. Smokers consumed a mean number of 20 cigarettes daily. Ex-smokers used to smoke a mean number of 19 cigarettes daily and quit smoking 1 year or more prior to the study. Never-smokers had never smoked on a daily basis for a month or more. A visual oddball task was used to elicit ERP responses. Visual P3 amplitude was significantly lower in current smokers compared to never-smokers, while ex-smokers did not differ from never-smokers (Figure XVI-5.7). In addition, there were smaller but significant reducing effects of alcoholism and family density of alcoholism (FDA) on P3 amplitude. However, the effect of alcoholism (and other substance dependence) was no longer significant after adjusting for current smoking and FDA, while the FDA effect remained significant after controlling for smoking and alcoholism. In fact, smoking and family density of alcoholism diminished P3 amplitude in an additive fashion. Psychopathology, drinking and use of medication did not affect P3. The researchers speculate that P3 amplitude reduction may be a consequence of chronic tobacco smoking and may reflect a reversible change in brain function. Alternatively, low P3 amplitude may indicate greater vulnerability to nicotine addiction and, specifically, a reduced ability to quit smoking. Low P3 may therefore be associated with smoking persistence and dependence rather than with smoking initiation itself. Regardless of which possibility is true, a potentially grave implication for alcoholism research follows from these results: Since alcohol and nicotine dependence are highly comorbid (as was also the case in this study), uncontrolled nicotine co-dependence might be responsible for a substantial part of the P3 amplitude reduction seen in alcoholics or other substance-dependent individuals.

Knott *et al.* (1999) found no P3 alterations in a small sample of smokers from a similar age range (18–81 years). However, in contrast to Anokhin and colleagues, these researchers used an auditory ERP paradigm, and this difference in task modality may account for the divergent findings. Indeed, a similar failure to detect P3 decrements after auditory, but not visual, paradigms is observed in studies of alcohol-dependent or FHP individuals.

EEG

The EEG effects of both current smoking and short-term smoking abstinence have been studied. Knott and colleagues (Knott and Harr 1996, 1997) have examined spontaneous EEG activity in their sample of 18- to 81-year-old smokers and non-smokers presented above. The EEG was recorded before and after sham smoking in non-smokers and cigarette smoking in smokers. Smokers evinced higher beta activity and interhemispheric alpha coherence. The beta findings are reminiscent of similar findings in alcohol and cocaine users. The same group as well as other researchers have also studied the EEG within the first 2 days of smoking abstinence and found an EEG slowing characterized by increased slow wave power and a decrease of dominant alpha frequency (Pickworth *et al.*, 1989; Knott, 1990). This EEG slowing continued throughout 10 days of abstinence, and returned to pre-quit levels after resumption of smoking (Pickworth *et al.*, 1989). Only one well-controlled study monitored physiological and subjective changes during a longer period of abstinence. Gilbert *et al.* (1999) studied male smokers using EEG, mood questionnaires, a visual rapid information-processing task, and measurements of heart rate during eyes-closed and eyes-open relaxation and stress conditions. Subjects were first measured at baseline and were then randomly assigned to a quit vs. a continue-to-smoke group. Quitters and smokers were later reassessed on the same days, corresponding to days 3, 10, 17 and 31 of abstinence for the quitters. Compared to smokers, quitters showed increased delta, theta, slow alpha and slow beta power as well as a decrease in mean alpha frequency. The slow alpha difference was most pronounced over the posterior scalp. Post-quit heart rate was decreased in the quitters. Importantly, these

changes did not resolve across 31 days of abstinence. In general, EEG, heart rate and mood changes were highly dependent on condition (eyes closed vs. eyes open, stress vs. relaxation). Pre-quit nicotine exposure was positively correlated with alpha slowing on day 3, but not later. Higher trait depression was correlated with increases in left-greater-than-right slow alpha power on days 3 and 31.

The authors draw several conclusions from their findings. EEG slowing lasted for the entire 31 days of abstinence, indicating that it may be permanent or recover very slowly. The correlation of pre-quit nicotine exposure with EEG slowing on day 3 suggests that the initial portion of EEG slowing may reflect a transient abstinence effect. The correlation of trait depression with hemispheric alpha asymmetry on days 3 and 31, on the other hand, indicates a sustained effect. Subjects with high trait depression had greater left than right-frontal alpha power during abstinence along with increased depressiveness. Relatively decreased activity in the left compared to the right-frontal cortex has also been found in clinical depression and depressive mood states. These findings therefore support the hypothesis that smoking abstinence results in frontal EEG asymmetries that predispose depression-prone individuals to depression. Conversely, there is evidence that nicotine might attenuate depressive and other negative affective states by reducing right-frontal and enhancing left-frontal information processing.

Startle Reflex

The startle reflex is a response to a sudden, intense stimulus that can be observed across different species and sensory modalities. In humans, presentation of a loud startling noise (pulse) leads to a sharp eye-blink response. Pre-pulse inhibition (PPI) of the startle reflex refers to a reduction in the magnitude of the eye blink when the startling pulse is preceded by a weak pre-pulse, which itself does not evoke a measurable response. PPI is thought to reflect a pre-attentive sensorimotor gating process that serves to filter out irrelevant stimuli and protect against stimulus overload. Deficits in PPI have been found in disorders in which sensorimotor gating is thought to be deficient, such as schizophrenia.

There are few studies of the startle reflex and PPI in smokers. They showed that nicotine dependence has no effect on the magnitude of the startle response (Mueller *et al.*, 1998), but strong relative to weak dependence is characterized by reduced PPI (Kumari and Gray, 1999). Mueller and colleagues compared healthy male and female non-smokers to smokers after either prolonged, brief or no smoking deprivation in experimental sessions at baseline, after smoking-cue exposure and after cigarette smoking. No group differences in startle magnitude emerged for any session. Kumari and Gray studied healthy male smokers after overnight smoking withdrawal. Smokers with high nicotine dependence scores had significantly less PPI than those with low nicotine dependence.

Three explanations for these findings are conceivable. Increasing levels of nicotine dependence could lead to increasing cumulative levels of nicotine-induced neurobiological alterations that may manifest in decreased PPI. Alternatively, the heavily nicotine-dependent subjects may have suffered more severe withdrawal symptoms after overnight smoking withdrawal, which may have been reflected in lower levels of PPI. Finally, heavy nicotine intake may be an attempt to restore some trait-like cognitive deficit through self-medication, and this deficit could be indexed by deficient PPI. In this case, reduced PPI would reflect a premorbid characteristic of smoking dependence, similar to that hypothesized for alterations of P3 and EEG beta in other substance abusers. Future research will show which one of these alternatives is most likely.

AMPHETAMINES

ERP

While some studies have examined ERPs, we were unable to find studies of EEG in subjects dependent on amphetamine or its close congeners (the amphetamine derivative MDMA will be covered in the next section). There is a research tradition concerned with the study of methamphetamine dependence and psychosis in Japan. That country has seen two epidemics of methamphetamine abuse, the first shortly after the Second World War, the second starting in the 1970s. Iwanami and colleagues conducted several studies to assess the effects of chronic methamphetamine-induced psychosis on ERP measures (Iwanami *et al.* 1991, 1994, 1995, 1998). In their earlier studies (Iwanami *et al.*, 1991, 1994), these researchers found delayed P3 latency and reduced frontal and central, but not parietal, auditory P3 amplitudes in remitted methamphetamine psychotics receiving antipsychotic medication. A later study (1995) in unmedicated subjects revealed no significant delays in P3 latency and only a non-significant decrease in P3 amplitude. However, this study found a reduced area under the negative-difference wave spanning the period of 0 to 400 ms after stimulus onset. This wave is associated with attention to stimuli in the attended channel, and its reduction may indicate impaired auditory stimulus processing in methamphetamine psychotics. Recently (1998), these authors extended their investigation to the frontal P3a component of the ERP and found reduced P3a amplitude and area with delayed P3b latency, but normal P3b amplitude, in neuroleptic-maintained methamphetamine abusers abstinent for at least 2 weeks. P3a amplitude reductions are also present in patients with prefrontal lesions, and may thus indicate frontal damage in methamphetamine psychotics. However, the fact that Iwanami and colleagues have found P3 deficits only in medicated patients indicates that they may be related to antipsychotic medication rather than to chronic methamphetamine abuse. On the other hand, the abnormalities in the attention-related negative-difference wave are consistent with similar findings in amphetamine-dependent patients. The Australian researchers McKetin and Solowij (1999) compared highly and weakly dependent amphetamine users on ERP measures in a selective attention task that involved detecting infrequent long-duration target tones presented among short-duration tones that varied in location and pitch. Highly dependent amphetamine users were found to have reduced early processing negativity and peak N1 amplitudes. Early processing deficits were correlated with poor task performance and with poor attention and concentration. Taken together, these findings are consistent with the view of a dysfunction in selective attention in severe chronic amphetamine or methamphetamine abusers. Iwanami and colleagues also point out the similarities between their findings and those in schizophrenic patients, which support the hypothesis that methamphetamine psychosis may be a model for paranoid schizophrenic psychosis.

MDMA ('ECSTASY')

MDMA (3,4-methylenedioxymethamphetamine) is the main component of the drug 'ecstasy', which is predominantly consumed in clubs and at weekend 'raves' where users dance to 'techno' music. In public and scientific circles, MDMA has generated interest mainly due to its possible harmful effects. In particular, a wealth of animal studies showing that high or repeated doses of MDMA can cause massive depletion of central serotonin and damage to serotonergic cell bodies have raised concern over the possibility of similar neurotoxic damage in human MDMA users. While to date these concerns have mainly been addressed and supported in neuropsychological, neuroendocrinological and neuroimaging research, there are a few relevant psychophysiological studies.

ERP

Tuchtenhagen *et al.* (2000) recorded auditory evoked potentials with stimuli of increasing intensity in abstinent 'ecstasy' users with concomitant cannabis use, and in two matched control groups of cannabis users and non-users. 'Ecstasy' users had consumed about 3.5 tablets per month over an average period of 27 months, which is not a very heavy use, and were abstinent for a mean duration of 3 weeks. The intensity dependence of the tangential auditory evoked N100/P200 dipole source activity was assessed. This measure is analogous to the amplitude–intensity (A/I) gradient that we discussed in the section on alcohol. As we saw, alcoholics show increasing VEP responses to increasing stimulus intensities (positive A/I slopes), while non-alcoholic controls do not. This response is typical for so-called augmenters, who tend to increase physiological reactions to increasingly intense stimuli. Furthermore, high-intensity dependence of the VEP and N100/P200 response is thought to reflect low functioning of serotonergic neurotransmission in the primary sensory cortex. Thus, this measure may shed light on the possibility of serotonergic dysfunction in 'ecstasy' users. Confirming this hypothesis, Tuchtenhagen and colleagues found that 'ecstasy' users exhibited an increase of the amplitude of the tangential dipole of the N100/P200 component with higher stimulus intensities, whereas both control groups failed to exhibit this feature. These findings support other evidence for an 'ecstasy'-induced impairment in serotonergic brain function. Another possibility to be considered is that these results reflect pre-existing personality traits such as high novelty-seeking and impulsivity which are typical in drug users. However, several arguments speak against this possibility: high-intensity dependence did not correlate with these personality features. Moreover, not only the 'ecstasy', but also the cannabis control group exhibited higher levels of these personality traits, without showing high-intensity dependence. Finally, the intensity dependence in the 'ecstasy' users was more pronounced than that reported in healthy subjects with high novelty-seeking. In conclusion, high-intensity dependence may be a result of chronic exposure to 'ecstasy', and may possibly indicate central serotonergic dysfunction in 'ecstasy' users.

EEG

Dafters *et al.* (1999) examined eyes-closed resting EEG in moderate recreational 'ecstasy' users abstinent for at least a week. No control group was included, and the analysis was focused on correlations of qEEG variables with variables of drug exposure, mood and cognition. The effects of the use of other drugs were statistically partialled out. 'Ecstasy' use during the previous 12 months was found to correlate positively with absolute power of global alpha and left-posterior beta, and to correlate negatively with relative delta power and with posterior EEG coherence for the 1–20 Hz frequency range. There were no significant correlations with the severity of depression and several cognitive measures of memory and intelligence. The general dose dependence of the EEG findings supports the notion that increasing 'ecstasy' use produces increasing changes in brain function. Dafters and colleagues point out that the pattern of spectral EEG changes is reminiscent of the normal effects of ageing on the EEG. The exposure-dependent decrease of EEG coherence, a measure of the synchronization of firing between paired cortical locations, may indicate dysfunctional brain connectivity as evidenced in studies of dementia, ageing, depression and HIV-related cognitive impairment. Gamma *et al.* (2000) compared moderate-to-heavy 'ecstasy' users and control subjects on measures of mood and resting EEG activity recorded from 31 channels during open and closed eyes. The EEG was analysed by means of conventional power analysis as well as source localization using low-resolution brain electromagnetic tomography (LORETA; Pascual-Marqui *et al.*, 1994; Pascual-Marqui, 1999). While conventional analysis revealed no main effects of group for global absolute power, LORETA indicated increased global power of theta, slow alpha and fast beta. In an analysis of channel-wise absolute power, fast beta power was found to be higher at all recording sites during open, but not closed, eyes. Furthermore, both conventional power analysis and LORETA showed a significant increase in fast alpha power in the right temporo-occipital cortex during both open and closed eyes (Figure XVI-5.8). 'Ecstasy' users had higher levels of depressiveness which highly correlated with increases in beta power (Gamma *et al.*, unpublished data). The elevations of absolute alpha and beta power are in line with the findings of Dafters and colleagues. In contrast to the latter study, however, EEG variables were not correlated with the level of MDMA exposure (unpublished data), while beta changes were related to higher depressiveness. This finding is particularly intriguing in the light of consistent findings of increased beta activity in depressed patients. Together with the fact that increased beta is also common to alcohol and cocaine dependence, these results support the view that beta hyperactivity is a premorbid characteristic of individuals predisposed to substance abuse.

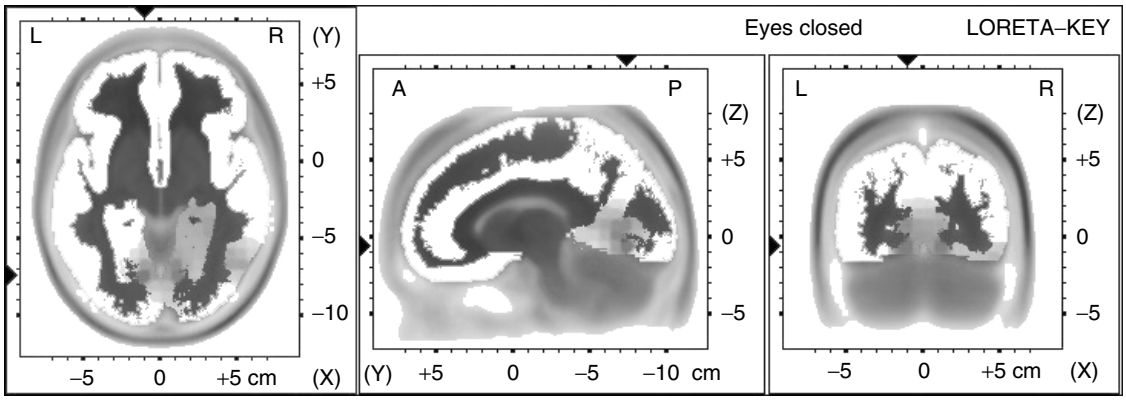

Figure XVI-5.8 Tomographic images displaying differences in fast alpha activity between MDMA ('ecstasy') users and control subjects. 'Ecstasy' users show significant increases (orange colour) in right temporo-occipital fast alpha power during closed eyes. Images have been computed from 31-channel scalp EEG recordings using low-resolution brain electromagnetic tomography (LORETA). Data from Gamma *et al.* (2000) (See Colour Plate XVI-5.8)

OTHER DRUGS

The psychophysiological literature on the chronic use of other widespread psychoactive drugs such as benzodiazepines and hallucinogens is virtually non-existent. Abraham and Duffy (1996) recorded EEG and sensory evoked potentials in LSD users suffering from hallucinogen persisting perceptual disorder (HPPD). HPPD is characterized by chronic visual hallucinations persisting beyond acute drug effects. HPPD subjects evinced faster alpha frequency and shorter visual EP but prolonged auditory EP latencies compared to a matched control group. These differences were most prominent in temporal and left parietal scalp areas. According to the authors, these changes possibly reflect cortical disinhibition and demonstrate differential effects of LSD use on auditory vs. visual systems.

Severely benzodiazepine-dependent patients were assessed during slow withdrawal by Hallstrom and Lader (1981). Heightened levels of anxiety and perceptual disturbances as well as decreases in EEG beta activity occurred during the withdrawal period but subsided after 2 weeks.

CUE REACTIVITY

Studies of cue reactivity in substance users are, to a large degree, conceptually independent of other types of psychophysiological substance use research. Moreover, these studies concern reward and motivational mechanisms that — as evidenced by a growing body of literature (see other chapters in this book) — may operate in a similar fashion across different drugs, owing to a common neurochemical basis. Therefore, we will treat this topic in one place only, integrating the findings from different drugs of abuse.

In the present context, cue reactivity refers to subjective and physiological reactions to visual, auditory or behavioural cues related to the use of a drug. These cues can be photographs, video or audio material, or they may involve the presence of drug paraphernalia or require subjects to engage in drug preparation rituals. It has been proposed that these reactions are conditioned responses in the classical Pavlovian sense, whereby environmental stimuli closely associated with the act of using a drug become capable by themselves of eliciting some of the subjective and physiological changes that normally occur in response to the drug. Such conditioned responses are considered important because it is believed that they constitute a major motivation for readministering the drug and may thus increase the risk for relapse and treatment failure. Although this putative conditioning occurs in the user's natural environment and cannot therefore be directly observed in the laboratory, it is possible to obtain experimental evidence that can be seen as counting towards the conditioning hypothesis. If cue reactivity in fact reflects past conditioning, then drug abusers should show stronger subjective and autonomic responses to drug-related cues than non-drug controls, and should show stronger responses to drug-related cues than to neutral cues or to cues related to substances other than the drug of choice.

The available studies (reviewed in Niaura *et al.*, 1988) have in fact consistently produced these results. With regard to autonomic reactivity, alcoholics frequently showed greater increases in heart rate, skin conductance and salivation in response to drinking-related cues than to other cues or compared to control subjects. Occasionally, increased changes in blood pressure, skin temperature and alpha waves were also reported. Increased autonomic reactivity to drug-related cues has also been found in smokers (Rickard-Figueroa and Zeichner, 1985; Saumet and Dittmar, 1985) and opioid addicts (Childress *et al.*, 1986; O'Brien *et al.*, 1986). Cocaine abusers showed increased skin conductance and heart rate, and decreased skin temperature following exposure to cocaine-related cues compared to non-drug controls (Negrete and Emil, 1992; Robbins *et al.*, 1997). These cues included the presentation of video tapes showing the buying and administering (e.g. 'free-basing' = smoking) of cocaine, audio tapes featuring users talking about their experiences with using cocaine, or even required subjects to engage in the preparation of surrogate cocaine powder for smoking. The same kind of cues related to other drugs, e.g. opiates, as well as neutral cues were not or much less effective in triggering autonomic responses in cocaine abusers (Childress *et al.*, 1988; Ehrman *et al.*, 1992).

With regard to subjective responses, craving or the urge to use the drug of choice as well as withdrawal symptoms are typically observed after cue exposure in substance-abusing or dependent individuals (Rickard-Figueroa and Zeichner, 1985; Ehrman *et al.*, 1992; Powell *et al.*, 1992; Robbins *et al.*, 1997). Reports of emotional or affective alterations are also frequent, although less consistent in nature. Interestingly, both dysphoria or distress (Powell *et al.*, 1992; Negrete and Emil, 1992) and euphoria or 'high' (Kranzler and Bauer, 1992; Robbins *et al.*, 1997) can be elicited by the cues.

While these findings are clearly compatible with a conditioning account of cue reactivity, they do not resolve the controversy that surrounds the issue of whether these cue responses are drug-like (i.e. similar to the actual effects of the drug) or drug-opposite in nature. There are different conditioning models that make different predictions as to the direction of these responses and how they may motivate continued drug-taking behaviour and relapse (reviewed in Niaura *et al.*, 1988).

Wikler developed a model that postulates that, apart from its direct reinforcing effect, a drug also has indirect reinforcing properties by suppressing acute withdrawal symptoms (such as craving) that have arisen from the previous drug ingestion. Since these withdrawal symptoms reliably follow drug ingestion and the environmental cues associated with it, they can become conditioned responses to those environmental cues (which themselves become conditioned stimuli).

A similar model by Siegel proposes that the conditioned responses to drug preparation stimuli are anticipatory compensatory reactions to counteract the pharmacological impact of the drug and maintain a homeostatic balance. In the absence of actual drug ingestion, these reactions will be experienced as drug-opposite changes including craving and dysphoria. Thus, both the 'withdrawal' and the 'compensatory' models have in common that conditioned responses to drug-related cues are not similar to the effects of the drug itself.

A third model favoured by Stewart and others posits that conditioned drug-related stimuli elicit a neural state and a concomitant positive appetitive response that are similar to those elicited by the drug itself. Thus, the drug itself or stimuli that have been paired with its motivational effects stimulate drug craving and drug seeking. This appetitive motivational model does not exclude drug-opposite cue responses, but it postulates that they do not contribute to relapse directly, but by virtue of becoming conditioned stimuli associated with the positive effects of the drug. In Wikler's and Siegel's models, drug-opposite reactions motivate drug use or relapse by a desire to use the drug to overcome these adverse or withdrawal-like effects.

Some studies have directly tested predictions made by these different models. Kranzler and Bauer (1992) have presented video tapes featuring neutral or cocaine-related material to cocaine-dependent male patients. Compared to the neutral material, the cocaine-related film material elicited significantly greater craving and drug-like euphoria, but not withdrawal symptoms. The degree of craving significantly correlated with euphoria but, again, not with withdrawal symptoms. These results clearly support an appetitive motivational model of drug effects. Reviews of the earlier literature focusing on alcohol and smoking (Niaura *et al.*, 1988) and on opiates and stimulants (Stewart *et al.*, 1984) also come to the conclusion that an appetitive conditioning account fits

better with the available data. More recent studies, however, do not unequivocally support this conclusion. For example, Ehrman *et al.* (1992) found much less evidence for a cocaine 'high' than for withdrawal and craving responses. Robbins *et al.* (1997) examined a large sample of male cocaine abusers and observed both withdrawal and high responses to cocaine-related audio and video tapes and to faked drug preparation rituals. Neither response, however, correlated with cue-induced craving, lending support to neither of the conditioning models.

Inconsistencies like these (and perhaps also general reservations regarding behaviouristic explanations) may make it worthwhile to look for alternative, non-conditioning theories of cue reactivity. One such alternative is derived from social learning theory and emphasizes cognitive over conditioned determinants of drug-taking behaviour and relapse (reviewed in Niaura *et al.* 1988). According to this model, there are stressful situations that place abstinent drug users at increased risk for relapse. These high-risk situations may or may not be drug related (i.e. may or may not include symptoms of withdrawal or high). If an individual's resources do not provide for a non-drug-related way of coping with the situation, this will decrease his or her confidence in being able to efficaciously handle the situation. Such negative self-attributions together with positive outcome expectancies regarding drug use (e.g. the expected pleasurable effects and the relief from withdrawal) will increase drug craving and the risk for lapse or relapse.

The data from several studies are in line with this model. Positive outcome expectancies in response to drinking-related cues were found to significantly correlate with increased desire for a drink in alcoholics and social drinkers (Cooney *et al.*, 1987). Moreover, positive outcome expectancies during detoxification correlated with relapse and the amount of drinking in detoxified alcoholics (Brown, 1985). In adolescents, the belief that alcohol would improve social competence was associated with drinking (Christiansen and Goldman, 1983). A more recent study by Powell *et al.* (1992) explicitly compared experimental predictions made by conditioning accounts with those following from a social learning model. These researchers exposed opiate addicts detoxified for at least 1 week to photographs of opiate ingestion and drug paraphernalia, including a fake packet of heroin with either injection equipment (needle and syringe) or foil and matches for smoking, depending on the subjects' preferred route of administration. Self-reports of cue-induced craving, physical symptoms, affect and positive outcome expectancies regarding emotional relief, hedonism, lifestyle, social relations and pain/withdrawal relief were collected. The opiate cues induced significant craving, dysphoria and withdrawal symptoms, but no increase in drug-positive effects. Craving significantly correlated with dysphoria, withdrawal, drug-positive effects and positive outcome expectancies, particularly with emotional relief. When all these variables together were entered into correlational analysis, craving no longer significantly correlated with withdrawal and drug-positive effects. The correlation of craving with dysphoria is consistent with the withdrawal and compensatory conditioning models proposed by Wikler and Siegel. The correlation with positive outcome expectancies, on the other hand, is clearly in accord with social learning models. The appetitive conditioning model received the least support in this study.

We can conclude that social learning models are a valid alternative to conditioning models, but that the final word about which theory is the right one, or whether the truth is not rather a new theory or a combination of existing models, is far from being spoken. These uncertainties stand in marked contrast to the consistency of cue-evoked subjective and autonomic responses in individuals dependent on a variety of substances. This consistency has fuelled the hope that cue desensitization or extinction may be effective complementary strategies in drug treatment, in particular relapse prevention (Childress *et al.*, 1987; O'Brien *et al.*, 1990).

PSYCHOPHYSIOLOGY OF SUBSTANCE ABUSE: FINDINGS, INTERPRETATIONS AND A MODEL

Findings and Interpretations

Table XVI-5.1 presents a qualitative and non-exhaustive overview of the psychophysiological findings among chronic substance abusers. As can be seen, many different measures and paradigms have been used, but few of them have been pervasively employed, making comparisons across substances difficult. Exceptions are P3 paradigms and resting EEG recordings, which reveal consistent decrements in P3 amplitude and increases in EEG beta activity in individuals abusing a variety of substances. Little evidence exists for drug-specific effects, which is in part a consequence of the scattered use of methods and paradigms. One such case is reduced alpha activity that appears to be a specific effect of alcohol not shared by other substances. Rather than further detailing these findings, the remainder of this section will be concerned with the question how these (primarily electrophysiological) findings can be interpreted in terms of their underlying brain substrate and functional significance.

Brain Substrates

Inferences to the brain loci underlying observed scalp EEG/ERP/EP changes are a notorious problem in electrophysiological research. This so-called 'inverse problem' states that it is impossible to unambiguously identify the intracerebral location/distribution of neuroelectrical activity that underlies a given scalp EEG recording. This means, for example, that, although the P3 is typically maximal over parietal leads, we cannot infer that it is generated in the parietal cortex. Likewise, the fact that P3 differences between alcoholics and controls are largest over posterior sites does not mean that this group difference emerges from posterior brain areas. While such inferences may have a certain validity in dense electrode array recordings, they are hopeless in the majority of studies reviewed here that used very few recording sites. Thus (with exceptions noted below), EEG/ERP studies by themselves cannot provide conclusive, only supportive, evidence for the brain location of observed electrophysiological changes. Such evidence could become more conclusive to the extent that other research, e.g. neuroimaging or lesion studies, would independently pinpoint the brain sources of (alterations of) EEG rhythms or ERP components. For example, the conjecture that decreases in P3a amplitude (which shows a frontal scalp distribution) in alcoholics may reflect frontal cortex damage gains substance through the independent finding that P3a amplitude decrements can also be found in patients with prefrontal lesions.

While this situation has looked like an impasse to some researchers, recent progress in EEG source localization techniques may provide a partial remedy. Using biologically plausible assumptions, it has become possible to compute the intensity and distribution of intracerebral neuroelectrical activity from scalp-recorded multi-channel EEG (see, for example, the LORETA method: Pascual-Marqui *et al.*, 1994, 1999). To the extent that methods like LORETA are validated, they allow us to infer, with a given precision, the brain loci generating the brain wave activity we record on the scalp and, consequently, to localize the brain areas underlying EEG/ERP changes observed in substance abusers.

Functional Significance

Assessing the functional significance of the electrophysiological findings in substance abusers has also been problematic. In our

Table XVI-5.1 A selection of psychophysiological findings in chronic substance abusers and individuals with a positive family history of alcoholism

	Alcohol	Cocaine	Opiate	Nicotine	Cannabis	Amphetamines	MDMA/'ecstasy'	Positive FHA
EEG								
Delta activity	↑	↓	–	↑	–		–	—
Theta activity	↑	—	–	↑	↑		↑	—
Alpha activity	↓↓	↑	–	↑	↑		↑	↑↓ –
Beta activity	↑↑	↑	↓[a]	↑	–		↑	↑
ERP								
P3 amplitude	↓↓	↓↓	↓	↓	↓	↓		↓↓
P3 latency	↑	↑	–	–	↑	↑		—
N100 amplitude	↓↓					↓		
N200 latency	↑↑							
MMN amplitude	↑↓							
EP								
P50 suppression		↓			↓			
VEP P100 latency	↑		↑					
VEP N75 latency	–		↑					
A/I slope	↑						↑	
BAER latency	↑							
Other								
Startle amplitude				–				
PPI				↓				
Cue reactivity	↑↑	↑↑	↑↑	↑↑		↑		

Doubled symbols represent robust findings, i.e. reported by the majority of a larger number of studies. Single symbols represent less robust findings, i.e. either positive reports by a small number of studies, or mixed reports by a larger number of studies. ↑ / ↓= increase/decrease; – = no change; empty cell = not reported; A/I = amplitude/intensity; BAER = brain stem auditory evoked response; FHA = family history of alcoholism; MMN = mismatch negativity; PPI = pre-pulse inhibition of the startle reflex; VEP = visual evoked potential.
[a]Beta decreased with increasing duration of use (Bauer, 2001c).

view, the problems originate from the nature of the findings and from confusions in the use of concepts such as 'deficit'. For example, many researchers, possibly aware of the tenuous nature of inferences to brain loci or brain damage, have interpreted electrophysiological changes in terms of some unspecified kind of brain 'dysfunction', 'deficit' or 'impairment'. However, used in this way, such terms are practically void for vagueness, unless we consider any functional change as a deficit, which will render the term meaningless. Further difficulties arise when changes of a given kind and magnitude are interpreted as deficits in some groups, but not others. An example of this are P3 decrements which can be found both in substance abuse populations compared to non-drug controls, and in healthy men compared to women. In the case of substance abusers, these decrements are labelled deficits, but usually not in the case of healthy men and women. Regarding ERP measurements, the problematic use of concepts connects with problems in the nature of the data. ERPs have widely accepted functional interpretations in terms of attentional resource allocation, context updating and the like. Thus the abundant demonstrations of altered ERP characteristics in substance abusers are usually interpreted as deficits of attentional or cognitive information processing. However, very often these alterations are not accompanied by deficits in attentional performance in the task used to elicit the ERP responses. Moreover, in many studies of substance abusers, deficits in attentional and cognitive performance are not even found in neuropsychological tasks that are much more difficult and cognitively demanding than ERP tasks.

A plausible explanation for this seemingly paradoxical situation is that the ERP may be a much more sensitive measure than task performance, and may therefore uncover even subtle, subclinical attentional changes that escape measurements of task performance. But to the extent this is true, the functional significance of such changes becomes more difficult to assess, since then these changes stand isolated from any overt clinical, behavioural or subjective consequences. It further becomes questionable whether we should label such changes 'deficits'. Finally, also the EEG is not spared from interpretive problems. The functional significance of EEG rhythms is still a chapter strewn with question marks. Neither clear nor unique higher-level functions have been identified for the different frequency bands. Alpha rhythms may serve as an example. Increases in alpha activity have been associated with such diverse states and processes as relaxed wakefulness, drug-induced euphoria, normal ageing and HIV brain pathology, to name just a few. Matters are further complicated by the fact that a small percentage of the normal population does not show detectable alpha rhythms — apparently without functional impairments. Unfortunately, the other frequency bands do not fare much better, making functional interpretations of EEG frequency changes fairly speculative.

The foregoing considerations are by no means intended to be a comprehensive treatment, but they point out some important limitations that afflict inferences from and interpretations of psychophysiological data. Currently, EEG/ERP/EP evidence regarding the biological underpinnings (i.e. the nature and location of brain changes) is essentially supportive rather than conclusive, and its functional interpretation is problematic. Ways to overcome these limitations include the use of sophisticated topographical analyses and advanced source localization methods. In addition, other research disciplines must continue to contribute to the elucidation of the brain sources and functional roles of EEG frequency bands and ERP/EP components. These efforts to strengthen and revitalize EEG technologies will be worthwhile, since the EEG has several crucial advantages over other brain-imaging methods, notably its superior time resolution, cheapness, high portability and ease of use.

A Model for Substance Abuse

The fact that certain electrophysiological findings such as P3 decrements and beta hyperactivity in substance abusers persist even with prolonged abstinence, are shared across different drugs (see Table XVI-5.1) and are found in unaffected relatives and offspring of affected individuals supports the view that these are premorbid characteristics not primarily related to the pharmacological effects of a given drug. Low P3 in particular meets the criteria for a psychophysiological endophenotype marking the predisposition for a range of psychopathology, including externalizing and substance-related disorders. Genetic epidemiological studies further support the view of a common heritable predisposition for the abuse of a range of different psychoactive substances, including cannabis, sedatives, stimulants, opiates and psychedelics. The same conclusion is bolstered by a growing body of neuroscientific studies that locates a common neural basis for the abuse of different substances in brain systems for reward, motivation and learning (see later chapters in this book).

Data from large-scale longitudinal family studies such as the Collaborative Study on the Genetics of Alcoholism (COGA) and the Minnesota Twin Family Study (MTFS) have provided invaluable clues regarding the nature of this predisposition. Iacono *et al.* (1999, 2000) have developed a model of substance abuse based on several important findings from the MTFS. The MTFS showed that in both adolescent boys and their fathers higher levels of externalizing disorders (CD in boys, ASPD or ASP features in fathers) are associated with substantially higher levels of substance abuse. Moreover, paternal alcoholism status also had a significant influence on the occurrence of substance use and externalizing tendencies in the boys, in that boys with alcoholic fathers had more externalizing and drug use symptoms than boys with non-alcoholic fathers. The largest increase in these symptoms, however, was seen in those boys whose fathers had an undersocialized form of alcoholism characterized by ASPD or ASP features, comorbid illicit drug abuse and low constraint. What these findings indicate is that the presence of substance use in adolescent boys appears to be largely dependent on the presence of concomitant externalizing features, both in themselves and in their fathers. The common denominator of these externalizing symptoms appears to be a form of behavioural disinhibition which manifests in a spectrum of impulsive, unconstrained, aggressive, but also drug-taking behaviours. Given these findings, and also taking into consideration the ample evidence for the heritability and genetic basis of these deviant behaviours, we arrive at a formulation of the first part of the model endorsed by Iacono and colleagues: *The propensity for substance abuse is part of a heritable predisposition for behavioural disinhibition, which is expressed as a spectrum of externalizing and substance use behaviours*. A similar view regarding alcoholism is held by Begleiter and co-workers from the COGA project (Begleiter and Porjesz, 1999). These researchers equate the inherited predisposition for alcoholism with a premorbid state of CNS disinhibition or hyperexcitability. This disinhibition or hyperexcitability can be temporarily alleviated by the consumption of alcohol, which facilitates the development of chronic alcohol use, and finally, physical dependence. Additionally, this disinhibited CNS state may manifest as disinhibited externalizing behaviours unrelated to substance use.

The second part of the model derives from psychophysiological research indicating that certain heritable psychophysiological changes are associated with substance use and externalizing behaviours as well as with the underlying neuronal/behavioural disinhibition, and may well share a common genetic basis with them. Thus, these psychophysiological changes qualify as endophenotypes marking the genetic risk for disinhibited psychopathology. We have seen that a low P3 is currently the best candidate for such an endophenotype. There are also other, less promising, candidates, including excessive beta activity or a poor modulation of electrodermal responses to predictable aversive stimuli (Taylor *et al.*, 1999; Iacono *et al.*, 2000). We can now formulate the second part of the model as follows: *Certain heritable psychophysiological abnormalities, in particular a low P3 amplitude, are endophenotypes marking the genetic predisposition for the development of substance abuse and externalizing disorders*. The complete model is depicted in Figure XVI-5.9. Figure XVI-5.9 also shows important influences and relations we have neglected so far. Thus, although the major portion of the genetic risk for substance abuse may originate from a common set of genes and may be expressed in a predisposition shared by all forms of substance abuse, there are also unique genetic contributions specific to a given substance. Furthermore, environmental influences, both within and outside the family, are as important as genetic factors in determining the risk for substance abuse. To put it in more popular terms, nature and nurture each contribute equally to the development of substance use, abuse or dependence.

It has also become increasingly clear in recent years that genes and environment interact and that these interactions are essential in understanding the aetiology of many disorders (see chapter XVII-10). A final important consideration is that the model is not intended to apply to all types of substance abuse. There is considerable heterogeneity in substance abuse populations with regard to patterns of abuse, time course and severity of the disorder, as well as gender, socio-economic background, symptom profiles and personality traits of the abusing individual. The scope of the model by Iacono and colleagues presented here is restricted to a type of substance abuse characterized by early onset and undersocialized or antisocial behaviour. This type is based on typologies proposed by Cloninger (1987) and other investigators for alcoholism. Integrating neurobiological and psychosocial data, Cloninger identified two basic types of alcoholism. Type I occurs in both genders, usually begins after age 25 and is entertained by rigid, apprehensive and emotionally dependent individuals with low novelty-seeking, high harm avoidance and high reward dependence. Type II occurs predominantly in males before age 25 and is entertained by impulsive, uninhibited, independent individuals characterized by high novelty-seeking, low harm avoidance and low reward dependence. Type II individuals are prone to antisocial personality features and aggressive acting out. It has been estimated that type II alcoholics make up about 80% of patients presenting for hospital treatment. Furthermore, as asserted by Cloninger, low P3 amplitude may be present only in antisocial type II, but not type I, alcoholics. Thus, the model of alcohol and drug abuse we present here may only be valid for a subset of disinhibited male substance abusers with antisocial tendencies. In women, internalizing rather than externalizing psychopathology, particularly mood and anxiety disorders, is associated with the risk for the development of substance abuse. Also, the genetic and neurophysiological basis of substance abuse may be quite different in women compared to men.

Despite these restrictions, the proposed model is an important step towards integrating our knowledge on the neuronal, genetic, psychosocial, behavioural and psychophysiological processes underlying a major type of substance abuse. This and similar models primarily reflect our advancements in the work based on the second major rationale for psychophysiological research on substance-related disorders, namely to find biological markers that identify individuals at risk for these disorders. Work based on the first rationale, which is the identification of the biological substrates and functional significance of psychophysiological changes in substance abusers, has faced more difficulties, as we have seen in the previous section. Nevertheless, the psychophysiological research into substance abuse and dependence, as reviewed in this chapter, has made significant advancements within the past three decades. Eventually, these advancements will also contribute to the development of more specific and effective treatments for

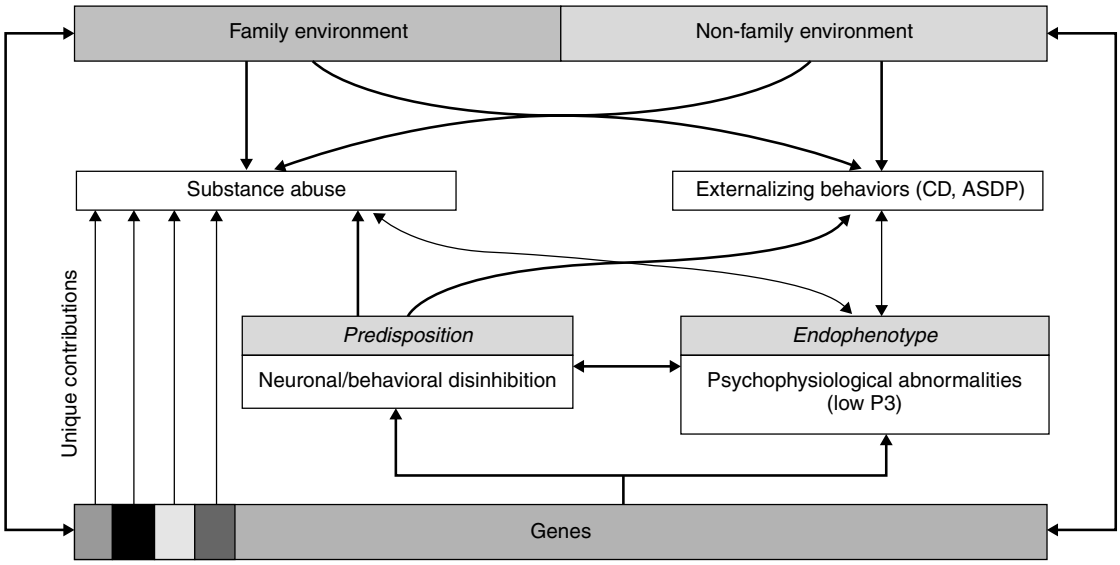

Figure XVI-5.9 A model depicting genetic, environmental and neural influences that jointly determine the risk for developing substance abuse or dependence. A common set of genes produce both a neural/behavioural disinhibition that predisposes for substance abuse and externalizing behaviours, as well as associated psychophysiological changes such as a small P3 amplitude. These changes can be conceptualized as an endophenotype that marks the predisposition for substance abuse and related externalizing pathology. The endophenotype can be used to identify unaffected carriers of high-risk genes, and hence to increase the power of genetic linkage and association analyses. Apart from common genetic factors, the development of substance abuse is also dependent on substance-specific genetic contributions, family and non-family environment and gene–environment interactions. After Iacono, Begleiter and co-workers

adult substance abusers and of prevention programmes that target high-risk children and adolescents before the critical age of substance abuse.

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XVI-6

Neuropsychology of Substance Abuse

M. Lacy and L. Sworowski

INTRODUCTION

In this chapter the neuropsychological aspects of substance abuse will be reviewed. The lifetime prevalence rate for any substance use disorder is 16.7% (Regier *et al.*, 1990). The DSM-IV (1994) lists 11 classes of substance-related disorders: alcohol, amphetamines, caffeine, cannabis, cocaine, hallucinogens, inhalants, nicotine, opioids, phencyclidine, and sedatives, hypnotics and anxiolytics. This classification system further breaks down the diagnosis into two categories: substance-induced disorders and substance use disorders. Cognition difficulties thought to be related to substance use are addressed under the category of substance-induced disorders. These include substance-induced delirium, substance-induced persisting dementia and substance-induced persisting amnestic disorder. The substance use disorders must be classified as either substance abuse or dependence conditions. There are no criteria for cognitive dysfunction in either substance use disorder. When cognitive problems are associated with these disorders, the diagnosis of substance-related disorder not otherwise specified may be used. Parsons (1996) has argued that while only 10% of alcohol-using patients have diagnosable organic disorders, another 50–85% of patients have mild to moderate cognitive deficits that do not meet the diagnostic criteria for either substance-induced dementia or persisting amnesia. Thus, the majority of studies in this area, with the exception of some alcohol-related research, have focused on investigating this latter group. Specifically, in the current body of literature most studies address the early neurocognitive effects of abuse and dependence, with few longitudinal studies, and even fewer studies addressing substance-induced disorders. Thus, this chapter will focus on reviewing these studies. As it is beyond the scope of this chapter to review all 11 classes of drugs, the neuropsychological aspects of some of the most commonly abused drugs among adults and adolescents — alcohol, cannabis, cocaine, 'ecstasy', ketamine, opiates and LSD — will be presented in the following sections. The neuroimaging, psychophysiological and functional imaging of substance abuse is reviewed in other chapters of this book and thus will not be addressed here in any detail. Currently, the most research in substance use disorders is directed at understanding the neurocognitive effects of alcohol use.

ALCOHOL

Alcohol is the most commonly used drug (DSM-IV, 1994). According to the National Institute of Alcoholism and Alcohol Abuse (NIAAA www.niaaa.nih.gov/publications), the prevalence of alcohol abuse and dependence is 7.41% for adults, with 18- to 29-year-olds having a prevalence of 15.94% and older adults, aged 65 and older, having the lowest prevalence at 0.64%. Approximately 10–14% of adults abuse alcohol, with at least 6% meeting diagnostic criteria for dependence. Alcohol-related problems begin early in life. Epidemiological studies indicate that over 50% of 14-year-olds in 2000 reported drinking at least once in their life, with 14.1% acknowledging binge drinking, defined as five drinks in the prior 2-week period. Over 71.4% of 16-year-olds acknowledge drinking in the past, with 26.2% admitting binge drinking. Approximately 80% of 18-year-olds acknowledge drinking alcohol, with 30% reporting an episode of binge drinking (NIAAA, 2001).

Given the early abuse and continuous widespread use of this drug, understanding the neuropsychological effects is imperative. While alcohol is involved in numerous medical conditions, this chapter will review the cognitive profiles associated with Wernicke–Korsakoff syndrome, alcohol-related dementia, alcohol abuse/dependence and to a lesser extent the scant research on social drinkers.

Neurocognitive Effects of Wernicke–Korsakoff Syndrome

In DSM-IV, Wernicke–Korsakoff Syndrome (WKS) is typically diagnosed as an alcohol-induced delirium followed by an alcohol-induced persisting amnestic disorder. The term WKS often refers to a two-phase syndrome, with an acute Wernicke encephalopathy (WE) followed by a persisting Korsakoff syndrome (KS). In the acute phase, patients may present with confusion, ocular difficulties (e.g. nystagmus) and cerebellar dysfunction (e.g. gait ataxia), although the sensitivity of this triad is questionable. In 131 WKS cases diagnosed at necropsy, Harper *et al.* (1986) found only 16% displayed this triad and 19% had no clinical symptoms. The prevalence of WE has been difficult to determine. A retrospective study (Torvik *et al.*, 1982) revealed that only 20% of post-mortem confirmed cases (12.5% of all alcoholics) were clinically diagnosed. Harper *et al.* (1995) found the prevalence of WKS to be 0.4–2.8% of autopsies regardless of clinical history. In the past, WKS has been associated with other nutritional conditions, such as self-starvation, intravenous feedings, persistent vomiting, malignancies, and carcinomas of the oesophagus, intestines and stomach. Recently there have been few unequivocal cases of amnestic syndromes from non-alcoholic depletion (see review, Kopelman, 1995). Wernicke patients who die in the acute phase show symmetrical lesions in the paraventricular regions of the thalamus, hypothalamus and mammillary bodies, along with less frequent lesions in the periaqueductal region of the midbrain, floor of the fourth ventricle and superior cerebellar vermis (Kopelman, 1995). Many patients who survive this stage often continue to have significant pathological changes and cognitive deficits (Colchester *et al.*, 2001; Park *et al.*, 2000; Victor *et al.*, 1971). Pathological studies suggest lesions must be present in the mammillo-thalamic circuits, with the anterior thalamic nuclei being the critical area for a resulting amnestic syndrome characteristic of KS (Cole *et al.*, 1992; Harding *et al.*, 2000).

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The prevalence and mode of onset of WE prior to KS are unclear. In the classic 1971 Victor *et al.* pathology studies, 96% of KS patients had histories of WE, with only 10% having an insidious onset. In contrast, Cutting (1978) found more heterogeneity in patients evaluated by a psychiatric service, with more frequent cases of insidious onset especially for females. The portion of patients with a documented history of WE is less frequently documented in the literature than seen previously and recent studies suggest KS may occur without clear history of documented WE (Butters, 1985; Jauhar and Montaldi, 2000). However, autopsy studies suggest that WE is often underdiagnosed (e.g. Harper *et al.*, 1986) and thus the interaction between WE and subclinical episodes prior to KS is unclear.

Clinically, KS is characterized by persistent and disproportionate memory deficits, in contrast to generally intact intellectual functioning (Lezak, 1995) thought to be related to or the direct result of thiamine deficiency, although this latter finding is debatable (Butters, 1985; Molina *et al.*, 1994; Thomson, 2000). To investigate memory processes across dementing conditions, Delis *et al.* (1991) examined the memory performances of Alzheimer (AD), Huntington and KS patients using a 16-item list-learning task. The AD and KS groups did not significantly differ on immediate free recall, learning slope, semantic clustering, primacy–recency effects, delayed free recall, recognition discriminability, false positives and response bias indices. Using percentage of cued intrusion errors and recognition discriminability versus initial learning, discriminant function analysis classified 100% of the KS patients as AD and correctly classified 85% of the AD patients. Thus, KS patients displayed a pattern of memory failure seen in other cortical, rather than subcortical, dementias.

While explicit memory processes are impaired in this population, other aspects of memory may be preserved. The classic studies of amnestics by Warrington and Weiskrantz (1982) revealed adequate speed of access to semantic and phonological knowledge, some degree of improvement on retesting, and differential improvement in retention when tested by cued recall and a prior learning effect. More recently, Heindel *et al.* (1993) compared memory performances of KS, AD, Huntington and Parkinson patients. The KS group performed more similar to the AD group on tests of explicit memory, but displayed normal performances on priming tests, indicative of intact implicit memory abilities. In addition to intact implicit memory performances, KS patients also demonstrate average working memory and average forgetting rates/storage when encoding is equated (Kopelman, 1985).

While explicit memory failure has traditionally been attributed to encoding disruption and diencephalic disruption, several researchers have suggested that the memory disturbance in WKS is at least partly attributable to retrieval failures reflective of frontal lobe dysfunction (Noel *et al.*, 2001). To examine this possibility, Noel *et al.* (2001) presented the case of GS, a patient with prior WE symptoms. The patient completed several tests of verbal memory, which included recognition paradigms. GS displayed primary retrieval deficits on all tests of verbal memory. The authors cite this case as an example of the heterogeneity in this population and the possibility of frontal dysfunction, reflective in retrieval rather than encoding deficits, underlying the memory disturbance in some patients. However, GS performed within normal limits on a demanding list-learning task, raising doubts about the significance of the reported episodic memory deficit in this patient.

Along with anterograde memory loss, KS patients also display a significant retrograde amnesia, steeper than that seen in AD (Kopelman, 1989). These patients have difficulty recalling events prior to the onset of the condition, on a steep temporal gradient. Patients have been noted to confabulate in the presence of this dense amnesia, although this appears rare and more often in the acute phases (Victor *et al.*, 1971). When present in the more chronic condition, several researchers argue that this may reflect inaccurate recall of real memories out of temporal context, reflective of frontal pathology (Kopelman, 1987, 1991a, 1991b). In fact, several studies have shown that KS patients have poor temporal orientation and judgement of time (Mimura *et al.*, 2000; Taylor and O'Carroll, 1995) along with consistent deficits across studies on measures of executive functioning, including problem solving, metamemory, proactive interference and insight (Janowsky *et al.*, 1989; Jacobsen, 1990; see review, Lezak, 1995).

As noted previously, typically KS perform similarly to chronic alcoholics on tests of generalized intelligence. Overall, they display better verbal intelligence, but impaired speeded visuospatial organization and perceptual skills. On simple tests of attention, such as digit span, scores are usually in the average range, but deficits emerge as more complex sustained and divided attention is required (Lezak, 1995).

In summary, the neuropsychological profile of KS includes impairments in recent and remote memory, along with milder deficits in executive functioning. In contrast, KS patients demonstrate relatively intact overall intelligence, implicit memory and rates of forgetting.

Neuropsychological Profile Associated with Alcohol-Induced Dementia

Based on DSM-IV criteria, alcohol-related dementia falls under the diagnosis of substance-induced persisting dementia. To combat the often vague and subjective aspects of the DSM-IV criteria, Oslin *et al.* (1998) proposed a more defined set of criteria (Table XVI-6.1) for alcohol-related dementia (ARD) similar to existing criteria for Alzheimer and vascular dementias. ARD is defined as a syndrome with multiple possible aetiologies, including ethyl alcohol, nutritional deficiencies, metabolic disturbance, immune-related injury, trauma and vascular injury. The use of these more defined criteria may allow for improved validity and reliability of the diagnosis across studies. To date, few published studies have utilized these criteria.

Possibly because of the vagueness in the DSM-IV criteria, the prevalence of ARD is unclear. Most studies list alcohol use as a comorbid condition to dementia, rather than a causative factor. When studies directly address alcohol use and cognition, the issue of dementia is rarely addressed. Despite these methodological problems, it is estimated that 22–29% of dementia cases are related to alcohol dependence. Alcohol abuse or dependence is 1.5 times more common in elderly demented patients. Dementia is 4.6 times more likely to occur in older men with histories of heavy drinking, and increasing alcohol consumption has been associated with a worsening performance on dementia screening scales (Oslin *et al.*, 1998). Finlayson *et al.* (1988) found 23% of elderly patients presenting for alcohol treatment met criteria for dementia. Approximately 29% of institutionalized elderly patients have alcohol-induced dementia. The only longitudinal study to examine the duration of heavy drinking as a risk factor for developing dementia revealed a 5-year history of drinking as a risk factor (Saunders *et al.*, 1991).

The neuropsychological profile in ARD has received much less attention in the literature than the subtler deficits associated with abuse or dependence. Research available suggests deficits in memory functioning, along with visuospatial and executive abilities (Cummings and Benson, 1992). While early studies have suggested deficits in encoding, more recent investigations utilizing recognition paradigms suggest retrieval deficits may underlie the memory impairment. For instance, Saxton *et al.* (2000) compared AD, non-demented elderly alcoholics, ARD elderly and normal controls. The alcoholic patients were matched on liver disease, nutritional status, number of head injuries and number of periods of abstinence. Compared to the AD patients with similar premorbid IQ estimates

Table XVI-6.1 Classification of alcohol-related dementia

A. Probable alcohol-related dementia includes the following:

1. A clinical diagnosis of dementia at least 60 days after the last exposure to alcohol.
2. Significant alcohol use as defined by a minimum average of 35 standard drinks per week for men (28 for women) for greater than a period of 5 years. The period of significant alcohol use must occur within 3 years of the initial onset of dementia.

B. The diagnosis of alcohol-related dementia is supported by the presence of any of the following:

1. Alcohol-related hepatic, pancreatic, gastrointestinal, cardiovascular or renal disease, i.e. other end-organ damage.
2. Ataxia or peripheral sensory polyneuropathy (not attributable to other specific causes).
3. Beyond 60 days of abstinence, the cognitive impairment stabilizes or improves.
4. After 60 days of abstinence, any neuroimaging evidence of ventricular or sulcal dilatation improves.
5. Neuroimaging evidence of cerebellar atrophy, especially of the vermis.

C. The following clinical features cast doubt on the diagnosis of alcohol-related dementia.

1. The presence of language impairment, especially dysnomia or anomia.
2. The presence of focal neurological signs or symptoms (except ataxia or peripheral sensory polyneuropathy).
3. Neuroimaging evidence for cortical or subcortical infarction, subdural haematoma, or other focal brain pathology.
4. Elevated Hachinski Ischemia Scale score.

D. Clinical features that are neither supportive nor cast doubt on the diagnosis of alcohol-related dementia included:

1. Neuroimaging evidence of cortical atrophy.
2. The presence of periventricular or deep white matter lesions on neuroimaging in the absence of focal infarct(s).
3. The presence of the apolipoprotein e4 allele.

The diagnosis of possible alcohol-related dementia may be made when there is:

1. A clinical diagnosis of dementia at least 60 days after the last exposure to alcohol.
2. *Either*: Significant alcohol use as defined by a minimum average of 35 standard drinks per week for men (28 for women) for 5 or more years. However, the period of significant alcohol use occurred more than 3 years but less than 10 years prior to the initial onset of cognitive deficits.
   Or: Possibly significant alcohol use as defined by a minimum average of 21 standard drinks per week for men (14 for women) but no more than 34 drinks per week for men (27 for women) for 5 years. The period of significant alcohol use must have occurred within 3 years of the onset of cognitive deficits.

Mixed dementia

A diagnosis of mixed dementia is reserved for clinical cases that appear to have more than one cause for dementia. The classification of probable or possible should continue to be used to convey the certainty of the diagnosis of ARD. The classification of mixed dementia should not be used to convey uncertainty of the diagnosis or to imply a differential diagnosis.

Alcohol as a contributing factor

The designation of alcohol as a contributing factor is used for the situation in which alcohol is used, but not to the degree required or within the time required to meet the classification of probable or possible alcohol-related dementia. This designation should not preclude the use of probable vascular dementia or probable dementia of the Alzheimer type.

and current MMSE scores, the ARD group exhibited significantly more deficits on tasks of executive and visuoconstruction ability. In contrast, the AD group displayed worse semantic memory abilities as displayed on tests of confrontational naming, recall and recognition memory. The ARD group performed worse than the non-demented alcoholic group across the test battery, but were only significantly different on two measures. They performed slower on a measure of fine motor control and on tests of initial learning and delayed free recall, but displayed similar recognition memory compared to normal controls and non-demented alcoholics, indicative of retrieval problems underlying memory impairment. The non-demented alcoholics and controls were only significantly different on immediate and delayed recall of visual material, possibly reflecting the early impact of alcohol on visuospatial functioning (Sher *et al.*, 1997). Data on the performances between these two groups on the recognition paradigm of this memory test were not presented and thus the nature (encoding, storage or retrieval) of the visual memory impairment is unclear.

Neuropsychological Functioning in Alcohol Abuse and Dependence

Over the last 25 years, Parsons and his colleagues have conducted the most extensive research on recently detoxified alcoholics, which they term intermediate-stage alcoholics (Parsons, 1998). Using longitudinal and cross-sectional studies, they have also been able to investigate the long-term effects of alcohol abuse. This extensive body of literature suggests that intermediate-stage alcoholics display deficits across neuropsychological test batteries, with lower scores on tests of memory, learning, abstracting, verbal problem solving, visuoperceptual analysis, perceptual motor speed and mental processing speed (Parsons, 1998). Along with being less accurate, other studies by this group of researchers (i.e. Glenn *et al.*, 1992) have suggested that alcoholics take a longer time to complete these tasks, resulting in deficits in efficiency.

In terms of severity, inspection of scores between the patient and control groups suggests only mild difficulties. For example, in one study (Parsons, 1998) alcoholics obtained the following mean domain T scores: verbal mean 48.5, visuospatial mean 48.8, perceptual motor mean 48.9 and semantic memory mean 48.2; compared to the following scores for the control group: verbal mean 52.2, visuospatial mean 51.8, perceptual motor mean 51.5 and semantic memory mean 52.6. All of these T scores reflect performance within the average range. Parson suggests that intermediate-stage alcoholics display a neurocognitive profile similar to mild brain-injured patients, with scores approximately one half a standard deviation or more below the mean. Thus while recently detoxified alcoholics display lower performance on neuropsychological measures, data suggest only subtle differences. In fact, in a study by a different group of researchers (Horner *et al.*, 1999), examining mild to moderate alcoholics without history of inpatient admissions or withdrawal symptoms, the alcohol group performed in the average range, despite the noted averse relationship between cognition performance and recent drinking. Further supporting the hypothesis of only minimal disturbance with alcohol abuse, Parsons reported significant overlap between

alcoholics and controls on most neurocognitive measures. Thus, the neuropsychological impact of alcohol abuse is subtle and diffuse in chronic alcohol abuse.

In terms of aetiology, despite examining numerous behavioural and medical variables, Parsons and colleagues (e.g. Nixon *et al.*, 1995), along with others (e.g. Ham, 2000), have shown that depression and history of childhood behavioural disorders reliably account for only a portion of the differences between patients and controls on cognitive measures. Nixon *et al.* (1995) found that while alcoholics reported more childhood behavioural disorders and residual attention deficit disorder, regression analysis indicated that alcohol abuse was the best predictor of cognitive efficiency. Ham (2000) found that even subclinical levels of depression and anxiety, along with childhood ADD, may contribute to the magnitude of the obtained impairment in cognition, although differences remain when these effects are partialled out. Thus, while childhood disorders (e.g. ADD) and emotional distress may contribute to the neurocognitive difficulties documented in alcoholics, they do not appear to solely account for these deficits. Parsons (1998) has suggested that individual differences may be related to a vulnerability factor. In fact, Di Scalfani *et al.* (1998) examined this hypothesis, which suggests that individuals with larger premorbid brains may have a later onset or lessened severity of cognitive impairment, which may account for the variability across subjects. They investigated the relationship between brain volume based on magnetic resonance imaging and neuropsychological performance in 28 cocaine–alcohol-dependent individuals, 19 cocaine-dependent only subjects, and 19 controls. Intracranial volume accounted for 20% of the variance in cognitive performance. While this study examined data primarily in cocaine-dependent individuals, it suggests the same may be true in alcoholic-only individuals.

Recovery

Parsons and others have also examined the issue of recovery, by employing cross-sectional and longitudinal studies. In 1983, Fabian and Parsons compared 40 alcoholics, sober for 4 years, 40 alcoholics sober for 1 month and 40 controls on a battery of neuropsychological tests. As seen previously, the recently detoxified patients performed significantly differently from the control group, while the long-term sober group performed similarly to the control group. This finding was replicated by a different group of researchers. Rourke and Grant (1999) found that age, length of abstinence and interim drinking affected long-term neurocognitive outcome. They examined 97 alcoholics shortly after detoxification (mean 29.7 days) and again 2 years later. At 2 years, 62 subjects resumed drinking, while 35 maintained abstinence. These patients were compared to 29 age-, education- and years of drinking-matched long-term abstinent alcoholics (mean years = 4.3) and 49 non-alcoholic controls. Results indicated that the group that maintained sobriety for 2 years displayed improved abstraction abilities, albeit still below long-term abstainers. Furthermore, those over the age of 50 who continued drinking displayed deteriorating abstraction abilities. Also, those patients who continued drinking developed worsening motor skills, regardless of age. In summary, the authors conclude: 'What the results from the current study may suggest is that certain neuropsychological abilities may become more susceptible to the toxic effects of alcohol as a person ages, and that the recovery of certain abilities in chronic alcoholics may require protracted periods of abstinence for normalization as a persons increases in age' (p. 242). Consistent with other investigations (e.g. Yohman *et al.*, 1985; Glenn *et al.*, 1994), it is clear that neuropsychological deficits deteriorate with continued alcohol abuse, but may recover with prolonged abstinence.

Horner *et al.* (1999) examined 69 mild to moderate alcohol-dependent outpatients without any inpatient history screened for other psychiatric diagnosis, medical condition or medication which could affect cognition. Examinations occurred approximately 4 days after abstinence or after withdrawal symptoms subsided. Patients were divided into groups based on recent alcohol consumption. Patients with higher consumption in the last 90 days performed significantly below low-consumption users on tests of delayed verbal memory and conditioned reaction time. Linear correlations using the entire sample revealed negative correlations between alcohol consumption and abstract reasoning, inhibition of automatic responses, condition reaction time and memory. Examination of years of drinking revealed no association with neuropsychological performance. Thus, in this group of high functioning mild to moderate drinking alcoholic outpatients, cognitive performance was related to recent drinking rather than long-term history of abuse.

Social Drinking

Finally, Parson and Nixon (1998) addressed the issue of neurocognitive deficits in social drinkers. In this review, 10 of 17 studies failed to demonstrate cognitive deficits associated with 'social' alcohol consumption. However, the negative study subjects averaged only 16.4 weekly drinks, while the positive studies subjects averaged approximately 42 drinks weekly. Further review suggested that consuming 21 or more drinks per week may result in the emergence of subtle cognitive difficulties.

Summary

In summary, the neurocognitive impact of alcohol abuse/dependence is diffuse, affecting memory, perception, construction, visuospatial, abstraction, executive and motor functioning. Inspection of data comparing normal controls and alcoholics indicate mild to subclinical deficits. This relative impairment is not solely accounted for by premorbid variables such as childhood behavioural disorders, or by current depression. Individual differences may reflect a vulnerability factor. Long-term abstinence may result in almost full recovery, especially in younger patients. Long-term abuse may result in continuing decline, with the possible development of an alcohol-related dementia. However, the amount drunk per week may have a greater impact on outcome than chronicity. Social drinkers who drink 21 drinks per week are at risk for developing cognitive dysfunction.

CANNABIS

Cannabis, the most widely used street drug in the world, contains over 60 cannabinoids. The pharmacological properties of these cannaboids are mostly unknown, although the most potent agent, tetrahydrocannabinol (THC), has been identified and examined in numerous studies. Unfortunately, the increasing THC content in the average marijuana cigarette over the last several decades has made it difficult to compare studies across time. For instance, in the 1970s the average marijuana cigarette contained 10 mg THC, while today the THC level can range from 20 to 300 mg depending on cultivation and other breeding techniques (Aston, 2001). Along with this drastic increase in potency, there has been a significant increase in the prevalence of cannabis use over the last decade. For instance, 30–40% of 15- to 16-year-olds in the UK and the USA reported trying marijuana, with 60% of university students in the UK reporting regular use of marijuana.

Studies investigating the acute impact of cannabis use on cognitive functioning have resulted in mixed results. In their 1995 review, Pope *et al.* concluded that there was significant data to indicate that there were acute, but not residual effects of marijuana on cognition. Since then, there continue to be studies raising questions regarding the reliability of acute effects of marijuana on

cognition. For instance, Fant *et al.* (1998) had 10 male participants smoke a single joint containing high and low doses of THC and then complete various cognitive tasks. In contrast to several earlier studies, results revealed no acute effect on cognition, regardless of potency. In 2001, Pope *et al.* examined 63 current heavy smokers, 45 'former' (abstinent 3 months) smokers and 72 controls at 0, 3, 7 and 28 days after admission. Results revealed that the current smokers performed worse on a list-learning task only on day 7, with performances similar to controls on nine other neuropsychological measures, including tests of attention, visual memory and executive functioning. Thus, recent studies raise questions regarding even reliable acute effects. Differences across studies in terms of study design (e.g. naturalistic versus laboratory), period of abstinence, potency of drug, years of abuse, premorbid intelligence and other substance use may underlie conflicting findings.

In summary, there are conflicting findings regarding acute cognitive effects related to marijuana use. When effects are found they appear to be subtle, affecting attention and memory, and resolving quickly with abstinence. There is no reliable evidence of permanent cognitive impairment in cannabis users.

COCAINE

Cocaine, in the form of cocaine hydrochloride powder, is one of the most frequently used drugs in the world. In 1999, NIDA reported that the rate of use in 17- to 18-year-olds was 9.8%, compared to 5.9% in 1994. In the last year, 6.2% of 18-year-olds reported using cocaine at least once, while 2.6% used it in the last month. Almost 5% of 13- to 14-year-olds have used cocaine at least once, 2.7% in the last year and 1.3% in the last month. In 1995, it was estimated that 1.5 million Americans used cocaine, with half a million using the drug weekly. In 2000, 12.7% of adults over the age of 26 reported trying cocaine in their lifetime.

Cocaine continues to be a widely used drug, with significant central nervous system complications. Cocaine use has been associated with seizures, ischaemic stroke, intracerebral haemorrhage, cerebral vasculitis, vascular spasm and transient ischaemic attacks (Lezak, 1995). Neuroimaging studies, including single photon emission tomography (SPECT) and positron emission tomography (PET), have revealed decreased cerebral metabolism and blood flow in prefrontal cortex associated with cocaine use (Rogers and Robbins, 2001). Given the possible impact on brain functioning, and the frequent complaint of cognitive difficulties among abusers (Washton and Tatarsky, 1984), it is important to examine the current state of the literature examining the neuropsychological impact of cocaine abuse/dependence.

Horner (1999) outlined eight factors to consider in evaluating cocaine studies: (1) specific measures used, (2) sample size, (3) length of abstinence at time of evaluation, (4) monitoring of abstinence, (5) severity and type of use, (6) concurrent abuse of other substances, (7) neuromedical, developmental and psychiatric histories, and (8) comparability of control groups. While not specifically addressed by Horner, it is also important to consider estimates of premorbid intelligence in assessing the comparability of control groups, a factor rarely addressed in this body of literature. Finally, an additional methodological problem, seen in all studies examining abuse variables, is the reliance on self-report regarding such important variables as prior intensity, frequency and duration, along with polysubstance abuse, including nicotine. Over the last several years, while better methodological criteria have been employed, problems still exist that thwart a solid understanding of the neurocognitive impact of cocaine use. The following will review the most recent studies, which employ better research design.

Rosselli and Ardila (1996) compared 61 cocaine-dependent Colombian subjects to 63 controls. Using the conservative alpha level of 0.01, significant differences were seen on measures of WMS Logical Memory Immediate Recall and Mental Quotient. Significant differences were also seen on all measures derived from the Wisconsin Card Sorting Test (WCST), a test of executive functioning. Length and frequency of use correlated with level of impairment. No differences were seen on tests of sustained attention, fluency or naming, with only modest trends ($p < 0.05$) seen on measures of delayed visual and verbal memory, visuoconstruction and working memory. While this study suggests mild difficulties on immediate attention and executive functioning, the drug group had lower IQ scores and higher rates of history of anxiety and suicide attempts, raising questions regarding the comparability of the groups and the aetiology of the impairments.

Gillen *et al.* (1998) compared 19 cocaine-dependent and 16 non-cocaine-dependent men. All subjects were screened for history of other psychiatric or major medical conditions, childhood attentional difficulties, mild head injuries, learning disabilities and recent psychoactive medication. For the both groups, other substance abuse/dependence was also an exclusionary criterion, except current alcohol abuse and alcohol dependence in remission in the cocaine group. Cocaine use during the preceding month had to exceed 4 g, with the average 15.8 g. Comparisons between the groups revealed equivalent age, education, socio-economic status and racial composition. The cocaine group was statistically different on symptoms of depression, alcohol use and family substance abuse history compared to the control group. Cocaine abusers were tested approximately 7–8 days after entering the study and half were taking carbamazepine. No toxicology screens were conducted during the week prior to testing. No differences were found between the two cocaine groups and thus the data were grouped.

Tests results were interpreted as revealing group differences on Information, Vocabulary, and Picture Arrangement subtests of the WAIS-R (Weschler, 1981), with a trend for delayed visual memory and phonemic fluency. However, given that 29 comparisons were conducted, the more stringent criteria for significance would suggest only significant differences on the WAIS-R subtests Vocabulary and Picture Arrangement, with the cocaine group actually performing better on Trails A. As the authors note, the differences on the information and vocabulary subtests may reflect differences in premorbid IQ, as the control group had slightly higher education and socio-economic status and these tests are most resistant to brain changes and are highly correlated with premorbid intelligence. It is also noteworthy that all scaled scores on the WAIS-R were within the average to low average range (8–10) for the cocaine group, again suggesting no significant impairment. Thus, this study again suggested no significant differences between a small group of recently abstinent cocaine-dependent individuals, despite histories of alcohol abuse, mild depression and possibly lower premorbid IQ compared to a slightly better-educated control group.

Selby and Azrin (1998) examined 60 cocaine-abuse/dependent incarcerated felons, abstinent for an average of 36 months, compared to 138 non-cocaine-abusing felons. Cocaine abusers averaged over 8 g per week for longer than 12 months, for an average of 13 years of use and were abstinent for 36 months, although screening of current use was not reported. The groups were similar in age, education, race and intelligence, and screened for comorbid conditions. Overall analysis revealed that the cocaine group performed similar to the control group on tests of short-term and long-term memory, executive and visuomotor functioning. Thus, this study demonstrated that after long abstinence individuals with a history of cocaine abuse/dependence do not display significant neurocognitive deficits compared to well-matched controls.

Robinson *et al.*'s (1999) study compared 30 cocaine abusers, 30 cocaine and alcohol abusers and 30 age-, education-, race- and sex-matched controls on an extensive battery of neuropsychological tests. Cocaine abusers reported using approximately 11.9 g per month over 4 years, with 67% freebasing and 33% inhaling.

Patients reported abstinence for a mean of 146 days. Current alcohol use was unavailable, but patients were excluded if they met diagnostic criteria for abuse or dependence. Neuropsychological data were converted to age-, education- and sex-corrected *T* scores based on Heaton normative data (Heaton *et al.*, 1992), rather than using the control group as a reference. Results indicated that all groups obtained similar full-scale IQ and Heaton Reitan Average Impairment ratings. In contrast, on the Global Deficit Score, reflective of tests on which subjects performed two standard deviations below the mean, a higher percentage of cocaine users (27%) scored in the impaired range. Inspection of tests revealed differences on the Psychomotor, Simple motor and Attention domains, with no differences found on verbal, executive, learning, incidental memory, explicit memory and sensory abilities. Specifically, patients performed significantly worse on digit symbol, picture arrangement, Trails A, Speech Perception and grip strength. Impairment on these four cognitive tasks may reflect attentional difficulties. In contrast, they performed equally as well as controls on the Tactile Performance Test, Object Assembly, Block Design, Digit Span, Arithmetic, Picture Completion, Rhythm and tests of speeded dexterity. Thus, patients reporting abstinence for over 4 months demonstrated only inconsistent deficits on speeded motor-based attention tasks, along with reduced grip strength, with intact overall IQ, memory and abstraction.

While Robinson *et al.*'s study suggests some aspects of attentional processing may be impaired in abstinent cocaine users, consistent with evidence of frontal lobe dysfunction from neuroimaging studies, Horner's (1999) review of the literature on attentional processing in cocaine abusers did not support a consistent attentional deficit in this population. Horner identified seven well-designed studies that tested patients after several weeks of abstinence and controlling for confounding variables (Herning *et al.*, 1990; O'Malley *et al.*, 1992; Berry *et al.*, 1993; Roberts and Bauer, 1993; Bauer, 1994; Beatty, 1995; Van Gorp *et al.*, 1995). Despite improvements in methodology, mixed findings persisted across studies. Including the above-cited studies and others, Horner concluded, 'Thus, based on the evidence accumulated to date, it would be premature either to accept or reject the hypothesis of attentional impairment associated with cocaine dependence' (p. 30).

In one of the few longitudinal studies of cocaine abusers, Van Gorp *et al.* (1999) examined 37 cocaine abusers with a 1-year history of abuse, 13 years of education and almost daily use of cocaine, with recent use over 4 g in the last month. Subjects were compared to age- and education-matched controls within 72 hours of admission, and then 10, 21 and 45 days later on tests of declarative and procedural memory. Patients were monitored for substance use during the course of the study. Results revealed no significant differences on tests of verbal delayed memory at any testing time, although drop-out rates may have influenced this finding. On a measure of non-verbal delayed memory (Rey Complex Figure), the cocaine group performed significantly and consistently worse on the delayed free recall subtest at each test session. In contrast, this group displayed a faster rate of learning on the procedural memory task, even when the positive effect of nicotine was controlled. Thus, in this longitudinal study of relatively 'new' (use < 2 years) cocaine abusers, they were able to learn and recall verbal information in a normal fashion and learned at a faster rate on a procedural memory task, but displayed consistently worse delayed visual memory. One possible explanation for this finding is that patients with a prior history (greater than 6 months) of alcohol abuse were not excluded from this study. Selby and Arzin (1998) found that patients with a history of polysubstance (alcohol plus cocaine) abuse performed worse on the same measure of visual memory, while cocaine-only abusers performed in a similar fashion to controls.

To address the issue of dose-related effects, Bolla *et al.* (1999) compared 30 abstinent cocaine-abusing and 21 non-abusing well-matched controls, screened for past and present psychiatric and neurological illnesses, including other substance abuse. Cocaine subjects used cocaine by any route for at least 1 year, at least four times a month, and were using at the time of admission to the study. The cocaine group was given a comprehensive neuropsychological battery on the 28th or 29th day after entering into the inpatient unit. They received no treatment or medication during their inpatient stay prior to testing and randomly underwent urine screens to confirm abstinence. Controls and subjects were matched on premorbid intelligence, education and age. Multiple regression analyses indicated that the cocaine group performed better than the control group on tests of immediate memory (RAVLT Trial 1) and visuoperceptual/construction problem solving (Block Design), while performing worse on a measure of reaction time (go/no-go paradigm). This is consistent with other findings of other researchers, who have shown better performance on certain neurocognitive tasks in cocaine groups (e.g. verbal fluency, O'Malley *et al.*, 1992; attention, Gillen *et al.*, 1998; visuospatial learning and recall, Manschreck *et al.*, 1990).

Regression analysis revealed dose-related effects on tests of memory, executive, visuomotor, psychomotor speed and manual dexterity. Intensity of use, defined as grams of cocaine used per week, rather than duration or weekly frequency, was the most consistent predictor of performance. Bolla *et al.* hypothesized that neurobehavioural effects would only be seen at high levels of use. High-abusing patients (>2 g per week) were compared to low-abusing patients. Results indicated that the high-use patients performed worse than the low-use patients. However, while there were significant differences between the abusers, analyses comparing the high-dose users and the control group were not discussed. Inspection of available mean data indicated few differences on several of the measures. Specifically, the high-abusing group performed worse on only two tests, with slower reaction times and slower Trails B performance. Thus, these data suggest deficits on speeded cognitive flexibility and reaction time with dose-related effects for heavy cocaine-smoking individuals, but otherwise intact performance on other cognitive domains.

Bolla *et al.* (2000) also examined the impact of dose-related effects of cocaine and cocaine plus alcohol, on cognition. The study examined 29 chronic (mean 8 years) cocaine abusers, without history of alcohol abuse, and 27 cocaine abusers who also reported consistent abuse of alcohol (mean 15 years), in a treatment facility at 1–3 days of abstinence and again after 4 weeks. At time 1, regression analyses revealed dose effects on learning and memory measures for those who used 3 g or more of cocaine per week. Digit symbol also was negatively related to cocaine dose. Performance on the go/no paradigm revealed more false positive errors and less accuracy with higher cocaine use. After 4 weeks, partial variance regression analyses revealed dose-related effects for tests of mental processing speed in those reporting mid-range use of substances. Those who performed better at time 1 showed less of a dose-related effect, supporting a cognitive reserve hypothesis. There was also a dose-related effect for delayed verbal memory, with those that abused more cocaine performing worse at 4 weeks than low-dose users. Thus, this study supports the hypotheses of dose-related effects on certain measures of cognition, consistent with Jasiukaitis and Fein (1999). Those authors also found dose-related effects on cognition, with low-using patients ($22 000 spent per year) performing worse than high-using subjects ($64 000 spent per year). However, the subjects in this study used alcohol along with cocaine. While statistically controlled, this is not a clear assessment of the role of dosage on cognition in a group of cocaine-alone abusers.

Together these recent investigations indicate the impact of recent and long-term abuse of cocaine is minimal when present, though

possibly more likely when high doses are consumed, but may be compounded by the concurrent abuse of alcohol.

'ECSTASY'

'Ecstasy', ±3,4-methylene-dioxymethamphetamine (MDMA), is a ring-substituted amphetamine derivative with both stimulant and low-level hallucinogenic properties that was originally used for potential chemical warfare by the US Army in the 1950s. Psychotherapists later used it in the 1970s as an adjunct to psychotherapy because of its properties of increasing self-awareness and empathy (Rodgers, 2000). At present, the consumption of MDMA is primarily by adolescents and young adults in many Western countries (Bolla *et al.*, 1998; Morgan, 2000; NIDA, 2001). A recent Norwegian survey indicated that more than 3% of adolescents aged 14–17 have used ecstasy, with twice as many boys using the drug than girls, and in the UK between 200 000 and 5 million tablets are taken each weekend (Pedersen and Skrondal, 1999; Rodgers, 2000). In the USA, ecstasy use is associated with 'rave' culture and dance parties, and there are escalating numbers of both high school and college students who use MDMA at increasingly higher doses in other social contexts. For example, the rate of use of MDMA increased from 1999 to 2000 for students ages 13–18 (NIDA, 2000). The National Institute on Drug Abuse review of drug abuse trends among US grammar school and high school students showed that lifetime use of MDMA among 13- to 14-year-olds increased from 1.7% in 1999 to 3.1% in 2000. Similarly, there was a rise in lifetime use for 15- to 18-year-olds from 1999 to 2000 (i.e. 4.4% to 5.4% and 5.6% to 8.2%, respectively). African American high school senior students demonstrate lower rates of MDMA use (1.3%) compared to Hispanic (10.6%) and Caucasian students (7.6%) (NIDA, 2000).

This trend of increased ecstasy use among students and young adults is alarming given that research with laboratory animals has demonstrated neurotoxic effects of MDMA, including serotonergic neurodegeneration, decreases in brain serotonin and reductions in 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (Steele *et al.*, 1994; Morgan, 2000). Specifically, in rats and non-human primates, MDMA produces reduced serotonin levels and lesions in the cingulate, hippocampus, striatum, neocortex and thalamus (Ricaurte *et al.*, 1992, 1993). Investigating the possible long-term effects of MDMA exposure, Hatzidimitriou *et al.* (1999) found that squirrel monkeys exhibit persistent, decreased serotonergic innervation of all neocortices, hippocampal CA1 and CA2 regions (with evidence of some recovery in CA3), dentate gyrus, subiculum, caudate, putamen and the amygdaloid complex (except the central nucleus which fully recovers), 7 years after treatment with MDMA. Functional imaging studies (i.e. PET and SPECT) conducted with humans who use ecstasy have shown reduced glucose metabolism in the hippocampus, parahippocampal gyrus, insula, thalamus, amygdala, posterior cingulate bilaterally and portions of neocortex (i.e. decreased serotonin receptor binding in primary sensory and occipital cortices), as well as hypermetabolism in the ventromedial prefrontal cortex, ventral anterior cingulate, inferior temporal lobe and medial occipital lobe (Reneman *et al.*, 2000; Gamma *et al.*, 2000; Obrocki *et al.*, 1999; Semple *et al.*, 1999). However, some discrepancies exist (e.g. Gamma *et al.*, 2001) such that ecstasy use is not always associated with statistically significant differences in regional blood flow relative to healthy control subjects.

Although the primary focus of the current chapter is to review the possible neuropsychological effects of ecstasy use, it is notable that MDMA has been associated with particular personality variables such as impulsivity, sensation seeking and novelty seeking (for a review, see Morgan, 2000). While inconsistencies exist (e.g. McCann *et al.*, 1994) elevations on various measure of trait impulsivity (e.g. Impulsiveness, Venturesomeness and Empathy Questionnaire) have indicated that heavy ecstasy users demonstrate greater impulsivity than non-drug-using controls (Parrot, 2000). Overall, it appears that specific personality factors such as impulsivity or sensation seeking may be associated with increased risk for heavy ecstasy use, which may subsequently place users at risk for experiencing neurotoxic effects of MDMA.

A number of studies have examined the possible neuropsychological sequelae related to MDMA use given its demonstrable effects on serotonergic systems in animals and indirect effects observed in humans, likely affecting limbic and basal forebrain structures. Perhaps the most reliable finding regarding cognitive functioning among ecstasy users is that memory performance is often somewhat lower when compared with ecstasy-naïve control subjects (for a review see Morgan, 2000; Bhattachary and Powell, 2001; Gouzoulis-Mayfrank *et al.*, 2000; Zakzanis and Young, 2001; Morgan, 1999; Rodgers, 2000). For example, Morgan (1999) examined immediate and delayed recall among polydrug users who use ecstasy (>20 times) ($N = 25$), polydrug users who do not use ecstasy ($N = 22$), and healthy controls ($N = 19$). Polydrug users who also take ecstasy had not used ecstasy for an average of 65 days prior to participating in the study. Results indicated that the ecstasy group recalled fewer details of a story during immediate and delayed recall trials (delayed recall was administered approximately 40 minutes after initial presentation) compared to polydrug users and non-drug-using controls. The verbal recall performance of the ecstasy users was markedly decreased compared to participants in the other two control groups. Cannabis consumption did not differ significantly between the two drug-using groups, and when the two drug-using groups were combined cannabis consumption was significantly negatively correlated with immediate recall scores but not delayed recall. The two drug-using groups did differ on estimated IQ scores and duration of LSD use; however, only duration of LSD use proved to be a significant covariate and group differences on immediate and delayed recall performance remained significant when LSD use was statistically controlled. The author also examined the relationship between time since last use of ecstasy and memory performance and found that individuals who abstained from using ecstasy for more than 6 months had significantly better immediate and delayed recall scores compared to those who had used it within the past 6 months. Degree of memory impairment for individuals who had used the drug within the past 6 months was not reported. It is not clear if those individuals who did not use ecstasy in over 6 months also abstained from other or all drugs, and therefore it is difficult to determine whether the better performance was due to the lack of ecstasy use only or abstinence in general. Nonetheless, these data suggest that there is likely some recovery of memory functioning in moderate ecstasy users who refrain from taking it for a significant amount of time.

In a similar study, Rodgers (2000) compared the performance of 15 regular ecstasy and cannabis users, 15 cannabis users only and 15 non-drug-using controls on a broad battery of memory tests (i.e. Wechsler Memory Scale–Revised). Results indicated that both drug-using groups performed more poorly than the non-drug users on the General Memory Index and immediate and delayed story memory. Although statistically significant, a review of the index scores revealed that the MDMA and cannabis users' performance ranged from low average to average (i.e. not within a clinically impaired range), while the control group was average to high average. Only the ecstasy group demonstrated poorer delayed recall on a verbal-paired associates task and a visual-paired associates task relative to the cannabis and non-drug-using groups. Groups in this study did not differ on a composite index of immediate visual memory scores or subtests assessing attention and concentration. Differences in story recall were found in both the MDMA–cannabis group and the cannabis-only group; thus these results suggest that a deficit in story memory may be due to long-term cannabis use rather

than ecstasy use. However, ecstasy consumption may be associated with additional memory deficits, such as impairment in the ability to remember complex information (i.e. randomly organized material) across multiple cognitive domains (verbal and visual).

Other researchers have also found support for memory deficits among ecstasy users associated with amount of time since last use as well as total lifetime consumption of MDMA (Bhattachary and Powell, 2001; Gouzoulis-Mayfrank *et al.*, 2000; Zakzanis and Young, 2001; Bolla *et al.*, 1998). Compared to non-drug-using controls, Bhattachary and Powell (2001) showed that users of MDMA demonstrated poorer immediate and delayed prose recall compared to non-users. In this study, memory scores were not correlated with cannabis use; the apparent discrepant findings relative to the Rodgers (2000) study may be at least partially attributable to the variability and degree of cannabis use between these studies. Specifically, the cannabis group in the Rodgers (2000) study was using cannabis, on average, 4 days per week for a period of 11 years, whereas Bhattachary and Powell (2001) included individuals who had used cannabis two to four times in the 30 days prior to testing and their entire cannabis history was not reported. Regarding the effects of degree of ecstasy use, Bhattachary and Powell (2001) found that heavier users of MDMA obtained the worst memory performance relative to 'novice' and 'regular' users. Total lifetime consumption of ecstasy was the strongest predictor of memory scores, accounting for 34% and 36% of the variance of immediate and delayed recall, respectively. Time since last use predicated an additional 5% of variance in immediate recall and 10% of variance in delayed recall. When studied longitudinally, verbal memory performance has been shown to decline over a 12-month period with continued MDMA use (Zakzanis and Young, 2001). The authors of this study demonstrated that ecstasy users using the drug at a rate of 2.4 times per month display poorer memory performance on immediate and delayed prose recall tasks, 1 year following their initial baseline assessment. Measures of simple facial recognition memory, object memory and prospective memory were not significantly different over time (Zakzanis and Young, 2001).

None of the studies reporting poor verbal memory performance by ecstasy users employed methods of separating different memory processes. Without such investigation, it is difficult to determine whether ecstasy users exhibit poor encoding and consolidation processes or instead display impaired retrieval strategies that are more closely associated with executive dysfunction implicating a disruption of frontal–subcortical circuits. Some support for the latter hypothesis was provided by the Rodgers (2000) study, which found that ecstasy users show greater memory impairment when recalling complex, randomly arranged information (verbal or visual-paired associates). These tasks place high demands on one's ability to organize information and employ self-generated mnemonic strategies for optimal performance, an ability considered to be mediated by frontal cortical regions. Damage to frontal cortex or its associated connections to other cortical regions and subcortical structures can impair one's capacity for generating learning strategies as well as retrieving information previously learned. Future studies should include a recognition paradigm when assessing memory functioning in order to clarify the type of possible memory impairment and other cognitive deficits in individuals who use ecstasy.

In addition to decreased memory performance, recent research has shown that individuals who use ecstasy demonstrate poorer performance on measures of 'executive functioning' relative to controls. Specifically, some studies have demonstrated that working memory, divided attention, information-processing speed, motor speed and problem-solving skills may be affected by consumption of MDMA. For example, Croft *et al.* (2000) found that users of both MDMA and cannabis demonstrate slower processing speed (Stroop Word Reading), impaired memory span (digit span forward and backward), decreased verbal fluency ('Animals') and decreased manual dexterity for the left hand than individuals who do not use cannabis or MDMA. The authors cautioned that the differences between groups could be attributable to the cannabis use rather than ecstasy, as only processing speed (Stroop Word Reading) differences remained significant when cannabis use was held constant.

Other researchers have demonstrated additional evidence for relative decrements among ecstasy users (after controlling for cannabis use) on measures examining components of executive functioning. Wareing *et al.* (2000) found that both previous (i.e. those who had not used in at least 6 months; time since last use average, 323 days) and current users of MDMA demonstrate difficulty with tasks that place a high demand on cognitive resources. One measure administered in this study was the random letter generation test, a task that requires subjects to speak aloud consonants (i.e. no vowels) in a random sequence, avoiding repetitions of the same letter or using an alphabetical sequence, at different rates for each letter (1 second to 4 seconds). On this task, ecstasy users generated fewer letters and exhibited a greater degree of redundancy during the fastest letter rate presentation. Ecstasy users also had more vowel intrusions compared to non-users at the 1-, 2-, and 4-second letter rates. On a measure of information-processing speed, in which subjects were asked to determine whether two groups of letters were the same or different, the ecstasy groups processed information as quickly as non-users; however, they committed more errors when judging two groups containing nine letters. Increased error rates were not noted on the simpler trials involving three- and six-letter groups. Current users of MDMA reported more anxiety than non-users, while previous ecstasy users did not differ from controls on level of anxiety. The authors conducted covariance analyses to control for the effects of potentially confounding variables (e.g. anxiety, LSD use, physical health) and the results indicated that nearly all group differences on the letter generation task and the processing speed task remained significant. This study lends support for subtle impairments in some aspects of executive functioning among ecstasy users. Based on evidence from the previous ecstasy-using group (average time since last use, 323 days), the cognitive changes identified in this study may persist for almost 1 year following abstinence from MDMA.

In a study comparing ecstasy–cannabis users, cannabis-only users and non-drug-using controls, Gouzoulis-Mayfrank *et al.* (2000) did not find support for decreased accuracy on speeded information-processing measures; however, ecstasy users did exhibit longer response times on a test of selective visual attention (i.e. go/no-go) than the cannabis group or non-drug group. On a divided attention task involving both visual and auditory processing, MDMA users evidenced significantly longer reaction times than the two control groups. Auditory working memory capacity (digit span backward) was also poorer among ecstasy users relative to non-drug-using controls. When assessing the effects of moderate versus heavy ecstasy consumption, Verkes *et al.* (2001) showed that moderate and heavy MDMA use is associated with poorer visual working memory ability on the Corsi Block Tapping Test, although group differences did not reach statistical significance. On a test similar to a Sternberg paradigm (i.e. presentation of six words or figures and subsequent presentation of five new items and one previously presented), both moderate and heavy ecstasy users demonstrated difficulty recognizing previously presented material (working memory deficit). Reaction times on simple and choice attention tests were slowest in the heavy ecstasy-using group; however, these differences were diminished after controlling for level of education and score on the Beck Depression Inventory. Serotonergic functioning, measured by cortisol and prolactin response to dexfenfluramine challenge test, was also examined in this study and results indicated compromised neuroendocrine functioning among both moderate and heavy MDMA users relative to non-using controls. Serotonergic functioning was

significantly related to performance on the Corsi Block Tapping Test such that individuals with higher cortisol responses during the challenge test obtained better scores on the Corsi task. Overall, the effects of heavy ecstasy use may be associated with some slight inefficiency in working memory, processing speed and reaction time, although elevated levels of depression and lower levels of education may also explain or contribute to these findings.

In summary, the literature on the neuropsychology of MDMA consumption is somewhat variable but generally suggests that prolonged, moderate to heavy, regular use of ecstasy may produce subtle cognitive changes that affect one's memory capacity and executive functioning, and the changes may persist after abstinence from MDMA. Importantly, the majority of the studies reviewed above did not indicate individuals using MDMA obtained scores on cognitive tests that were within the very significantly impaired range, but rather scores generally represented low average to mildly deficient performance (e.g. Rodgers, 2000). Based on these results it is difficult to clearly determine to what extent MDMA use affected performance as well as other possible factors such as premorbid functioning, IQ, other drug use, personality traits and alcohol use.

KETAMINES

Ketamine is a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist that interferes with the action of the excitatory amino acids glutamate and aspartate, and its primary medical purpose is for veterinary anaesthesia (Anis *et al.*, 1983; Curran and Morgan, 2000). Anatomically, NMDA receptors are found in regions of the cerebral cortex and hippocampus and are associated with the modulation of dopamine concentration in the striatum (Curran and Morgan, 2000; Smith *et al.*, 1998). Blockade of NMDA receptors is associated with inhibition of long-term potentiation (LTP) in the hippocampus, impairing one's ability to encode new information (Kandel *et al.*, 2000). A functional imaging (PET) study indicated that subanaesthetic doses of ketamine are associated with increased extracellular dopamine concentrations in the prefrontal cortex and striatum (Smith *et al.*, 1998). According to the Drug Abuse Warning Network (DAWN) the recreational use of ketamine is common among young adults and teenagers and is considered a 'club drug' because of its association with 'raves' and dance clubs. Since 1994, use of ketamine has increased dramatically and emergency medical treatment for unexpected reactions or overdoses related to ketamine has also increased. Specifically, in 2000, there were 263 mentions of ketamine-associated reactions or overdoses in emergency room reports across multiple cities (NIDA Advanced Report, 2001). Of the 263 mentions, 70% were individuals under the age of 25.

Studies examining the acute effects of ketamine on healthy subjects have shown that ketamine intoxication is associated with decreased attentional capacity, reduced learning and memory functioning, poor novel-problem solving and impaired working memory ability (Hetem *et al.*, 2000; Krystal *et al.*, 2000; Adler *et al.*, 1998). For example, Hetem *et al.* (2000) showed that individuals learning words before and after infusion of ketamine ($0.5\,mg\,kg^{-1}$) recalled significantly fewer words administered during the ketamine phase than subjects who received a placebo. However, individuals who received the ketamine did not demonstrate deficient recall for words given prior to the drug administration, suggesting ketamine impairs encoding processes but not retrieval processes. During ketamine infusion, subjects also exhibited lower scores on attention tasks, including digit–symbol coding and digit cancellation. In a similarly designed study using the WCST, Krystal *et al.* (2000) found that subjects receiving a ketamine infusion obtained significantly fewer categories (1.5 versus 5.1) and committed more perseverative errors (69.2 versus 46.0) when compared to subjects who did not receive the drug. Individuals who initially received a placebo during the first administration of the WCST did not perform more poorly on the second WCST trial when they were given ketamine, indicating that ketamine likely interferes with the acquisition of functions related to abstract learning. It has also been demonstrated that acute effects of ketamine adversely affect working memory capacity assessed by a one-back and two-back paradigm (Adler *et al.*, 1998).

The possible persistent neurocognitive effects of recreational ketamine use have not been studied in large group investigations involving abstinent ketamine users. One study examining the relatively short-term effects have shown that ketamine-using individuals exhibit impairments in working, episodic and semantic memory 3 days after using ketamine compared to individuals with minimal ketamine histories but otherwise similar drug and alcohol histories (Curran and Morgan, 2000). On day three, ketamine users recalled less information from a prose passage following a 15-minute delay than non-ketamine-using controls. Ketamine users also generated fewer items on a semantic fluency task as well as committed more errors on a speeded task involving judgement of the semantic accuracy of sentences 3 days after ketamine intoxication. Groups did not differ on day three on a digit cancellation task, word-stem completion task, serial sevens or phonemic fluency. Examining the effects of frequent ketamine use, Curran and Morgan (2000) showed that frequent users (more than twice a month) evidence marked impairments in delayed recall of verbal information relative to infrequent users (less than twice a month) 3 days after taking ketamine. Frequent users also obtained significantly lower scores on phonemic and semantic fluency on day three; however, speeded comprehension of sentences, serial sevens and digit cancellation performance between groups did not differ. Although the long-standing effects of ketamine use are not clear, these studies provide evidence for an obvious and dramatic effect of acute ketamine intoxication on cognitive functioning as well as short-term changes in memory capacity.

OPIATES

Heroin use is on the rise, especially in Europe, the USA, and Australia. Along with increased prevalence, there has been a significant increase in the purity and availability of this drug. Subsequently, the rate of heroin non-fatal overdoses in addicts is estimates to be as high as 40%. Polysubstance abuse is also extremely high in this population (Gossop *et al.*, 1996). There are only a few studies examining the neurocognitive effects of opiates on cognition. Hill and Mikhael (1979) found that opiate users, primarily heroin addicts, demonstrated deficits on the Tactual Performance memory subtest and a tapping test, but generally intact executive functioning, consistent with other studies (Bruhn and Maage, 1975; Rounsaville, 1982). However, Ornstein *et al.* (2000) found evidence of executive deficits in heroin addicts. In this study 22 heroin-dependent and 23 amphetamine-dependent subjects were compared to age and premorbid IQ matched controls on several neuropsychological tasks. Results indicated that the heroin group performed significantly worse on the IDS subtest of the CANTAB and on a visuospatial strategy task, suggestive of possible frontal dysfunction. This finding is consistent with the widely held hypothesis that opiates most likely exert their reinforcing effects by increasing the mesolimbic DA system, which projects to the nucleus accumbens, prefrontal cortex and amygdala (Koob and Bloom, 1988). Additional evidence of possible frontal dysfunction comes from SPECT studies that have shown altered cerebral blood flow in the frontal and parietal cortices in patients with chronic opiate dependence. In addition to executive deficits, Darke *et al.* (2000) compared methadone maintenance

patients to a matched sample of non-heroin-using controls and found significant impairments in attention, memory and processing speed. Thus, recent studies examining the effects of heroin and possibly methadone on cognition have found evidence of at least acute effects following short-term abstinence. The impact of other substance abuse and non-fatal heroin overdoses may exemplify some of the findings as found in the Darke study.

Studies examining morphine use have shown little cognitive effects. For instance, Walker and Zacny (1998) examined the cognitive effects of 20–40 mg of morphine on 12 healthy volunteers. The drug did not affect performance on attention, memory and reasoning tests, consistent with other researchers (O'Neill *et al.*, 2000).

LSD

Lysergic acid diethylamide (LSD) is a synthetic ergoline compound that distorts cognitive, sensory and perceptual processes and was accidentally discovered by the chemist Albert Hoffman in 1943 (Hartmann, 1995; Aghajanian and Marek, 1999). LSD has been widely used for its psychedelic properties since the 1960s and its use has continued to rise among teenagers and young adults who are associated with the dance ('rave') scene. A recent survey conducted in Edinburgh, Scotland, showed that approximately 30% of participants (sample $N = 122$) using drugs at dance events use LSD, and its consumption ranks fifth (behind ecstasy, amphetamine, cannabis and cocaine) among the drugs most frequently used by drug-using individuals who attend rave parties (Riley *et al.*, 2001). Riley *et al.*'s (2001) survey also showed that the majority of these individuals use other drugs in addition to LSD and that 37.8% of those who use LSD take the drug at least once a month.

Aghajanian and Marek (1999) reviewed the effects of hallucinogens, including LSD, and their relationship to serotonergic activity within the central nervous system. Specifically, the effects of LSD are related to serotonergic mechanisms, and its hallucinogenic properties in particular are associated with its affinity for 5-HT_{2A} (and 5-HT_{2C}) receptors primarily within areas of the cerebral cortex and locus coeruleus (Aghajanian, 1994; Egan *et al.*, 1998). The locus coeruleus is responsible for maintaining vigilance and responsiveness to novel stimuli (Kandel *et al.*, 2000). It receives afferent input from sensory neurones carrying somatic, visceral and other sensory information and it contains spontaneously active neurones that project diffusely throughout the central nervous system (Aghajanian and Marek, 1999). The administration of LSD decreases the spontaneous activity within the locus coeruleus that ultimately results in increased activation of locus coeruleus neurones due to the stimulation from sensory afferents (Aghajanian and Marek, 1999). Within the cerebral cortex, LSD has a predominantly excitatory effect at 5-HT_{2A} receptors, which are densely located within the region of the medial prefrontal cortex (Aghajanian and Marek, 1999). Aghajanian and Marek (1999) suggest that the prolonged excitation of cortical regions, through glutamatergic transmission by hallucinogens, may contribute to the sensory, perceptual and cognitive distortions produced by drugs such as LSD. At 5-HT_{1A} receptors in the raphe nucleus, LSD is a potent agonist that has a direct inhibitory effect on the raphe neurones. LSD's activity at the 5-HT_{1A} receptors is not correlated with hallucinogenic properties but rather has similar anxiolytic effects to other 5-HT_{1A} agonists (Aghajanian and Marek, 1999).

The extant literature regarding the neuropsychological effects of long-term LSD use is mixed. Halpern and Pope's (1999) review of studies examining the possible residual effects of hallucinogen use indicated that some of the early studies, despite various methodological concerns (e.g. failure to control for current or past use of drugs other than LSD), demonstrated subtle neurocognitive impairments. Specifically, slightly decreased performances on tests of spatial abilities, sustained attention and processing speed, abstraction ability and cognitive flexibility have been indicated among LSD users; however, these results have not been consistently replicated. Culver and King (1974) attempted to control some of the potentially confounding variables such as premorbid intellectual abilities, personality functioning and alcohol history, and their results showed that when compared to non-LSD users individuals who used LSD performed similarly and generally within normal limits on a cognitive battery. However, performance on the Trail Making Test were significantly lower among the LSD group than the control groups' performance, even when alcohol consumption was held constant (as cited in Halpern and Pope, 1999). Overall, Halpern and Pope (1999) concluded that 'the [possible] residual neuropsychological effects of chronic hallucinogen use, if present, are modest'; many findings have not been replicated by subsequent studies and significant methodological concerns limit the interpretability of the majority of results.

Persistent perceptual disturbances associated with LSD use are rare but the consequences of chronic LSD use have included prolonged psychotic reactions, depression, flashbacks, exacerbation of pre-existing psychiatric illness and post-hallucinogen perceptual disorder (PHPD) (Smith and Seymour, 1994). PHPD is a disorder in which individuals reportedly experience trails of light or after-images following hand movement as well as increased psychological difficulties such as anxiety, depression or panic (Smith and Seymour, 1994; Hartmann, 1995). PHPD is a chronic condition that may persist for years following abstinence from LSD. Some cases have been treated successfully with selective serotonin reuptake inhibitors, benzodiazepines and naltrexone (Hartmann, 1995; Lerner *et al.*, 1997).

CONCLUSION

The majority of neuropsychological studies in this area have examined the effect of alcohol use on cognition. In general, these studies have indicated acute and residual cognitive deficits associated with abuse. Research on other drugs has been limited and often hindered by methodological flaws (e.g. failure to address premorbid intelligence and polysubstance abuse). At present, there is some support to suggest acute effects on cognition associated with cannabis, cocaine, ecstasy, ketamine, LSD and opiates, although these deficits are often minimal. The long-term effect on cognition is still unclear and warrants well-designed longitudinal studies.

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Functional Anatomy of Substance-Related Disorders

R.A. Wise and E.L. Gardner

For the most part, the functional anatomy relevant to substance abuse disorders remains to be identified. One problem is that the field of substance-related disorders is not yet well defined. For the purposes of the present chapter, the substances under discussion will be drugs of potential abuse. However, the category of 'food' includes at least two classes of substances — sweets and lipids — that can also be abused. The case of food underlines an important principle: substance abuse need not involve the kinds of physiological dependence that have been assumed in the case of drug addiction. Food is 'abused' only when it is taken above and beyond any obvious physiological need. Current evidence suggests significant overlap in the brain circuitry relevant to foraging for food and foraging for drugs of abuse. For the most part, such circuitry has been identified as critical for the substance of interest to serve as a reward or 'reinforcer'. The terms reward and reinforcer are descriptive, not explanatory; they merely identify substances or events that are habit-forming.

The concept of habit is central to the topic of substance abuse. Again, the term is descriptive rather than explanatory. All substance abuse reflects the *habitual* intake of a substance. Some habits are benign, such as the habit of eating a small portion of pastry or ice cream following a meal. We invoke the notion of substance abuse in relation to habits that are pursued compulsively despite negative consequences. The consequences most obviously associated with substance abuse are addiction and obesity. The transition from recreational drug use to addiction is gradual, just as is the transition from overweight to obese, and we have no evidence to suggest that the mechanisms of modest food or drug intake differ in anatomical loci from those of excessive intake. Thus our current knowledge of the neuroanatomy of substance abuse is based largely on our understanding of why individuals take food and drug substances in the first place.

In one sense, the task of identifying the neuroanatomy of drug abuse is an easier one than that of identifying the neuroanatomy of feeding: most drugs of abuse act at receptors that have been identified and localized in the depths of the brain. Unlike foods, drug rewards do not rely on peripheral sensory pathways to reach the brain mechanisms serving motivational function. While addictive drugs act on multiple receptor populations, and while only a fraction of these populations serve motivational functions, by injecting drugs directly into the brain and comparing the effectiveness at different injection sites, we are able to identify chemical trigger zones for the habit-forming actions of several drugs of abuse. These trigger zones and the pathways that connect them can serve as a first step towards identifying the brain structures involved in substance-related disorders.

A classic issue in the field of drug abuse is the role of drug dependence. Clear-cut dependence syndromes can develop with chronic use of opiates, alcohol, barbiturates and benzodiazepines (Kalant, 1977), and more subtle dependence syndromes can develop with use of nicotine (Shiffman, 1979), cannabis (Jones, 1980) or cocaine (Jones, 1984). Addiction theory has, at least until recently, been dominated by the assumption that the self-medication of withdrawal distress differentiates between addiction and the so-called 'recreational' use of drugs. The present chapter thus begins with a discussion of some of the issues and neuroanatomy of drug dependence before turning to the topic of the neuroanatomy of drug reward.

NEUROANATOMY OF DRUG DEPENDENCE

Drug dependence develops with chronic drug use and is revealed in 'withdrawal symptoms' that are seen upon interruption of drug use. There is no common denominator of the range of drug dependence phenomena; the symptoms produced by withdrawal from a given drug depend on the specific actions of that drug. Generally, the withdrawal symptoms associated with a given drug are opposite to the symptoms of intoxication with that drug. Thus withdrawal from stimulants is generally associated with depression and withdrawal from depressants is generally associated with agitation. As a general rule, dependence is seen as resulting from 'compensatory' mechanisms: mechanisms that resist intoxication and tend, over time, to re-establish normal equilibrium. These compensations also result in tolerance (progressive desensitization) to the intoxicating effects of the drug and when the drug is withdrawn the unopposed compensations produce the objective signs of the withdrawal syndrome. If a system has no tonic endogenous input, it is possible to observe tolerance of the response to an exogenous drug in the absence of any overt symptoms when the drug is withdrawn. Usually, however, tolerance and dependence go together.

To the degree that drugs of different classes share common actions, they tend to establish overlapping tolerance and dependence syndromes and they are at least partially effective at alleviating each other's withdrawal symptoms. The ability of one drug to cause tolerance that extends to another drug is termed 'cross-tolerance'; the ability of one drug to alleviate the withdrawal symptoms associated with another drug is termed 'cross-dependence'. Even drugs such as opiates, alcohol and barbiturates, which produce partial cross-tolerance and partial cross-dependence, have some actions — and thus cause some adaptations — that are not shared (Kalant, 1977). There is a good deal of cross-tolerance between depressant drugs (opiates, alcohol, barbiturates and benzodiazepines) but little if any between depressant drugs of abuse and stimulant drugs of abuse (Wise, 1987).

'Somatic' Dependence Signs

Pursuit of the anatomy of drug dependence mechanisms has been fuelled by classic addition theory, which held that the self-medication of withdrawal distress was a central if not a defining

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property of addiction (Tatum *et al.*, 1929; Himmelsbach, 1943; Lindesmith, 1947; Goldstein and Goldstein, 1961; Collier, 1966; Jaffe and Sharpless, 1968). Inasmuch as the withdrawal symptoms associated with such drugs as opiates and alcohol are unpleasant, and inasmuch as addicts report that they are 'sick' during withdrawal and that they take their drug to get 'well', this theory had obvious face validity. From the perspective of classic addiction theory, the anatomy of drug dependence should be the anatomy of the brain circuits regulating thermoregulation, autonomic function and the other somatic signs of drug withdrawal — the signs that addicts report as aversive and liken to some of the symptoms of influenza.

Our best knowledge of neuroanatomy of such dependence phenomena involves opiate dependence. In part, this is because the opiate withdrawal syndrome has frequently served as the model of other addictions. In part, it is because opiates can be injected directly into the brain sites where they have various actions and where they remain reasonably localized for many minutes. In part, it is because opiate withdrawal symptoms can be precipitated by administration of opiate antagonists, which can also be restricted to localized brain areas. Localization is relative, of course; drugs injected into the brain can diffuse to adjacent structures, to the ventricular system and into the circulation, from which they can reach distant targets of action. Adequate localization of the effects of direct drug injections into the brain requires the demonstration that higher doses are required to produce the same effects when the injections are in adjacent regions, into the ventricles or into the circulation. This is a requirement that is frequently not met in studies involving central drug injections.

Localized brain injections of opiates and opiate antagonists have been used to identify sites at which opiates act to cause the various symptoms of opioid intoxication and subsequent withdrawal, and where opiate antagonists precipitate the various somatic symptoms of the opiate withdrawal syndrome in dependent laboratory animals. The somatic symptoms include diarrhoea, ear blanching, ptosis, teeth chattering, 'wet dog' shakes, seminal emissions and in rats, attempts to escape from the test chamber. The human symptoms are similar: lacrimation, rhinorrhoea, yawning, sweating, restless sleep, dilated pupils, tremor, nausea and vomiting, diarrhoea, increased heart rate and blood pressure, chills, abdominal cramping, muscle spasms and sexual orgasm (both male and female).

The brain mechanism of a given somatic withdrawal symptom is the brain mechanism involved in the normal function that is distorted by intoxication and subsequent withdrawal. The diarrhoea during opiate withdrawal reflects an adaptation in the mechanism responsible for the constipation during opiate intoxication: the mechanism of μ-opioid inhibition of intestinal contractility. During intoxication the intestine relaxes; during withdrawal it contracts and expels the accumulated faeces. In the case of each withdrawal symptom, a mechanism specific to that symptom is involved. The sweats and chills of opiate withdrawal reflect the actions of opiates and opiate withdrawal on the mechanisms of regulation of body temperature. Thus the anatomy of opiate withdrawal is, essentially, the anatomy of opiate action. Not surprisingly, very many brain structures are implicated in the full range of opiate and other drug dependence symptoms.

Among the first opiate withdrawal symptoms to be associated with specific brain regions were the somatic signs of ptosis, teeth chattering, wet dog shakes, and escape behaviour. These symptoms can be precipitated in opiate-dependent rats by injections of opiate antagonists into the ventricular system of the brain (Laschka *et al.*, 1976a). If the cerebral aqueduct is blocked so that antagonists injected into the lateral or the third ventrical do not have easy access to the fourth ventral, it is injections into the fourth ventricle (Laschka *et al.*, 1976b) that are most effective. Injections into the nucleus locus coeruleus, at the lateral floor of the fourth ventrical, are similarly effective (Maldonado *et al.*, 1992). If the opiates themselves are infused locally into the periaqueductal grey matter or into the fourth ventricle, which is fed by the aqueduct, systemic naloxone precipitates the somatic signs of teeth chattering and escape behaviours (Wei and Loh, 1976; Wei, 1981; Bozarth and Wise, 1984). One hypothesis holds that these symptoms are responses to the fluctuations of body temperature that accompany opiate withdrawal and result from opiate-induced disturbance of temperature-regulating mechanisms (Wei *et al.*, 1974).

Of the sites that are readily reached by drug injections into the fourth ventricle, nucleus locus coeruleus and the periaqueductal grey have received most attention. The noradrenergic neurones of the locus coeruleus are inhibited by morphine; this inhibition undergoes tolerance with repeated application (Aghajanian, 1978). In opiate-dependent animals, the opiate antagonist naloxone precipitates accelerated firing of these neurones (Aghajanian, 1978); at least part of this hyperactivity appears to involve adaptations of the locus coeruleus itself (Ivanov and Aston-Jones, 2001). Part of this hyperactivity results from altered neuronal input to these cells (Akaoka and Aston-Jones, 1991). Thus while the cells of the locus coeruleus appear to be one anatomical substrate of dependence, the cells that provide input to the locus coeruleus are another. Presumably, the many cells that receive input from the locus coeruleus are yet other such substrates.

Indeed, most of the brain is involved directly or indirectly in the opiate dependence syndrome. Signs of abnormal activity in opiate-withdrawn animals are seen in the abnormal expression of early immediate genes in a variety of brain areas associated with autonomic function. Among these are the locus coeruleus and the A5 noradrenergic cell group, the nucleus of the solitary tract, the parabrachial nuclei, and the caudal and rostral ventrolateral medulla (Stornetta *et al.*, 1993). Moreover, it is not just autonomic control systems that are altered by opiate dependence. Indeed, studies of early immediate gene expression confirm that opiate withdrawal is accompanied by abnormal neuronal activity in multiple structures and at all levels of the neuraxis. Among additional sites where immediate early genes are activated during opiate withdrawal are the paraventricular nucleus of the hypothalamus, amygdala, ventral tegmentum, nucleus accumbens, neostriatum, cerebral cortex, hippocampus, thalamus, cerebellum and various layers of the spinal cord (Hayward *et al.*, 1990; Stornetta *et al.*, 1993; Beckmann *et al.*, 1995; Rohde *et al.*, 1996).

The fact that opiate withdrawal effects are widespread in the central nervous system makes a general search for an anatomical basis of opiate dependence a questionable enterprise. The problem is confounded when other drugs of abuse are considered, particularly because there is no common denominator to stimulant and depressant somatic dependence signs (Wise, 1987). The affected circuitry is not only widespread; it also differs from drug to drug. Even when we restrict consideration to the opiates, dependence appears to be more easily characterized in neurochemical than in anatomical terms. Opiate receptors are G-protein-coupled, and the exposure of G-protein-coupled receptors to agonists usually results in desensitization of the receptor (Ferguson *et al.*, 1998; Pitcher *et al.*, 1998). Other drugs of abuse also act on (e.g., cannabis) or via (e.g., cocaine, amphetamine and, more indirectly, nicotine) G-protein-coupled receptors. Rebounding supersensitivity of such receptors will, where the receptor usually receives endogenous input, result in withdrawal symptoms. This fact appears to confirm what has already been suggested: every site of opiate action can contribute to the complex phenomenon of opiate dependence. Localization of the mechanisms of opiate or other drug dependence thus requires a symptom-by-symptom characterization that may never be completed. Interest in drug withdrawal symptoms has thus tended to focus on the specific symptoms most likely to contribute to the motivation for drug self-administration.

Classic addiction theory — with its myriad withdrawal symptoms and syndromes — has always had as a primary weakness that it does not explain why non-dependent subjects initiate sufficient

drug intake to *become* dependent, nor does it explain why detoxified addicts are re-addicted so quickly. Broad dependence theory has thus been challenged by workers who hypothesize that it is the generation of drug-induced positive reinforcement and euphoria, rather than the alleviation of withdrawal-induced dysphoria, that motivates compulsive drug-seeking in addicts (McAuliffe and Gordon, 1974; Bijerot, 1980; Wise and Bozarth, 1987; Gardner, 1992; Robinson and Berridge, 1993; Di Chiara, 1998). This view gained support from the fact that stimulant drugs like cocaine, nicotine and cannabis can be taken compulsively despite withdrawal symptoms quite different from and much weaker than those associated with depressant drugs (Shiffman, 1979; Jones, 1980, 1984). Further support came from studies showing that lower animals would learn to take morphine, heroin, amphetamine, cocaine and related drugs compulsively even if they were not first made dependent. Indeed, animals will self-administer opiates in doses and limited access conditions that cause no overt somatic withdrawal symptoms (Deneau *et al.*, 1969; Woods and Schuster, 1971). Confirmation that opiates are strongly habit-forming independent of their ability to induce physical dependence came from the demonstration that rats self-administer morphine into the ventral tegmental area of the brain, a site at which infusions of doses that are effective elsewhere fail to produce somatic signs of dependence, but not into the periaqueductal grey, where infusions do cause somatic dependence signs (Bozarth and Wise, 1984). With the waning influence of dependence theory, interest in the neuroanatomy of somatic dependence signs has also waned, and dependence theory has come to focus on intoxication-induced neuroadaptations within the specific circuitry of drug reward.

Reward-Relevant Dependence Signs

With the realization that opiate dependence was not a necessary condition for compulsive opiate self-administration, the assumption that some form of physical dependence must explain compulsive self-administration of addictive drugs became less attractive and classic addiction theory became overshadowed by theories that focused on the positive reinforcing properties of drugs for non-dependent subjects (Fibiger, 1978; Wise, 1978, 1988; Wise and Bozarth, 1987; Di Chiara and Imperato, 1988; Gardner, 1992; Robinson and Berridge, 1993). The brain circuitry contributing importantly to the positive reinforcing properties of drugs of abuse has been a target of intensive investigation and is discussed in some detail in the next section. With the accumulating evidence as to the neural substrates of drug reward has come a focusing of dependence theory on those specific substrates. In the modern reformulation of dependence theory, neuroadaptations in the reward circuitry itself are posited to account for states of generalized depression and anhedonia and it is hypothesized that it is the self-medication of such states that adds the compulsive dimension to drug self-administration (Solomon and Corbit, 1973; Leith and Barrett, 1976; Kokkinidis *et al.*, 1980; Dackis and Gold, 1985; Kokkinidis and McCarter, 1990; Frank *et al.*, 1992; Koob and Bloom, 1988).

The first suggestion that drugs of abuse might desensitize the mechanisms of reward came from studies of the effects of drugs on rewarding electrical stimulation of the brain. While drugs of abuse usually potentiate the rewarding effects of lateral hypothalamic and related brain stimulation (Wise, 1996b), reward thresholds are elevated following withdrawal from chronic exposure to amphetamine (Leith and Barrett, 1976; Kokkinidis *et al.*, 1980; Wise and Munn, 1995), cocaine (Frank *et al.*, 1988, 1992; Kokkinidis and McCarter, 1990; Markou and Koob, 1991), ethanol (Schulteis *et al.*, 1995) and nicotine (Watkins *et al.*, 2000). The period of elevation lasts, however, only a few days, far short of the period when peak craving for opiates (Shalev *et al.*, 2001) or cocaine (Grimm *et al.*, 2001) occurs.

A variety of neuroadaptations have been found within the mesolimbic dopamine system and its primary terminal field, the nucleus accumbens. These loci are central to current knowledge of brain reward circuitry, and neuroadaptations in these loci are of potential relevance to drug dependence. The known neuroadaptations range from morphological changes (Beitner-Johnson and Nestler, 1991; Beitner-Johnson *et al.*, 1992; Sklair-Tavron *et al.*, 1996) to changes in neurotransmitter sensitivity (Bell *et al.*, 2000; Henry and White, 1991) and changes in intracellular signalling cascades and transcription factors (Cha *et al.*, 1997; Chen *et al.*, 1997; Fitzgerald *et al.*, 1996; Hope *et al.*, 1992; Kelz *et al.*, 1999; Nestler *et al.*, 1990; Terwilliger *et al.*, 1991). Some of these changes are associated with decreased sensitivity — tolerance — to the drug, while others are associated with increased sensitivity — sensitization. Mechanisms of tolerance are consistent with traditional dependence theory, but mechanisms of sensitization are opposite to those associated with dependence theory, and the finding that addictive drugs can sensitize the nervous system to the drug's rewarding action raises another challenge to dependence theory and the question of whether its anatomy is part of the anatomy of substance abuse.

Sensitization in Reward Pathways

Dependence theory is predicated upon the notion that increasing amounts of the drug are needed to ameliorate withdrawal symptoms. Dependence theory thus requires that the effects of repeated drug experience decrease the sensitivity of the brain to the addictive drug, and bias normal brain function in the opposite direction from the acute drug state (Solomon and Corbit, 1973; Koob *et al.*, 1989). Some drug effects — in particular those associated with somatic withdrawal symptoms — do appear to desensitize with chronic drug treatment. The reward-specific effects of amphetamine (Lett, 1989; Piazza *et al.*, 1990; Lorrain *et al.*, 2000), cocaine (Lett, 1989; Horger *et al.*, 1990; Shippenberg and Heidbreder, 1995) and morphine (Lett, 1989; Shippenberg *et al.*, 1996), however, appear to undergo sensitization, at least with intermittent repeated treatment. Thus while it is true that tolerance to the rewarding effects of cocaine can occur within a binge of cocaine self-administration (Emmett-Oglesby and Lane, 1992; Li *et al.*, 1994), sensitization to the rewarding effects of cocaine appear to occur *between* such binges. While tolerance usually decreases within a few days of the last drug administration, sensitization to intermittent psychomotor stimulants is very long lasting (Robinson and Becker, 1986).

The finding that between-session sensitization is much stronger and longer-lasting than any obvious signs of tolerance to the rewarding effects of these drugs has led to yet another positive reinforcement perspective on addiction (Robinson and Berridge, 1993). This perspective has again raised the question of whether drug dependence phenomena play the significant role in addiction that has been suggested by dependence theory. The sensitization-of-reward findings directly challenge all forms of dependence or opponent process theory and raise, again, serious questions about the relevance of the neuroanatomy of dependence to the neuroanatomy of substance abuse. While it is clear that there are drug-induced neuroadaptations within the reward system itself, the importance of drug-opposite or drug-like neuroadaptations for drug self-administration require direct experiments that have only recently begun (Carlezon *et al.*, 1998; Kelz *et al.*, 1999).

NEUROANATOMY OF DRUG REWARD

While it does not make obvious sense to discuss a 'trigger zone' for the rewarding effect of such natural incentives as food or sexual contact, it is well established that food, like the psychomotor

stimulants and a number of other drugs of abuse, depends upon dopamine neurotransmission for its rewarding effects (Wise *et al.*, 1978; Spyraki *et al.*, 1982; Ettenberg and Camp, 1986). The major dopamine systems originate in the midbrain and have long-axon projections to the forebrain. These neurones originate as one embryonic group, but the somata spread laterally from the midline and are somewhat arbitrarily subdivided into a nigrostriatal system, originating primarily in the substantia nigra and projecting primarily to the caudate nucleus, and a mesocorticolimbic system, originating primarily in the more medial ventral tegmental area and projecting primarily to the frontal and cingulate cortices, the septum, hippocampus, amygdala and the nucleus accumbens. Of the various dopaminergic terminal fields, the nucleus accumbens is most clearly seen as serving motivational function (Mogenson *et al.*, 1980). It appears to house trigger zones — regions where the drug binds and initiates its relevant pharmacological action — for the rewarding effects of at least three classes of addictive drug.

Nucleus Accumbens

The nucleus accumbens appears to be the primary site of rewarding action for amphetamine, one of two sites of rewarding action for cocaine and for phencyclidine, and one of two sites of putative rewarding action for addictive opiates. The mechanisms of rewarding action of amphetamine and cocaine are secondary to their actions at the dopamine transporter, by which they elevate nucleus accumbens dopamine levels, whereas phencyclidine and morphine have rewarding actions due to their actions at receptors for the neurotransmitter glutamate or for one or more of the endogenous opioid peptides, respectively. The rewarding action of each appears to result in decreased activity in the medium-sized spiny neurones that comprise the output neurones of the nucleus accumbens. While much less is known about the habit-forming actions of cannabis, alcohol, barbiturates, benzodiazepines and caffeine, there are reasons to suspect that the nucleus accumbens also plays a part in the habit-forming effects of these potentially addictive agents.

Phencyclidine

Phencyclidine (PCP) is a non-competitive antagonist at the NMDA-type excitatory amino acid receptor, where it blocks some of the excitatory effects of glutamate (Chaudieu *et al.*, 1989; Ohmori *et al.*, 1992). It is also, at higher concentrations, a dopamine uptake inhibitor (Gerhardt *et al.*, 1987). It is self-administered compulsively by a small but stable subset of humans (Crider, 1986); it is also self-administered by laboratory animals (Balster *et al.*, 1973), but not with the vigour or reliability that characterizes intravenous heroin or cocaine self-administration (Collins *et al.*, 1984). PCP has mixed rewarding and aversive actions when given systemically (Barr *et al.*, 1985; Crider, 1986; Iwamoto, 1986), but has straightforward rewarding actions when given directly into the shell portion of the nucleus accumbens (Carlezon and Wise, 1996). PCP shares this rewarding action with NMDA antagonists that do not affect dopamine uptake, and the rewarding action of these agents in the nucleus accumbens is not attenuated by sulpiride, a D_2 dopamine antagonist that attenuates the rewarding effects of nucleus accumbens injections of the dopamine uptake inhibitor nomifensine (Carlezon and Wise, 1996). Thus PCP has dopamine-independent rewarding actions at the NMDA receptor, where it binds at 10 times lower concentration than is required for its binding at the dopamine transporter (Chaudieu *et al.*, 1989; Ohmori *et al.*, 1992).

Before accepting the assumption that intracranial injections of a drug identify the systems where it acts, one should require convincing evidence that the drug is not diffusing from the site of injection to a distal site of action. A central drug injection can reach a distant site of action in three ways. First, it may simply diffuse through the extracellular space to adjacent structures. Only if injections into surrounding structures are less effective and occur with greater latency can we conclude that a drug is acting in a given target site. Second, drugs that readily cross the blood–brain barrier can reach distal sites of action via the circulation. The obvious safeguard against this possibility is the demonstration that the same low doses are ineffective when given intravenously. Finally drugs injected under hydraulic pressure can follow the pressure gradient up the cannula shaft, and, if the cannula penetrates a ventricle, to the ventricular pressure sink through which it can reach distal circumventricular organs. This artefact led to misinterpretation of several studies involving the effects of central hormone and neurotransmitter injections on drinking behaviour (Johnson and Epstein, 1975). In the case of intracranial self-administration of PCP, fluid egress up the cannula shaft is the most likely of these possibilities. The lateral ventricle is just above the nucleus accumbens, and a vertical penetration to the shell of the nucleus accumbens usually involves penetration of the ventricle, establishing a pressure gradient from the cannula tip to the ventricular system (where fluid flows naturally from the lateral to the third and fourth ventricles and cerebrospinal aqueduct. The demonstration that PCP is self-administered into the shell of the nucleus accumbens (Carlezon and Wise, 1996) has two controls that increase confidence that the drug's rewarding action is in the nucleus accumbens. First, an angled cannula was used to avoid penetration of the ventricle. Second, rats did not self-administer PCP to a site in the core of the nucleus accumbens, 1 mm dorsal and slightly lateral — along the same cannula track — to the positive reward sites in the shell of the accumbens. Such essential controls (Routtenberg, 1972; Wise and Hoffman, 1992) are lacking in most central injection studies, and this fact leaves interpretation of several central injection studies in doubt.

Amphetamine

Amphetamine binds to and reverses monoamine transporters, causing release of noradrenaline (norepinephrine), dopamine and serotonin. The first evidence of a role for dopamine in drug reward came from studies in which intravenous amphetamine self-administration was disrupted by treatment with dopamine-selective (but not noradrenaline-selective) receptor antagonists (Yokel and Wise, 1975, 1976; Risner and Jones, 1976). Lesions of the nucleus accumbens, like treatment with dopamine receptor blockers, attenuate intravenous amphetamine self-administration (Lyness *et al.*, 1979). Amphetamine is self-administered directly into the nucleus accumbens but not into the lateral ventricles (Hoebel *et al.*, 1983); this self-administration of amphetamine is not evident if amphetamine is co-injected with dopamine antagonists (Phillips *et al.*, 1994). Local injections of amphetamine into this region but not adjacent regions also establish conditioned place preferences (Carr and White, 1983). Thus the nucleus accumbens is a critical site for the habit-forming actions of amphetamine; amphetamine's rewarding action depends on the activation of local dopamine receptors by the extracellular dopamine that accumulates (Zetterström *et al.*, 1981) because of amphetamine's action at the dopamine transporter.

Once a period of amphetamine self-administration has been initiated, extracellular dopamine concentration appears to regulate the frequency of continued responding. Rats tend to respond to amphetamine until their nucleus accumbens dopamine levels are elevated three- to five-fold; they then wait until prior injections are partially metabolized and their dopamine levels have fallen to approximately twice-normal levels before responding again (Ranaldi *et al.*, 1999). Thus dopamine actions in the nucleus accumbens are not only critical for whether or not amphetamine is rewarding; they also appear to determine how long a given injection will satiate an animal.

Cocaine

Cocaine, like amphetamine, is rewarding through its ability to elevate nucleus accumbens dopamine levels (de Wit and Wise, 1977; Risner and Jones, 1980). Whereas amphetamine is a dopamine releaser, cocaine is a dopamine uptake inhibitor (Heikkila *et al.*, 1975a, 1975b). While cocaine appears to be more effective as a reinforcer when injected into the frontal cortex (Goeders and Smith, 1983), if animals are given sufficient exposure they will self-administer it directly into the nucleus accumbens (Carlezon *et al.*, 1995), where dopamine-selective lesions attenuate the rewarding effects of intravenous cocaine (Roberts *et al.*, 1977, 1980). Moreover, frontal cortex injections of cocaine appear to be rewarding because they trans-synaptically elevate nucleus accumbens dopamine levels (Goeders and Smith, 1993). Nomifensine, a more selective (Samanin *et al.*, 1975) and efficacious (Nomikos *et al.*, 1990) dopamine uptake inhibitor, is also rewarding in the shell (but not the core) of the nucleus accumbens, and its effectiveness is blocked by co-administration of the D_2 dopamine antagonist sulpiride (Carlezon *et al.*, 1995). This suggests that it is not the local anaesthetic action of cocaine (Ritchie and Greene, 1985) in nucleus accumbens that accounts for its rewarding actions there. Self-administered doses of cocaine, like self-administered amphetamine, elevate nucleus accumbens dopamine levels (Hurd *et al.*, 1989; Pettit and Justice, 1989) and maintain them at levels two or more times above normal baseline levels (Wise *et al.*, 1995b).

Opiates

It is also thought that opiates trigger rewarding actions in the nucleus accumbens. Morphine (Olds, 1982) and the endogenous opioid met-enkephalin (Goeders *et al.*, 1984) are reported to be self-administered into this region, and morphine injections into this region are reported to cause conditioned place preferences (van der Kooy *et al.*, 1982). Some concern must remain, however, as to whether opiate injections into this brain region have their rewarding actions locally (Wise, 1989). In studies where nucleus accumbens self-administration or conditioned place preference has been reported, anatomical controls that would rule out drug efflux to the ventricular system or adjacent sites have not been reported. This is troublesome because much lower doses of morphine are self-administered or cause conditioned place preferences when injected into other brain sites (Bozarth and Wise, 1981, 1982; Bozarth, 1987; Olmstead and Franklin, 1997). Moreover, some workers have reported nucleus accumbens opiates to be ineffective in each of these reward paradigms (Bozarth and Wise, 1982; Schildein *et al.*, 1998; Zangen *et al.*, 2000).

It is tempting, nonetheless, to suspect that opiates at high enough concentrations can induce rewarding effects at nucleus accumbens opiate receptors. There are several populations of opioid receptors in nucleus accumbens, and μ- and δ-opioids inhibit nucleus accumbens medium spiny neurones (Hakan and Henriksen, 1989; Jiang and North, 1992). There are μ-opioid receptors on the medium spiny output neurones of the nucleus accumbens (Wang *et al.*, 1997). Activation of these receptors is thought to inhibit medium spiny neurone activity much as does dopamine and systemic morphine (Hakan and Henriksen, 1989). In addition, there are both μ- and δ-opioid receptors on the presynaptic terminals of corticolimbic glutamate inputs to the nucleus accumbens. Actions at each of these receptor subtypes appear to inhibit release of the excitatory amino acid glutamate onto medium spiny neurones (Jiang and North, 1992), much as does blockade of NMDA receptors by PCP. The two compounds that have been reported to be self-administered into the nucleus accumbens, morphine and met-enkephalin, each activate both μ- and δ-opioids. There are also κ-opioid receptors in the nucleus accumbens, but κ-opioids are aversive and act at the dopamine transporter to decrease dopamine release (Thompson *et al.*, 2000).

Cannabinoids

While there are no behavioural studies in which local injections of cannabinoids were used to localize a rewarding site of action, there is evidence that cannabinoids can influence the mesolimbic dopamine system. The major psychoactive constituent of marijuana and hashish is Δ^9-tetrahydrocannabinol (THC). Partly for reasons of solubility, this substance appears to have its greatest abuse liability when smoked. Recently, however, intravenous self-administration of THC has been demonstrated in squirrel monkeys (Tanda *et al.*, 2000) and intravenous self-administration of a synthetic cannabinoid has been shown in mice (Martellotta *et al.*, 1998). Systemic treatment with THC can also establish conditioned place preferences (Lepore *et al.*, 1995; Valjent and Maldonado, 2000).

THC elevates nucleus accumbens dopamine levels when given either systemically (Chen *et al.*, 1990) or directly into the nucleus accumbens (Chen *et al.*, 1993), and it enhances potassium-induced dopamine release in much the same way as do dopamine uptake inhibitors (Ng Cheong Ton *et al.*, 1988). Such elevations appear to occur selectively in the shell of the nucleus accumbens (Tanda *et al.*, 1997a). When THC is given in combination with haloperidol, the dopamine release normally caused by haloperidol-induced blockade of dopamine autoreceptors is enhanced, as it is by dopamine uptake inhibitors (Gardner *et al.*, 1990). Finally, THC's effect on nucleus accumbens levels of the dopamine metabolite 3-methoxytyramine resembles the effects of a dopamine uptake inhibitor (Heal *et al.*, 1990) like cocaine or nomifensine (Chen *et al.*, 1994). It would thus seem that at least some part of the habit-forming effects of THC is triggered in the nucleus accumbens or somehow depends on nucleus accumbens dopaminergic function.

Alcohol

Ethanol elevates nucleus accumbens dopamine levels (Imperato and Di Chiara, 1986; Gonzales and Weiss, 1998; Weiss *et al.*, 1993), and intra-accumbens microinjections of the dopamine D_2 receptor antagonist raclopride produce dose-orderly decreases in ethanol self-administration (Samson *et al.*, 1993). Thus it seems likely that alcohol has habit-forming actions mediated in part by the nucleus accumbens. In this case, however, there is no evidence to suggest that alcohol's interface with drug reward circuitry is *within* the nucleus accumbens.

Caffeine

The only links between caffeine and drug reward circuitry are extremely speculative. There is no direct experimental evidence on caffeine's central sites of rewarding action. Indeed, although reports do exist of successful caffeine self-administration in laboratory rats (e.g., Collins *et al.*, 1984), monkeys (e.g., Deneau *et al.*, 1969) and baboons (e.g., Griffiths *et al.*, 1979), the bulk of the existing literature suggests that caffeine self-administration in laboratory animals is inconsistent and sporadic at best (see reviews by Griffiths and Woodson, 1988; Griffiths and Mumford, 1995; Howell *et al.*, 1997). More consistently, caffeine has been reported to augment the reinforcing properties (e.g., Schenk *et al.*, 1994; Shoaib *et al.*, 1999), prolong extinction of self-administration (e.g., Kuzmin *et al.*, 1999) and trigger reinstatement of extinguished self-administration (Schenk and Partridge, 1999) of *other* addictive drugs.

Caffeine is an antagonist at adenosine A_1 and A_2 receptors, and its behavioural effects appear to be mostly mediated by such actions (Fredholm *et al.*, 1999). As adenosine A_1 (Goodman and Snyder, 1982; Fastbom *et al.*, 1987) and A_{2A} (Dixon *et al.*,

1996; Rosin *et al.*, 1998) receptors are densely distributed in the nucleus accumbens, as A_{2A} receptors are extensively co-localized with dopamine D_2 receptors and preproenkephalin mRNA in accumbens medium spiny neurones (Svenningsson *et al.*, 1997, 1998), and as A_{2A} receptors are also co-localized (sparsely to be sure, but significantly above background levels) with dopamine D_1 receptors in the accumbens (Svenningsson *et al.*, 1997, 1998), it may be surmised that adenosine receptor activation in the accumbens modulates meso-accumbens dopaminergic function. Indeed, considerable evidence exists that A_1 or A_{2A} receptor activation inhibits dopaminergic function mediated through either the D_1 or D_2 receptor, and that adenosine antagonism *enhances* meso-accumbens dopaminergic function (e.g., Turgeon *et al.*, 1996; Le Moine *et al.*, 1997; Moreau and Huber, 1999; Svenningsson *et al.*, 1999; Poleszak and Malec, 2000). In a standard drug discrimination assay in monkeys, adenosine antagonism generalizes dose-dependently and completely to eight different dopamine receptor agonists that encompass a variety of mechanisms and sites of action, both pre- and postsynaptic (Holtzman, 1999). Further, the discriminative stimulus properties of the non-selective A_1/A_2 adenosine antagonist CGS-15943 are blocked dose-dependently and completely by the dopamine D_1 antagonist SCH-23390 and the D_2 antagonist eticlopride (Holtzman, 1999). Congruently, caffeine enhances dopamine turnover in the striatum (Waldeck, 1972, 1975; Watanabe and Uramoto, 1986) and enhances striatal dopamine release in freely moving rats as measured by *in vivo* voltammetry (Morgan and Vestal, 1989). Recent evidence suggests that adenosine and dopamine receptors form heterodimers in medium spiny neurones (Franco *et al.*, 2000). All of these interesting findings notwithstanding, it is only by tentative and arbitrary decision-making that we include caffeine with habit-forming drugs having rewarding sites of action in the nucleus accumbens. There is no evidence that caffeine has its rewarding action at trigger zones in the nucleus accumbens, and it is listed here simply because we could suggest no more relevant place for it.

Ventral Tegmental Area

Opiates

A good deal of evidence suggests that the primary site of rewarding action of opiates is in the ventral tegmental area, where μ-opioids disinhibit the mesocorticolimbic dopamine system by inhibiting the GABAergic cells that normally hold their dopaminergic neighbours under inhibitory control (Johnson and North, 1992). First, μ- and δ-opioids are self-administered into the ventral tegmental area but not into an area just dorsal to it (Bozarth and Wise, 1981; Welzl *et al.*, 1989; Devine and Wise, 1994; Zangen *et al.*, 2000). The μ-opioid DAMGO is 100 times more potent than the δ-opioid DPDPE. Ventral tegmental injections of these substances elevate nucleus accumbens dopamine levels (Leone *et al.*, 1991; Devine *et al.*, 1993), and DAMGO is here again 100 times more potent than DPDPE. Opiate antagonists injected into the ventral tegmental area decrease the rewarding effects of intravenous heroin, although there is disagreement as to whether they do so more potently than antagonists injected into the nucleus accumbens (Britt and Wise, 1983; Vaccarino *et al.*, 1985). Opioid doses that are ineffective in the nucleus accumbens establish intracranial self-administration and conditioned place preferences in the ventral tegmental area (Bozarth, 1987; Olmstead and Franklin, 1997; Zangen *et al.*, 2000). Indeed, in other behavioural tests opioids have stronger behavioural effects when injected into the ventral tegmental area than when injected into the nucleus accumbens (Broekkamp *et al.*, 1976; Kalivas *et al.*, 1983; Jenck *et al.*, 1987; West and Wise, 1988; Zangen *et al.*, 2000) unless the nucleus accumbens is pharmacologically or neurotoxically deprived of dopamine (Stinus *et al.*, 1985, 1986).

It is clear that self-administered intravenous doses of heroin are sufficient to activate the mesolimbic dopamine system, as self-administered intravenous heroin elevates nucleus accumbens dopamine much as do self-administered amphetamine and cocaine (Wise *et al.*, 1995a). Thus — since it is unlikely that medium spiny neurones in the nucleus accumbens can discriminate the dopamine overflow that results from heroin's action at the dopamine cell bodies from that resulting from amphetamine's or cocaine's actions at the dopamine transporter — it seems clear that opiate actions triggered in the ventral tegmental area must contribute significantly to the rewarding effects of intravenous heroin. This suggestion is strengthened by the fact that the initiation of the next within-session lever-press for intravenous heroin, like those for intravenous amphetamine and cocaine, can be predicted by the fall of nucleus accumbens dopamine levels to a trigger point about twice the normal dopamine concentration (Pocock *et al.*, 1994).

Nicotine

Nicotine also triggers its rewarding actions in the ventral tegmental area. Here the critical receptors are on the dopaminergic neurones themselves (Clarke and Pert, 1985). Intravenous nicotine self-administration is blocked by dopamine antagonists and neurotoxins as well as by chlorisondamine (Corrigall *et al.*, 1992), a nicotinic channel blocker that is taken up and concentrated within the dopaminergic cell bodies of the ventral tegmental area and substantia nigra (El-Bizri *et al.*, 1995). Nicotine increases dopaminergic cell firing (Grenhoff *et al.*, 1986; Mereu *et al.*, 1987) and elevates dopamine levels in the nucleus accumbens (Imperato *et al.*, 1986). Thus nicotine, like amphetamine, cocaine and morphine, appears to be habit-forming because it elevates dopamine levels in the nucleus accumbens.

Nicotine also elevates dopamine levels when infused directly into the nucleus accumbens, where the cholinergic agonist carbachol is self-administered (Ikemoto *et al.*, 1998a). However, as is the case with ventral tegmental and nucleus accumbens administration of opioids, nucleus accumbens actions of nicotine appear to be weaker than the ventral tegmental actions of nicotine (Benwell *et al.*, 1993; Nisell *et al.*, 1994) and thus seem unlikely to account for the rewarding effects of systemic nicotine (Corrigall *et al.*, 1992).

Cannabinoids

Like morphine, THC has actions in the ventral tegmental area (VTA) as well as in the nucleus accumbens. First, it stimulates dopaminergic cell firing and this effect is not blocked — while at least part of the elevations seen in dialysis studies are (Chen *et al.*, 1990; Tanda *et al.*, 1997a) — by opiate antagonists (French, 1997; Melis *et al.*, 2000). Second, local application of THC to the ventral tegmental area elevates ventral tegmental but not nucleus accumbens dopamine levels (Chen *et al.*, 1993). This effect is tentatively interpreted as a reflection of the inhibition of dendritically released dopamine; it is not known if this local effect of THC in the VTA is naloxone-sensitive. Local injections of cannabinoids into the VTA have not been tested in behavioural paradigms.

Alcohol

Ethanol increases the firing rate of dopamine neurones in the rat VTA (Gessa *et al.*, 1985; Brodie *et al.*, 1990) and it is self-administered directly into the VTA (McBride *et al.*, 1993; Gatto *et al.*, 1994; Rodd-Henricks *et al.*, 2000). This intracranial self-administration of ethanol was seen in selectively bred alcohol-preferring and ordinary Wistar rats, but not in selectively bred alcohol-non-preferring rats. The animals performing

such intracranial ethanol self-administration show lever discrimination, extinction of self-administration when vehicle is substituted for ethanol, and reinstatement of self-administration responding on the active lever when ethanol is once again made available (Rodd-Henricks *et al.*, 2000). Sites dorsal to the VTA do not support ethanol self-administration (Gatto *et al.*, 1994). In a mapping study, posterior ventral tegmental sites were found to support ethanol self-administration; anterior ventral tegmental sites did not (Rodd-Henricks *et al.*, 2000). As noted by McBride *et al.* (1999), intracranial ethanol self-administration may involve brain serotonin (5HT) mechanisms, especially $5HT_3$ receptors. Ethanol potentiates the depolarizing effects of 5HT at the $5HT_3$ receptor (Lovinger and White, 1991), and local $5HT_3$ agonist microinfusion increases somatodendritic dopamine release in the VTA (Campbell *et al.*, 1996), suggesting that activation of $5HT_3$ receptors in the VTA may activate meso-accumbens dopamine neurones. Most provocatively, local $5HT_3$ antagonist microinfusion prevents ethanol-induced somatodendritic dopamine release in the ventral tegmental area (Campbell *et al.*, 1996), suggesting that ethanol's action on meso-accumbens dopamine neurones is mediated via VTA $5HT_3$ receptors. Ethanol's actions on meso-accumbens dopamine neurones may also involve $GABA_A$-(Harris *et al.*, 1995) or NMDA-mediated (Weight *et al.*, 1991) mechanisms.

Barbiturates and Benzodiazepines

Barbiturates and benzodiazepines are self-administered and can be abused by humans. These drugs are also self-administered in animal models, more readily in primates than in rodents (Ator and Griffiths, 1987). Neither self-administration nor conditioned place preferences have been established with intracranial injections; thus notions of the sites of rewarding action for these drugs are largely based on evidence that barbiturates and benzodiazepines act via an interaction with $GABA_A$ receptors (e.g., Korpi *et al.*, 1997; Chebib and Johnston, 1999). Inasmuch as opiates are thought to have rewarding actions by decreasing $GABA_A$ inhibition of dopaminergic neurones in the VTA, one possibility is that barbiturates and benzodiazepines have dopamine-dependent rewarding actions in the VTA. This fits with the finding that dopaminergic lesions of the nucleus accumbens produced by microinjections of the dopaminergic cytotoxin 6-hydroxydopamine block diazepam-induced conditioned place preference (Spyraki and Fibiger, 1988). However, the GABAergic synaptology of the ventral region area is complex, as GABAergic neurones comprise not only afferents to but also efferents from this region. Recent evidence suggests that some GABAergic drug actions are reflected in dopaminergic output and others are reflected in non-dopaminergic output (Laviolette and van der Kooy, 2001).

Non-Dopamine Output from the VTA

The view that rewarding brain stimulation, natural rewards like those of food and sexual contact, and several drug rewards owe their ability to control behaviour to the fact that they elevate mesolimbic dopamine levels is widely but not uniformly accepted (Wise, 1996b; but see Koob and Goeders, 1989; Bechara *et al.*, 1998). Several recent findings suggest that the dopaminergic output of the VTA is not the only contribution of this region to reward function.

First, ventral tegmental microinjections of a D_1-type dopamine antagonist reduce the rewarding effects of intravenous cocaine (Ranaldi and Wise, 2001). D_1-type dopamine receptors are not expressed by the dopaminergic neurones of the VTA or adjacent substantia nigra themselves; rather, the D_1 receptor populations in this brain region are thought to be localized primarily to the terminals of GABAergic inputs to this region (Altar and Hauser, 1987; Beckstead, 1988). The evidence for this localization, however, is based on the adjacent substantia nigra, where D_1 receptor-expressing GABAergic terminals synapse primarily on GABAergic output neurones of the zona reticulata. There is dense expression of D_1 receptors in the zona reticulata of substantia nigra, and sparse expression in the zona compacta and VTA. This density parallels the density of GABAergic cell bodies and of GABAergic input in terminals that co-localize dynorphin, which identifies the D_1-expressing GABAergic medium spiny neurones of the striatum. This evidence suggests, very indirectly, that the D_1 receptors in the VTA and zona compacta are likely to be localized to GABAergic inputs to GABAergic rather than to dopaminergic targets. Ventral tegmental administration of D_1-type agonists causes increased glutamate as well as increased GABA efflux, however, raising the possibility that D_1 receptors may also be expressed on glutamatergic input to the VTA (Kalivas and Duffy, 1995).

The GABAergic anatomy of the VTA and substantia nigra is complex. The substantia nigra, and to a lesser extent the VTA, contains GABAergic neurones with dominant projections to the pedunculopontine tegmental nuclei (Gerfen *et al.*, 1982), and to the superior colliculus and medial thalamus (Williams and Faull, 1988). GABAergic cells of the VTA also project to the nucleus accumbens (Van Bockstaele and Pickel, 1995). GABAergic neurones also project to and inhibit (Johnson and North, 1992) their dopaminergic neighbours; it was originally suspected that this inhibition was accomplished by GABAergic local circuit neurones (Grace and Bunney, 1985), but recent evidence suggests that it is collaterals from GABAergic projection neurones that account for the tonic inhibition of nearby dopaminergic neurones (Tepper *et al.*, 1995).

Three lines of recent investigation suggest heterogeneity of GABAergic participation in reward function. First, Ikemoto *et al.* (1997b) have found that rats will self-inject the $GABA_A$ antagonist picrotoxin into the anterior but not the posterior VTA, and that this behaviour is disrupted by co-infusion of the $GABA_A$ agonist muscimol. However, when injected into the *posterior* VTA, muscimol is rewarding (Ikemoto *et al.*, 1998b). Zangen *et al.* (2000) also found evidence of rostral–caudal differences in ventral tegmental reward; they report that endomorphin-1, the endogenous ligand for the μ-opioid receptor (the rewarding effects of which are thought to be mediated by μ-opioid receptors on GABAergic neurones: Johnson and North, 1992), is vigorously self-administered into the posterior VTA but much less vigorously self-administered into the anterior VTA. Laviolette and van der Kooy (2001) have reported that the rewarding effects of ventral tegmental injections of the $GABA_A$ agonist muscimol are dopamine-dependent whereas the rewarding effects of ventral tegmental injections of the $GABA_A$ antagonist bicuculline are dopamine-independent.

Medial Prefrontal Cortex

Cocaine

Cocaine is self-administered directly into the medial prefrontal cortex (Goeders and Smith, 1983, 1986). It is difficult to imagine how injections into this area could diffuse to the nucleus accumbens, the other area implicated in cocaine reward. The self-administration of cocaine in this region is dopamine-dependent, as co-infusion of the D_2 dopamine antagonist sulpiride blocks the effect (Goeders *et al.*, 1986). It is not at all clear, however, that cocaine is effective in the frontal cortex — as it is in the nucleus accumbens — because of its ability to block the dopamine transporter. The dopamine transporter is sparsely distributed in the frontal cortex (Garris and Wightman, 1994; Sesack *et al.*, 1998), where it is the noradrenaline transporter that seems to be most strongly implicated in dopamine clearance (Tanda *et al.*, 1997b; Yamamoto and Novotney, 1998;

Morón *et al.*, 2001). The noradrenaline transporter has greater affinity for dopamine than does the dopamine transporter, and in the frontal cortex, unlike nucleus accumbens, it is abundant. Thus it is probably cocaine's ability to block the noradrenaline transporter in the frontal cortex that accounts for its self-administration into this area. Whatever the local site of action, self-administered cocaine in the frontal cortex causes elevation of dopamine levels in the nucleus accumbens, and this presumably trans-synaptic effect is thought to be necessary for cocaine's rewarding effects in the frontal cortex (Goeders and Smith, 1993).

Phencyclidine

Phencyclidine is also self-administered into the medial prefrontal cortex (Carlezon and Wise, 1996), where direct electrical stimulation is also rewarding (Routtenberg and Sloan, 1972; Corbett *et al.*, 1982). In this case the mechanism of action is not well understood, but it should be noted that the prefrontal cortex is intimately linked to the mesolimbic dopamine system. The ventral tegmental dopaminergic neurones project to the medial prefrontal cortex, where they synapse on GABAergic interneurones and on the pyramidal cells that are the cortical output neurones (Goldman-Rakic *et al.*, 1989; Sesack *et al.*, 1995). The prefrontal cortex neurones, in turn, project to both the nucleus accumbens and the VTA (Christie *et al.*, 1985; Sesack *et al.*, 1989; Sesack and Pickel, 1992). The primary neurotransmitter of these neurones is glutamate (Druce *et al.*, 1982; Sandberg *et al.*, 1985), and they co-localize the neuropeptide cholecystokinin (CCK) (Meyer *et al.*, 1982; Hökfelt *et al.*, 1988). These neurones are of interest because the release of CCK in both VTA and nucleus accumbens is more proportional to the strength of rewarding electrical stimulation of the prefrontal cortex than is the release of either dopamine or glutamate in either structure (You *et al.*, 1998).

Conceptual Nervous System of Addiction

While only a portion of the anatomy of substance abuse is known, a preliminary sketch of several important links in the rewarding effects of drugs can be suggested. At its core is the mesolimbic dopamine system with its cells of origin in the VTA and its most important terminals in the nucleus accumbens. The trigger zones for the habit-forming actions of nicotine and opiates are in the VTA; the critical receptors are expressed by dopaminergic neurones and GABAergic neurones, respectively. The endogenous ligand for the μ-opioid receptor in this region is endomorphin-1, which projects to the VTA from the cells in either the hypothalamus or the nucleus of the solitary tract (Martin-Schild *et al.*, 1999); the endogenous ligand for the nicotinic receptor in this region is acetylcholine, which is released in the VTA by projections from the pedunculopontine tegmental nucleus (Oakman *et al.*, 1995).

Trigger zones for the habit-forming actions of amphetamine, cocaine and phencyclidine are in the nucleus accumbens. The binding sites for amphetamine and cocaine are the dopamine transporters on dopaminergic nerve terminals in this region; the trigger zone for phencyclidine seems to be the NMDA receptor on medium spiny neurones. The endogenous transmitter mediating the rewarding effects of amphetamine and cocaine is dopamine; it appears to require synergistic effects (see, e.g., Clark and White, 1987) of both D_1-type and D_2-type receptors for dopamine to be rewarding (Ikemoto *et al.*, 1997a). The endogenous transmitter that, when blocked, accounts for the rewarding effects of phencyclidine would appear to be glutamate; glutamatergic afferents to the nucleus accumbens arise from a variety of cortical structures.

Additional trigger zones for cocaine and phencyclidine effects are found in the medial prefrontal cortex; the receptor mediating the rewarding effects of phencyclidine again appears to be the NMDA receptor. It is not clear what binding site mediates the rewarding effects of cocaine in the medial prefrontal cortex. While the rewarding effects of cocaine in this region appear to be dopamine-dependent, since they are blocked by the D_2 dopamine antagonist sulpiride and are eliminated by dopamine-specific neurotoxic lesions (Goeders *et al.*, 1986; Goeders and Smith, 1986), cocaine does not appear to have its rewarding effects as a result of actions at the dopamine transporter because this transporter is sparsely distributed in this region. Rather, cocaine's ability to elevate frontal cortex dopamine concentration appears to depend on its blockade of the noradrenaline transporter, which apparently takes up dopamine into noradrenergic nerve terminals.

The findings reviewed above suggest a common reward circuitry associated with a broad range of addictive drug classes. At the core of the circuitry are the mesolimbic dopamine system and the descending anatomical cascade of GABAergic neurones that dominate the efferent pathway from nucleus accumbens. While this unifying theme is attractive, the evidence for actions of different drugs in this system is consistent but minimal in several cases. A good deal of additional evidence will be needed, either to tie each of these drugs unequivocally to the suggested circuitry or to establish separate, parallel, reward circuitry for the drug classes for which current evidence remains limited. Moreover, we are hardly closer now than we were 20 years ago (Nauta *et al.*, 1978; Mogenson *et al.*, 1980) to understanding how the activity of these segments of motivational circuitry interfaces with and controls the mechanisms of behavioural action.

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XVI-8

Neuroimaging and Substance Abuse

R. Hitzemann, N. Volkow, J. Fowler and G.-J. Wang

INTRODUCTION

Substance abuse has played a prominent role in biological psychiatry. To a large extent, the mechanisms of action of abused drugs are well understood at the molecular, cellular and systems level. This level of understanding has been important to the development of our current hypotheses regarding the regulation of normal and abnormal behaviour. However, until quite recently, it was not possible to directly test (in humans) even relatively simple hypotheses such as whether abused drugs up- or downregulate their target receptors. The information that was available depended on either indirect measures of brain function, e.g. neuroendocrine challenge studies, or post-mortem analyses. While such techniques have provided important data, for a variety of reasons they are not entirely satisfactory. For example, post-mortem analyses depend on opportunistic samples where many critical variables, such as duration, amount and types of drugs abused, can only be partially known. Beginning in the mid-1980s, new techniques became commonly available that allowed the direct measurement of brain receptors and metabolism in the living brain. Two of these techniques — single photon emission computerized tomography (SPECT) and positron emission tomography (PET) — and their use in the study of substance abuse will be the subject of this chapter. PET and SPECT remain the only procedures available to directly measure neurotransmitter receptors in the living brain. Their disadvantage is that anatomical resolution is only moderate, and temporal resolution is poor. Thus, these techniques cannot be used to measure changes in receptor availability in the same temporal way that microdialysis is used to measure neurotransmitter release, nor can these techniques be used to measure receptor availability in some regions that are of interest for substance abuse, e.g. the shell of the nucleus accumbens. SPECT and PET have been, and continue to be, used to measure cerebral blood flow and metabolism. However, newer magnetic resonance-based techniques, such as functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS) and arterial spin perfusion, with much improved spatial and temporal resolution, are now generally preferred for such measurements. Details regarding SPECT and PET methodology are found in Chapter XI.

Figure XVI-8.1 uses a dopamine synapse to illustrate some of the data that can be obtained using PET. In addition to measuring the availability of D_2 dopamine receptors (^{11}C-raclopride) and dopamine transporters (^{11}C-methylphenidate), one can also measure the levels of monoamine oxidase A (MAO-A) (^{11}C-clorgyline), which is localized largely intraneuronally and monoamine oxidase B (MAO-B) (^{11}C-deprenyl), which is localized largely in glial cells and is involved in the metabolism of dopamine. Figure XVI-8.1 also illustrates that measurements of brain metabolism, e.g. with ^{18}F-deoxyglucose (FDG), can be used as a surrogate measure of the downstream postsynaptic events, at least in regions with known connectivity to the striatum. Not illustrated in Figure XVI-8.1, is a strategy used to measure changes in dopamine release, which has been adapted to both the SPECT and PET paradigms (e.g. Mach *et al.*, 1997). The strategy makes use of the competition between endogenously released dopamine and ligands for the D_2 dopamine receptor. When dopamine and the receptor ligands have relatively equal affinities for the receptor, then increases in dopamine release will be associated with a decrease in receptor binding. To increase dopamine release, methylphenidate or amphetamine is administered intravenously. The disadvantage to this technique is that very large increases in dopamine release (e.g. >500%) are required to produce relatively small changes in binding.

ALCOHOL

Given the well-known neuropathology and associated cognitive deficits associated with chronic alcohol abuse, it is not surprising that the first functional neuroimaging studies of substance abuse focused largely on alcoholism. Early studies (e.g. Berglund and Ingvar, 1976; Berglund and Risberg, 1977) used the xenon-133 technique to measure cerebral blood flow in chronic alcoholics. Elderly alcoholics were found to show a decrease in blood flow localized largely to the frontal and temporal cortical regions. The first PET study (see Samson *et al.*, 1986) used FDG to measure brain metabolism and noted only a small decrease in the mediofrontal area of the alcoholics; given the poor resolution of the early PET cameras, a more exact placement of the deficit was not possible. A survey of subsequent PET and SPECT studies of alcoholism follows. In reviewing this information, it is important to note that during the early period of alcoholism imaging research, there was a fundamental paradigm shift in our view of the mechanism(s) of alcohol action. Before the early 1980s, it was the consensus view that alcohol acted by perturbation of membrane structure and, thus, had a mechanism of action not dissimilar from the general anaesthetics (see Kresishman *et al.*, 1985). However, there were problems with this hypothesis that began with the discovery of benzodiazepine binding sites and the subsequent discovery that benzodiazepines acted by potentiating the effects of γ-aminobutyric acid (GABA) at the $GABA_A$ receptor (Braestrup *et al.*, 1978). These findings were problematic (from the perspective of understanding the mechanisms of alcohol action) since it was well known that alcohol and the benzodiazepines exhibited cross-tolerance and cross-dependence (e.g. Chan *et al.*, 1985; Chan *et al.*, 1988; Chan *et al.*, 1991). Thus, it seemed likely that ethanol caused a more specific effect than was originally considered and must interact in some way with the $GABA_A$ receptor. Subsequent research confirmed this hypothesis (Allan and Harris, 1986), and it is now known that ethanol has complex effects on a number of ligand-gated ion channels, including the $GABA_A$, *N*-methyl-D-aspartate (NMDA), Serotonin 5-HT_3, nicotinic acetyl choline (nACh) and glycine receptor arrays (Deitrich *et al.*, 1989). As will

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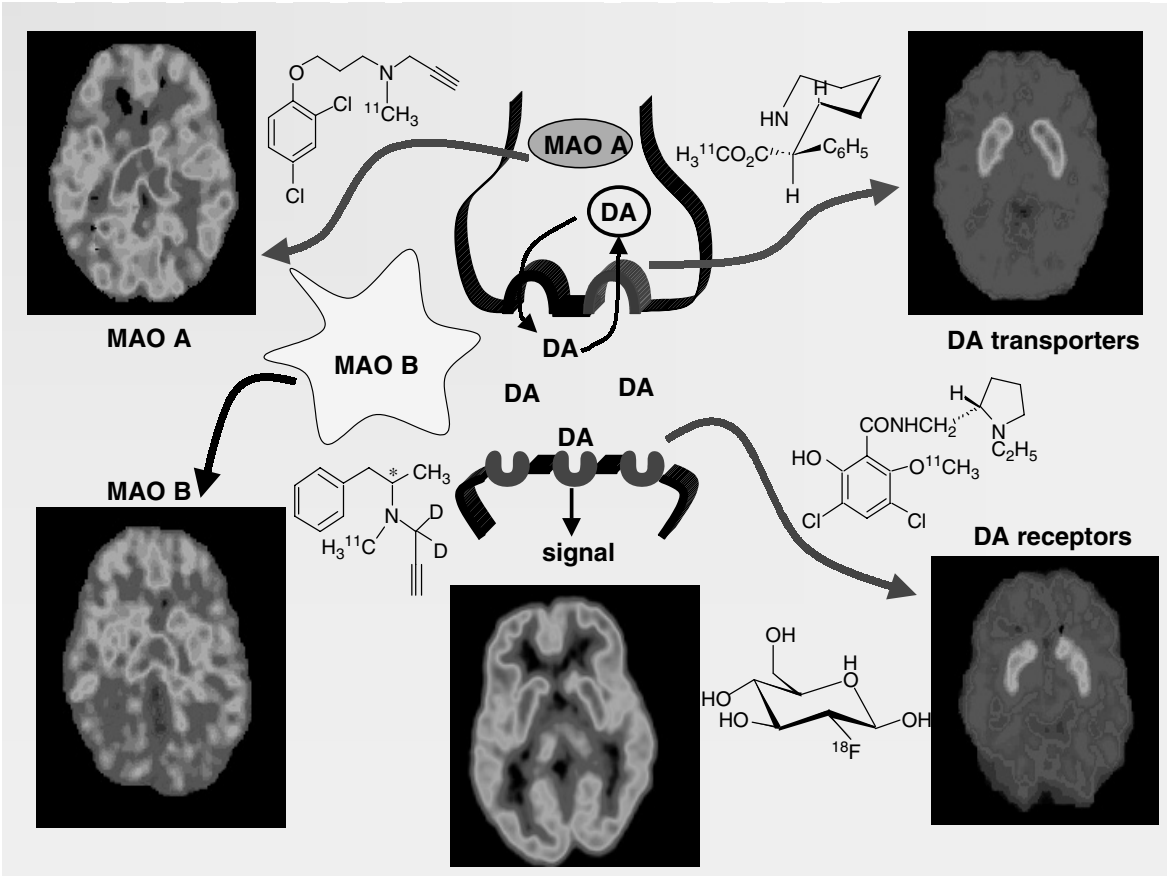

Figure XVI-8.1 Schematic of a dopaminergic synapse, indicating how PET can be used to evaluate dopaminergic function. The various ligands are ^{11}C-methylphenidate (dopamine transporter), ^{11}C-raclopride (D_2 dopamine receptor), FDG (metabolism), ^{11}C-deprenyl (MAO-B) and ^{11}C-clorgyline (MAO-A) (See Colour Plate XVI-8.1)

be noted below, this new information had a significant impact on the direction of imaging research.

Chronic Alcoholism: Cerebral Metabolism and Cerebral Blood Flow

Continuing the theme and direction of the earlier studies showing that brain metabolism and/or regional cerebral blood flow (rCBF) was decreased in chronic alcoholics, Sachs *et al.* (1987) examined a group of ten non-Korsakoff chronic alcoholics using PET and ^{11}C-deoxyglucose; the authors concluded that there were at least suggestive data pointing to lower glucose metabolism in the alcoholic brains, fewer region-to-region functional relationships, and significant impairment in right-hemispheric processing. Wik *et al.* (1988) followed ^{11}C-glucose metabolism in nine alcoholics and 12 controls; the authors observed that age decreased glucose metabolism, that this effect was greater in the alcoholics, and that the parietal cortex seemed to be the region most affected. Gilman (1988) found a significant decrease in cerebellar metabolism (PET–FDG) in patients with alcoholic cerebellar degeneration. In a subsequent study, this group observed that chronic alcoholics showed a decrease in the medial prefrontal region, which was independent of whether there was evidence of cerebellar degeneration with the associated decrease in cerebellar metabolism (Gilman *et al.*, 1990) Using SPECT and ^{133}Xe to measure rCBF, Melgaard *et al.* (1990) reported that blood flow was decreased in chronic alcoholics in both the anterior and posterior parts of the brain, and that the decrements were independent of morphological abnormalities. Using ^{99m}Tc-hexamethyl propylene amineoxime (HMPAO) rather than xenon, Erbas *et al.* (1992) found that 24 of 28 alcoholics showed abnormal rCBF, with most showing a significant decrement in the left hemisphere. Subsequent work (e.g. Adams *et al.*, 1993; Dao-Castellana *et al.*, 1998) illustrated that the prefrontal deficits are associated with significant neuropsychological impairments.

An important conclusion reached from these early imaging studies was that chronic alcoholism could have significant effects on cerebral metabolism and blood flow in the absence of marked anatomical changes (Wang *et al.*, 1993). Interestingly, even in patients with frank Korsakoff's syndrome, the metabolic deficit appears to be somewhat independent of the structural changes (Joyce *et al.*, 1994).

Although the data available suggested that cerebral metabolism was decreased in chronic alcoholics, the studies of Martin *et al.* (1992) and Volkow *et al.* (1992a) illustrated that the situation was not so straightforward. Using essentially identical experimental approaches (PET–FDG), Martin *et al.* (1992) did not detect an effect of chronic alcoholism on cerebral metabolism, while Volkow *et al.* (1992a) noted marked decreases in left parietal and right frontal cortex, including the orbitofrontal cortices and the cerebellum. Although the patients in the Martin *et al.* study had evidence of organic mental disorder attributable to alcoholism, they had been withdrawn for a significantly longer period than the Volkow *et al.* subjects, suggesting decreased metabolism was a state effect. This point was confirmed by Volkow *et al.* (1994) (see also Meyer *et al.*,

1985 and Ishikawa *et al.*, 1986), who examined detoxified alcoholics at 8–15, 16–30 and 31–60 days after the last use of alcohol. Recovery from the initial deficit in brain metabolism occurred primarily during the 16–30-day period. However, it was observed that brain metabolism in the basal ganglia remained persistently low. The clinical relevance of these findings should be interpreted cautiously. The group of alcoholics studied were neurologically intact and were in general good health; the question of whether a similar recovery would be noted in a more representative population of alcoholics is not known. Nicolas *et al.* (1993) examined cerebral blood flow (CBF) (^{99m}Tc-HMPAO–SPECT) in a group of 'healthy' alcoholics and found a significant decrement in CBF during the first week of abstinence, which returned to normal 2 months later. The initial decrement in CBF was correlated negatively with the amount of alcohol consumed during the previous month, but there was no relationship between the decrement in perfusion and performance on a battery of neuropsychological tests. Caspari *et al.* (1993) noted that perfusion (^{99m}Tc-HMPAO–SPECT) increased in the superior temporal cortex during withdrawal (weeks 1–4) but decreased in the basal temporal cortex. A small persistent decrement in basal ganglia perfusion was also noted, similar to the results of Volkow *et al.* (1994). More recently, Tutus *et al.* (1998) reported in recently withdrawn but stabilized alcoholics a persistent decrease of rCBF (^{99m}Tc-HMPAO–SPECT) in the temporal cortex.

Some studies on alcoholism and brain metabolism have examined longer periods of withdrawal. For example, Dupont *et al.* (1996) examined CBF (^{123}I-iodoamphetamine–SPECT) in a group of short-term and long-term abstinent alcoholics (mean abstinence = 25 days and 7.7 years, respectively). CBF and cognitive performance were improved in the long-term compared with the short-term group but remained significantly less than a comparison control group. Johnson-Greene *et al.* (1997) studied six alcoholics who received two scans with an interscan interval of 10–32 months. For four of the subjects who remained abstinent (or nearly abstinent), brain metabolism (PET–FDG) was improved at the second scan in some (but not all) frontal areas; cognitive performance also improved. For the two subjects who did not remain abstinent, brain metabolism decreased. Harris *et al.* (1999) noted a persistent cerebellar perfusion (^{99m}Tc-HMPAO–SPECT) in subjects who had been abstinent on average for >6 years. Finally, Gansler *et al.* (2000) found that in the left inferior frontal brain regions, alcoholics abstinent on average for 1.7 years showed a significant decrement in CBF from both normal controls and a group of alcoholics abstinent for an average of 10.5 years.

Gender appears to differentially influence the behavioural and neurochemical effects associated with chronic alcoholism (Gomberg, 1993), in particular the neurotoxic effects (Roman, 1988). However, Wang *et al.* (1998) did not detect a significant effect on brain metabolism in a group of chronic female alcoholics. The authors suggested that this lack of effect may be associated with the fact that female alcoholics generally consume less alcohol than their male counterparts.

The genetics of substance abuse are well established (see Chapter XVI-9). The question of whether there is a difference between family-history-positive and family-history-negative alcoholics on measures of metabolism and CBF has been examined. Mampunza *et al.* (1995) using ^{99m}Tc-HMPAO–SPECT found greater abnormalities of rCBF in family-history-positive compared with family-history-negative alcoholics. Volkow *et al.* (1995a) observed that family-history-positive (compared with negative) alcoholics had a lower cerebellar metabolism and a blunted response to a 30-$\mu g\,kg^{-1}$ lorazepam challenge (see also below). Adams *et al.* (1998) approached this issue from a different perspective. Noting that in at least some studies there is evidence of a reduced alcohol response in the sons of alcoholics compared with nonalcoholics (Schuckit, 1994), it followed that a positive family history might moderate the effects of chronic alcoholism on brain metabolism. However, no difference was detected in cerebral metabolism between (PET–FDG) family-history-positive and family-history-negative alcoholics.

There is considerable consensus that chronic alcoholism causes both short- and long-term decrements in brain metabolism. Differences between studies are noted in the brain regions affected and the duration of the persistent effects; such differences probably reflect differences in the patient samples and the neuroimaging techniques. However, the data point to persistent changes in brain metabolism and CBF, which are associated with both cognitive and psychiatric deficits. The question arises as to whether the pathology observed is specific to alcohol. As will be shown in subsequent sections, persistently decreased brain metabolism and CBF, especially in cortical areas, is a common feature of chronic drug abuse and is observed not only with alcohol but also with some central stimulants, e.g. cocaine.

Alcohol and Benzodiazepine Challenge Studies

Volkow *et al.* (1988a), following up on the earlier work of Newlin *et al.* (1982) and Mathew and Wilson (1986), measured CBF (PET and ^{15}O-labelled water) before and after an alcohol challenge (0.5 and 1.0 $g\,kg^{-1}$) in a group of social drinkers. At the high dose, alcohol decreased CBF in the cerebellum but increased flow in the right temporal and the prefrontal cortex. Interestingly, when the same group examined cerebral metabolism (PET–FDG), they found that the 1-$g\,kg^{-1}$ challenge decreased metabolism not only in the cerebellum but also in cortical areas, suggesting that in the latter there is an uncoupling between metabolism and flow (Volkow *et al.*, 1990b). It was also noted that recently detoxified alcoholics were more sensitive to the depressant effects of the ethanol challenge. Further, the depressant effects of ethanol were greatest in those regions with the highest density of benzodiazepine–$GABA_A$ receptors. It was suggested that the enhanced metabolic response in the alcoholics might result from an upregulation of benzodiazepine–$GABA_A$ receptors, for which there was some preclinical evidence (Ticku *et al.*, 1983). However, the available neuroimaging data suggest that this is not the case. Litton *et al.* (1993) reported no difference between controls and alcoholics in either the K_D or B_{max} for the binding of ^{11}C-flumazenil binding (a benzodiazepine receptor antagonist). Abi-Dargham *et al.* (1998) examined the receptor distribution volume (a measure of receptor availability) in a group of recently detoxified alcoholics; benzodiazepine–$GABA_A$ receptor availability (SPECT and ^{123}I-iomazenil) was decreased significantly in several brain areas, in particular the frontal, anterior cingulate and cerebellar cortices. Using a similar method, Lingford-Hughes *et al.* (1998) found that alcoholics had decreased receptor availability in the frontal, parietal and temporal cortices, and that some decrements were clearly not associated with grey matter atrophy. This same group also addressed the question of whether the decrement in receptor availability differed between men and women (Lingford-Hughes *et al.*, 2000); in a small sample, the authors observed that women tended to show decrements in the cerebellum, occipital lobes and parietal cortex and not in the frontal cortex. Another approach to examining benzodiazepine–$GABA_A$ receptor function is to challenge subjects with a benzodiazepine agonist and examine the effect on brain metabolism. Volkow *et al.* (1993a) found that alcoholics were less sensitive than controls in some brain regions to a lorazepam challenge (30 $\mu g\,kg^{-1}$ intravenously); the alcoholics showed a significantly reduced response in the thalamus, basal ganglia and orbitofrontal cortex. These data are particularly significant as they point to some components of the limbic-reward circuit as being affected by chronic alcohol administration. The use of the lorazepam challenge also illuminated changes in the thalamus, a region that is not greatly affected by an acute ethanol challenge (Wang *et al.*, 2000).

There are insufficient data to answer the question of whether it is the benzodiazepine–$GABA_A$ receptor system that primarily mediates the neuroadaptive and/or neurotoxic effects associated with chronic alcohol intoxication. Thus, the possibility must be considered that alcohol effects on other ligand-gated ion channels are also important. However, there is a general consensus that from the perspective of receptor binding and brain metabolism, the benzodiazepine–$GABA_A$ receptor system is attenuated in alcoholics, providing evidence of functional tolerance.

Dopamine

The role of brain dopamine systems, particularly the mesolimbic and mesocortical systems, in mediating substance (including alcohol) abuse is well established (Weiss, 2000). In a series of papers, Noble, Blum and colleagues (Blum *et al.*, 1990; Blum *et al.*, 1991; Noble *et al.*, 1991) put forth the proposition that there was an association between alcoholism and a Taq 1 polymorphism in DRD2 that affected D_2 dopamine receptor density. From the genetic perspective, the association between the Taq 1 polymorphism and alcoholism remains controversial (reviewed in Hitzemann, 1998). However, the question of whether there is an association between D_2 receptor density and alcoholism remains unanswered. Hietala *et al.* (1994) were the first to address this issue directly in the living brain. Using PET and ^{11}C-raclopride (a D_2/D_3 dopamine receptor ligand), these authors found that the striatum/cerebellar ratio of binding was lower in alcoholics compared with controls. Essentially, a similar result was found by Volkow *et al.* (1996a), although the magnitude of the decrement was greater (20% *v.* 12%). Repo *et al.* (1999) used SPECT and ^{123}I-epidepride (a D_2/D_3 dopamine receptor ligand, but with higher affinity than raclopride) to measure striatal binding and were unable to detect a difference between alcoholics and controls; however, these data must be interpreted cautiously since the ligand did not reach equilibrium during the experiment. Interestingly, this same group (see also Kuikka *et al.*, 2000) have suggested that extrastriatal D_2/D_3 receptors in the temporal pole are lower in alcoholics. Guardia *et al.* (2000) found no difference in the striatum between alcoholics and normal controls (SPECT and ^{123}I-iodobenzamide, also a D_2/D_3 dopamine receptor ligand). However, when the alcoholics were divided into those who relapsed and those who did not, the non-relapsing group had a significantly lower receptor density.

The question of whether the dopamine transporter (DAT) is altered in alcoholics has also been addressed. Tiihonen *et al.* (1995) found that DAT density (SPECT and ^{123}I 2-beta-carbomethoxy-3-beta-(4-iodophenyl)-tropane) (β-CIT)—a cocaine analogue) was lower in non-violent alcoholics compared with controls; interestingly, violent alcoholics had a DAT density somewhat higher than controls. Volkow *et al.* (1996a), is a small sample, detected no difference in DAT between alcoholics and controls (PET and ^{11}C-threomethylphenidate as the ligand). Laine *et al.* (1999) found that DAT density (SPECT and ^{123}I β-CIT) was lower in alcoholics, but in addition they noted that DAT density normalized as a function of duration of withdrawal. Finally, Gilman *et al.* (1998) found a significant reduction in the vesicular monoamine transporter (PET and ^{11}C-tetrabenazine) in the putamen of severe chronic alcoholics without Wernicke–Korsakoff disease.

Overall, the data are relatively consistent that both D_2 dopamine receptor and DAT density are lower in chronic alcoholics. The normalization of the DAT decrement with abstinence suggests that this is a 'state' effect. At this time, it is unknown whether the decrement in D_2 receptors normalizes with abstinence, or whether lower D_2 dopamine receptor density is a risk factor for alcoholism.

Serotonin

There is considerable evidence to suggest that a Serotonin (5-hydroxytryptamine, 5-HT) dysfunction is associated with alcoholism. For example, low 5-HT turnover has been associated with both alcoholism and impulse control problems (Linnoila *et al.*, 1983; Kruesi *et al.*, 1990; Higley *et al.*, 1992, 1996a; Higley *et al.*, 1996b). From the imaging perspective, interest has focused on the 5-HT transporter (5-HTT), for which there is a known naturally occurring variant with a relatively high gene frequency that is associated with reduced 5-HT capacity (Heils *et al.*, 1996; Lesch *et al.*, 1996). Heinz *et al.* (1998b) used SPECT and ^{123}I β-CIT (which, in the brainstem, measures binding largely to the 5-HTT) and found a reduction in the availability of 5-HTTs (−30%) in alcoholics; further, the reduction was correlated with lifetime alcohol consumption. In a subsequent study, this group found that the reduction was largely in those individuals homozygous for the ll-allele of the 5-HTT (Heinz *et al.*, 2000).

CENTRAL STIMULANTS: COCAINE AND METHYLPHENIDATE

For a variety of reasons, it is useful to compare cocaine and methylphenidate. Both drugs have similar mechanisms of action, although methylphenidate does not block the 5-HTT.

Pharmacokinetics

The synthesis of ^{11}C-cocaine (Fowler *et al.*, 1989) made it possible to determine the relationship between the euphoric effects of cocaine and the occupancy of the DAT. The initial findings pointed to a very high brain uptake (8–10% of the injected dose), a very rapid uptake (peaking 4–6 minutes after injection) and a rapid clearance (half-life in the brain of 20 minutes). These data suggested that pharmacokinetic parameters were the principal cause of the transient euphoric effects associated with cocaine administration. However, it was possible to test this hypothesis directly by comparing the uptake of cocaine with that of methylphenidate. Volkow *et al.* (1995b) compared the uptake of ^{11}C-cocaine and ^{11}C-methylphenidate (Figure XVI-8.2). The results obtained show that both drugs have a similarly rapid and high uptake into the brain; however, the clearance of methylphenidate from the brain is considerably slower (half-life = 90 minutes compared with 20 minutes for cocaine). Since the time course and intensity of the euphoric response were the same for both drugs, these data suggested that the euphoric response was associated with the rapid uptake, but that termination of the behavioural response was due to other factors (e.g. rapid adaptation at the dopaminergic synapse). The data in Figure XVI-8.2 also show the uptake of oral methylphenidate. The time to peak brain level is 60–80 minutes after drug administration; the slow uptake presumably accounts for the lack of euphoric effects from this route of administration.

The data illustrated in Figure XVI-8.2 also posed a new question: would the slow clearance of methylphenidate from the brain prevent the euphoria induced by a second administration of the drug? This was tested in a group of normal controls given two doses of methylphenidate (0.375 mg kg^{-1} intravenously) 60 minutes apart; DAT occupancy was measured using ^{11}C-methylphenidate (Volkow *et al.*, 1996b). The results obtained showed that the second drug administration produced a similar euphoric response to the first administration despite the fact that >80% of the transporters were still occupied from the first dose. Importantly, these data illustrated that a potential therapeutic strategy (DAT blockade) was not likely to be useful unless the transporters were completely or nearly completely occupied. Such a level of occupancy would likely be associated with significant side effects.

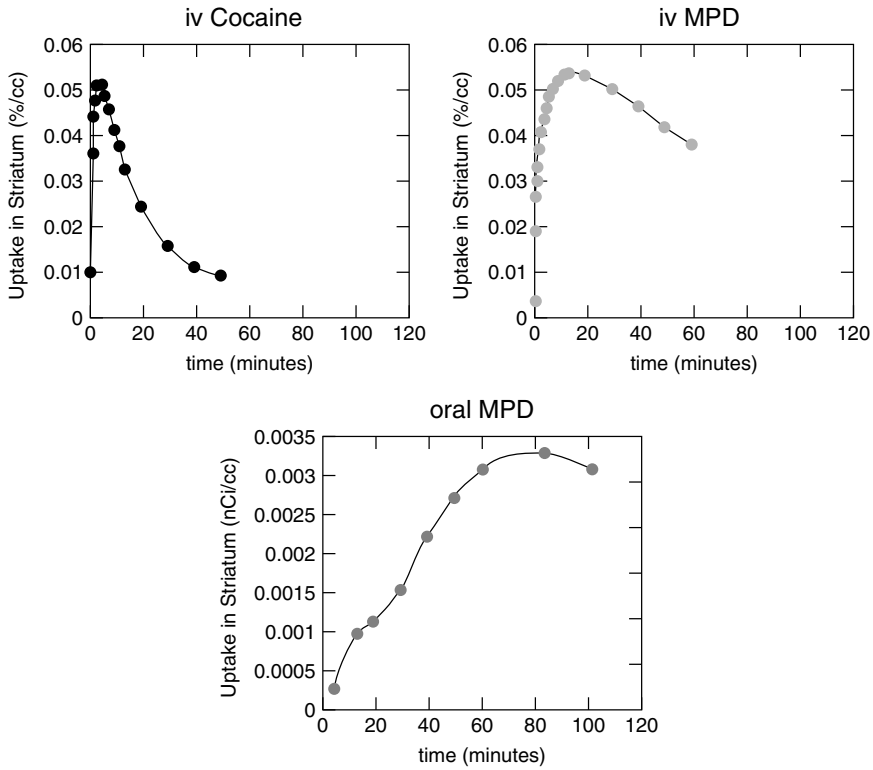

Figure XVI-8.2 Time courses for the striatal uptake intravenously administered ^{11}C-cocaine and ^{11}C-methylphenidate (MPD) and orally administered ^{11}C-methylphenidate. Data adapted in part from Volkow *et al.* (1995a) and Volkow *et al.* (1998)

The availability of ^{11}C-cocaine allows us to determine what is the threshold level of DAT blockade that is sufficient to induce a euphoric response (Volkow *et al.*, 1997a). Intravenous cocaine ($0.3-0.6\,\mathrm{mg\,kg^{-1}}$) blocked between 60% and 77% of DAT sites in the brain. The magnitude of the high was proportional to the level of DAT blockade, and at least 47% of the DAT sites had to be blocked to perceive the effects of cocaine. These data contrast with a subsequent report from this group (Volkow *et al.*, 1999a), in which it was observed that significant blockade (>60%) of DAT sites by methylphenidate was not associated with euphoria in four of eight subjects. The difference between the two studies was that the former used cocaine addicts and the latter used normal controls. The percentage of 'normals' who would not experience a high in response to cocaine, despite a significant DAT occupancy, is unknown. However, these data do point out that DAT occupancy alone is not sufficient to produce a euphoric response. What are the causes of such a marked intersubject variability? Understanding this issue is important not only in determining risk factors for substance abuse but also in developing effective therapies. At least two factors have been identified as important. One is the increase in synaptic dopamine (Volkow *et al.*, 1999a). Subjects were administered methylphenidate over the dose range of $0.025-0.5\,\mathrm{mg\,kg^{-1}}$; the amount of dopamine released was measured indirectly by monitoring changes in ^{11}C-raclopride binding. Methylphenidate-induced changes in ^{11}C-raclopride binding were associated significantly with self-reports of high and rush; importantly, these associations remained significant after accounting for differences in the dose and plasma concentrations of methylphenidate. Volkow *et al.* (1999a) suggested a mechanism to account for the intersubject variability in the reinforcing responses to methylphenidate. It was postulated that for equivalent levels of DAT blockade, the increases in dopamine induced by methylphenidate would be larger in subjects with a high dopamine tone compared with those with a low dopamine tone; this will result in a larger dopamine occupancy of D_2 dopamine receptors and a more intense high. The second factor that appears to be associated with the variability in methylphenidate response is the density of D_2 dopamine receptors. In a study of 23 normal controls administered $0.5\,\mathrm{mg\,kg^{-1}}$ methylphenidate, 12 reported the effects of methylphenidate as pleasant, nine described the effects as unpleasant, and two responded neutrally (Volkow *et al.*, 1999b). The subjects who reported the effects as unpleasant had a significantly higher level of D_2 receptors compared with the group that reported the effects as pleasant (Figure XVI-8.3). The causes of the variation in D_2 receptor density are unknown (see Hitzemann, 1998) but the range of variation is considerable.

Although the mechanism(s) of action of both cocaine and methylphenidate are well understood, it is clear that blockade of DAT sites is not sufficient to predict a euphoric or pleasant response to the drugs. Important variables appear to be the basal tone of the dopamine neurons and the density of D_2 dopamine receptors.

Chronic Cocaine Abuse, D_2 Dopamine Receptors, Dopamine Transporters and Dopamine Release

The obvious expectation from the abuse of central stimulants like cocaine is that dopamine receptors will be downregulated. Surprisingly, the preclinical data are not clear on this issue. Chronic cocaine administration to rats has been reported either to have no effect on D_2 receptor density (Stanwood *et al.*, 2000; Alburges *et al.*, 1993) or to transiently decrease receptor density (Goeders and Kuhar, 1987; Kleven *et al.*, 1990; Maggos *et al.*, 1998). Volkow *et al.* (1990a) using PET and ^{18}F-*N*-methylspiroperidol (NMS) found in a small sample of cocaine addicts that D_2receptor density was reduced after 1 week of abstinence from cocaine and was at

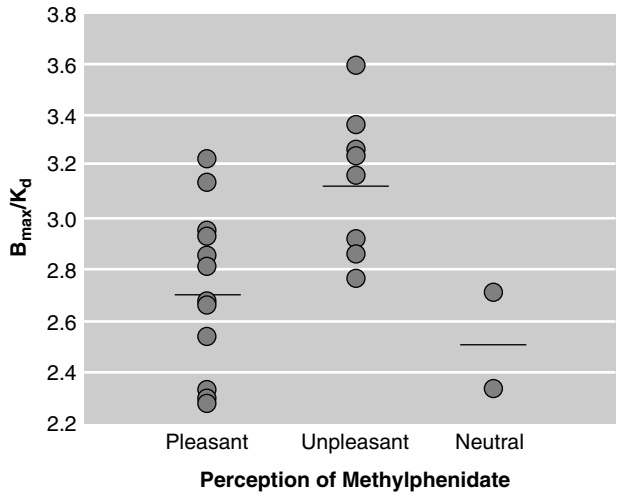

Figure XVI-8.3 Summary of the data illustrating the relationships between D_2 dopamine receptor density and response to methylphenidate. Note the large variation in receptor binding. Reproduced from Volkow *et al.* (1999a)

normal levels within 4 weeks of withdrawal. (It should be noted that NMS will bind with a high affinity to D_2, D_3 and D_4 receptors.) In a subsequent larger study of cocaine addicts ($n = 20$) (Volkow *et al.*, 1993b), significant receptor decrements were again found early in withdrawal. Furthermore, in this study, seven of the 20 addicts remained abstinent for 4 months and were rescanned. Although receptor binding had normalized, it was still significantly below normal levels. It was also noted that the decrements in striatal receptor binding were associated with decreased metabolism (PET–FDG) most markedly in the orbitofrontal and cingulate cortex. The authors suggested that dopamine dysregulation of these areas, which are known to be involved in impulse control, could be important to understanding drug-taking behaviour (see also Volkow and Fowler, 2000). Subsequent studies by this group, using ^{11}C-raclopride, have confirmed the lower receptor binding in cocaine addicts (e.g. Volkow *et al.*, 1997c). The post-mortem data are not clear on whether D_2 receptor binding is lower in cocaine addicts. Meador-Woodruff *et al.* (1993), using *in situ* hybridization, found that neither D_1 nor D_2 receptor expression was changed in the nucleus accumbens, caudate-putamen or substantia nigra of cocaine-exposed subjects. In contrast, Worsley *et al.* (2000), using a quantitative immunoblotting procedure for the measurement of D_2 protein levels in the nucleus accumbens, found a decrease in methamphetamine, cocaine and heroin users, although only the decrease in heroin users reached statistical significance. Segal *et al.* (1997) observed that the expression of the D_3 dopamine receptor is markedly increased (six-fold) in the nucleus accumbens of cocaine abusers. These data are of interest since under certain binding conditions, upregulation of D_3 receptors could obscure a downregulation of D_2 receptors.

Potentially, the DAT could be a site for neuroadaptation during chronic cocaine abuse. The brain uptake of ^{11}C-cocaine is reduced modestly (about 15%) in cocaine abusers, but the availability of DAT sites appears to be normal (Volkow *et al.*, 1996b). Interestingly, cocaine abusers do not show the normal age-related loss of striatal DAT sites, which may suggest that cocaine has some neuroprotective effects for dopamine neurons (Wang *et al.*, 1997).

To determine whether the increase in synaptic dopamine was different between controls and cocaine addicts, Volkow *et al.* (1997b) used a methylphenidate challenge technique which indirectly measures release by monitoring changes in the binding of ^{11}C-raclopride. Addicts showed a reduced release in the striatum and had a reduced high relative to controls. In contrast, the addicts showed an enhanced response in the thalamus, and the extent of the enhanced response was associated with drug-induced craving and was not seen in controls subjects. These results challenge the notion of an enhanced dopamine response in addiction and/or an enhanced induction of euphoria. Moreover, the data point to an important involvement of the thalamus in drug-seeking behaviour.

The data suggest that, at least within the striatal dopamine system, chronic cocaine intoxication is associated with an attenuated response to acute drug administration. Such a functional downregulation appears superficially to be the predicted and appropriate neuroadaptive response. However, for the cocaine addict, the reduction in the euphoric response is likely to trigger an increase in drug craving. In addition, some data point to adaptive changes in the striatal-thalamo-orbitofrontal circuit, which may contribute to increased craving and decreased impulse control (Childress *et al.*, 1999; Volkow and Fowler, 2000).

Other Transmitters

Although their precise role in addiction and drug craving is less clear, other neurotransmitters are affected by chronic cocaine intoxication. Two examples will serve to illustrate this point. Zubieta *et al.* (1996) used PET and the ligand ^{11}C-carfentanil to examine μ opiate receptor binding in a group of cocaine addicts and controls. The addicts showed increased receptor binding in several areas, including the caudate, thalamus, cingulate cortex and frontal cortex. The increase in binding was detected 1–4 days after cocaine withdrawal and persisted for at least 4 weeks. The initial increase in binding was correlated with the severity of drug craving. To determine whether the benzodiazepine–$GABA_A$ receptor system is affected by chronic cocaine intoxication, Volkow *et al.* (1998) challenged cocaine addicts and controls with lorazepam ($30\,\mu g\,kg^{-1}$) and examined both the behavioural effects and the effects on cerebral metabolism (PET–FDG). The results showed that despite significantly lower plasma drug concentrations, the addicts showed significantly greater decrements in brain metabolism, especially in the striatum, thalamus and parietal cortex. The addicts also reported significantly greater drug-induced sleepiness, which was correlated with the decrements in thalamic metabolism. Interestingly, this same group (Volkow *et al.*, 2000) found that cocaine addicts are less sensitive behaviourally and metabolically to an alcohol challenge, suggesting that alcohol and benzodiazepine affect the cocaine abuser's brain in a fundamentally different manner. Overall, these studies point out one of the major problems associated with trying to understand the mechanisms of drug addiction, the likely involvement of multiple neurotransmitter/neuropeptide systems.

Cocaine, Cerebral Metabolism and Cerebral Blood Flow

The initial interest in cocaine and CBF stemmed from the vasoactive properties of the drug and a marked increase during the late 1970s and 1980s in the number of cocaine-related cerebrovascular incidents (Brust and Richter, 1977; Schwartz and Cohen, 1984; Tuchman *et al.*, 1987). In addition, during this period there were an increasing number of reports of seizures associated with cocaine abuse (Baxter *et al.*, 1988). Volkow *et al.* (1988b) (PET–^{15}O-labelled water) found that cocaine abusers showed areas of decreased CBF as evidenced by 'patchy' areas where isotope accumulation was low; the prefrontal cortex appeared to be especially affected. Repeated scans after 10 days of withdrawal did not lead to a significant improvement in CBF. Holman *et al.* (1991) also noted patchy areas of reduced flow (SPECT–^{99m}Tc-HMPAO) in the inferoparietal, temporal and anterofrontal cortex and the basal ganglia; interestingly, there didn't appear to be a relationship between

these deficits and the mode, frequency or duration of cocaine abuse. The 'patchy' hypoperfusion has been confirmed in several studies (Miller *et al.*, 1992; Strickland *et al.*, 1993; Holman *et al.*, 1993; Bell *et al.*, 1994; Levin *et al.*, 1994; Levin *et al.*, 1995; Kosten *et al.*, 1998; Ernst *et al.*, 2000). Associated with perfusion deficits were deficits in cognitive function, including attention, concentration, visual and verbal memory, word production and visuomotor integration (Strickland *et al.*, 1993). The perfusion deficits may be worse in men than in women (Levin *et al.*, 1994), and they appear to be worse in multidrug users (Levin *et al.*, 1994; Kosten *et al.*, 1998). Interestingly, one group has reported improvements in CBF after treatment with buprenorphine (Levin *et al.*, 1995).

Paralleling the data on CBF, brain metabolism is reduced in cocaine addicts (Baxter *et al.*, 1988; Volkow *et al.*, 1991a; Volkow *et al.*, 1992b). Of particular interest is the observation by Volkow *et al.* (1992b) that cocaine abusers had significantly lower metabolic activity in 16 of 21 left frontal regions and eight of 21 right frontal regions. These decreases persisted for 3–4 months after drug withdrawal and were correlated with the dose and duration of cocaine abuse.

Although it is difficult to compare studies directly, the data suggest that for neurologically intact subjects, the decrements in CBF and brain metabolism in cocaine addicts are at least of a similar magnitude to those observed in alcoholics and, perhaps, are even more persistent. Importantly, it must be assumed that these decrements will slow the process of recovery and readaptation of the relevant neural networks.

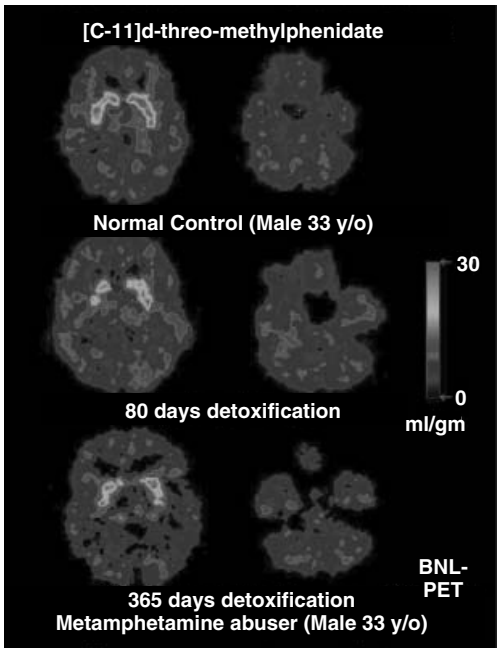

Figure XVI-8.4 Representative PET images showing ^{11}C-methylphenidate uptake to striatal dopamine transporter sites in a normal control and a methamphetamine abuser, scanned twice at 1 month and 4 months into withdrawal. Data adapted from Volkow *et al.* (2001a) (See Colour Plate XVI-8.4)

CENTRAL STIMULANTS: METHAMPHETAMINE

Dopamine Transporter and D_2 Dopamine Receptor

Methamphetamine has been abused for over 50 years. However, in recent years, the abuse appears to be increasing (Lukas, 1997). Methamphetamine and the related compound methcathinone can be made easily with relatively simple equipment, thus control of its production is difficult. Methamphetamine differs from cocaine and methylphenidate in that there is a considerable amount of animal literature showing that repeated high doses of the drug produce long-lasting depletions of both dopamine and serotonin (e.g. Seiden *et al.*, 1976; Ricaurte *et al.*, 1980). In non-human primates, two large doses of methamphetamine (2 mg kg^{-1}, 24 h apart) can reduce ligand binding to striatal DAT sites by >90% (Melega *et al.*, 2000). Although these doses are high, methamphetamine abusers are known to consume up to 10 g per day or approximately 120 mg kg^{-1} per day (Volkow *et al.*, 2001a) and, thus, some neuropathology would not be unexpected. Surprisingly, until recently, there was little human data on this issue. Wilson *et al.* (1996), in a post-mortem study of methamphetamine abusers, found reduced levels of dopamine, tyrosine hydroxylase and DAT sites in the nucleus accumbens, caudate and putamen; however, levels of DOPA decarboxylase and the vesicular monoamine transporter were normal. This type of neuropathology differs from that seen in Parkinson's disease and suggests that methamphetamine does not cause destruction of the dopamine neuron (e.g. see Melega *et al.*, 1997a; Melega *et al.*, 2000). McCann *et al.* (1998b) examined six methamphetamine/methcathinone addicts who had been withdrawn for an average of 3 years; they detected a significant decrement in DAT sites in both the caudate nucleus and putamen. These authors confirmed what had been observed previously in non-human primates: that the decrement was larger in the caudate compared with the putamen, thus differentiating the loss in addicts from that seen in Parkinson's disease patients. Volkow *et al.* (2001a) also noted a persistent reduction DAT availability (Figure XVI-8.4), with significant decrements being detected in subjects withdrawn for up to 11 months. These authors also found that the decrement in DAT availability was associated with deficits in both cognitive (auditory memory) and motor performance (grooved pegboard). A subgroup of these subjects (withdrawn for <6 months) were then retested 6–11 months later (Volkow *et al.*, 2001b); on retest, there was a significant improvement in the availability of DAT sites. Although some improvement was also noted on the cognitive and motor performance, these changes were not significant.

Finally, Sekine *et al.* (2001) examined DAT availability in a group of long-term methamphetamine addicts who also experienced significant psychiatric symptoms. Confirming previous findings, these authors noted a decrease in DAT sites in the methamphetamine users but also noted that the reduction in DAT sites appeared to be associated with positive psychotic symptoms.

Iyo *et al.* (1993) examined D_2 dopamine and serotonin 5-HT$_2$ receptors (^{18}F-NMS and PET) in six methamphetamine addicts who had also experienced psychosis. Five of the six subjects had been withdrawn from methamphetamine for more than 1 year. No difference in binding was detected in either the striatum (D_2 dopamine) or the frontal cortex (serotonin 5-HT$_2$). However, the authors did note a difference (decrease) in the ratio of binding between the striatum and frontal cortex. Volkow *et al.* (2001c) observed a modest but significant reduction in D_2 dopamine receptor availability (^{11}C-raclopride) in the caudate nucleus (−16%) and putamen (−10%). As in cocaine addicts, there was a significant relationship between receptor availability and metabolism in the orbitofrontal cortex.

Methamphetamine abuse differs from cocaine abuse in that for the former there appears to be a persistent presynaptic pathology, as evidenced by the decrease in the availability of DAT sites. The molecular mechanisms responsible for the loss of DAT sites are not clear; however, in non-human primates, the loss of DAT sites can be prevented by the concomitant administration of glial cell-line-derived neurotrophic factor (GDNF) (Melega *et al.*, 2000).

Methamphetamine abuse is similar to alcohol and cocaine abuse in that there is a significant and persistent decrease in D_2 dopamine receptor density.

Methamphetamine, Cerebral Metabolism and Cerebral Blood Flow

Iyo *et al.* (1997) noted marked focal vascular deficits (SPECT–^{99m}Tc-HMPAO) in the cortex of methamphetamine abusers that did not appear to be associated with strokes or cerebral atrophy. Further, these deficits were found in some individuals withdrawn from methamphetamine for up to 4 years. Alhasson *et al.* (2001), also using SPECT, found CBF to be lower in methamphetamine-dependent alcoholics (MDA) compared with cocaine-dependent alcoholics (CDA), who in turn were significantly lower than controls. The CDA group displayed marked regional abnormalities in the superior frontal region, which were not found in the MDA group (where the perfusion defects were diffuse). Volkow *et al.* (2001d) found an overall global increase in metabolism (PET–FDG), which was most notable in the parietal cortex (Figure XVI-8.5). After normalization of the data, significant decrements were detected in the thalamus and caudate nucleus. Pathological causes of increased metabolism have been associated with inflammation and gliosis (e.g. Roh *et al.*, 1998), and preclinical studies have documented that under certain conditions, methamphetamine can induce gliosis. Although there is no direct evidence that methamphetamine induces gliosis in humans, Ernst *et al.* (2000) have obtained MRS data in methamphetamine addicts consistent with this idea.

OPIOIDS

Despite the prevalence of the problem, and the enormous costs associated with treatment, there have been relatively few neuroimaging studies of opiate dependence. Early PET studies showed that the acute administration of morphine (London *et al.*, 1990) or buprenorphine (Walsh *et al.*, 1994) to healthy polydrug abusers decreased brain metabolism. A preliminary study of three post-addicts and two current addicts suggested that chronic heroin abuse produced a persistent decrease in brain metabolism (London, 1989). Van Dyck *et al.* (1994) examined the effects of naloxone-precipitated withdrawal from buprenorphine on rCBF (SPECT–^{99m}Tc-HMPAO) in 11 subjects; no naloxone-specific effects on rCBF were detected, although the severity of withdrawal showed a significant negative correlation with CBF in the anterior cingulate cortex.

Krystal *et al.* (1995) examined the effects of naloxone-precipitated withdrawal in a group of methadone-dependent

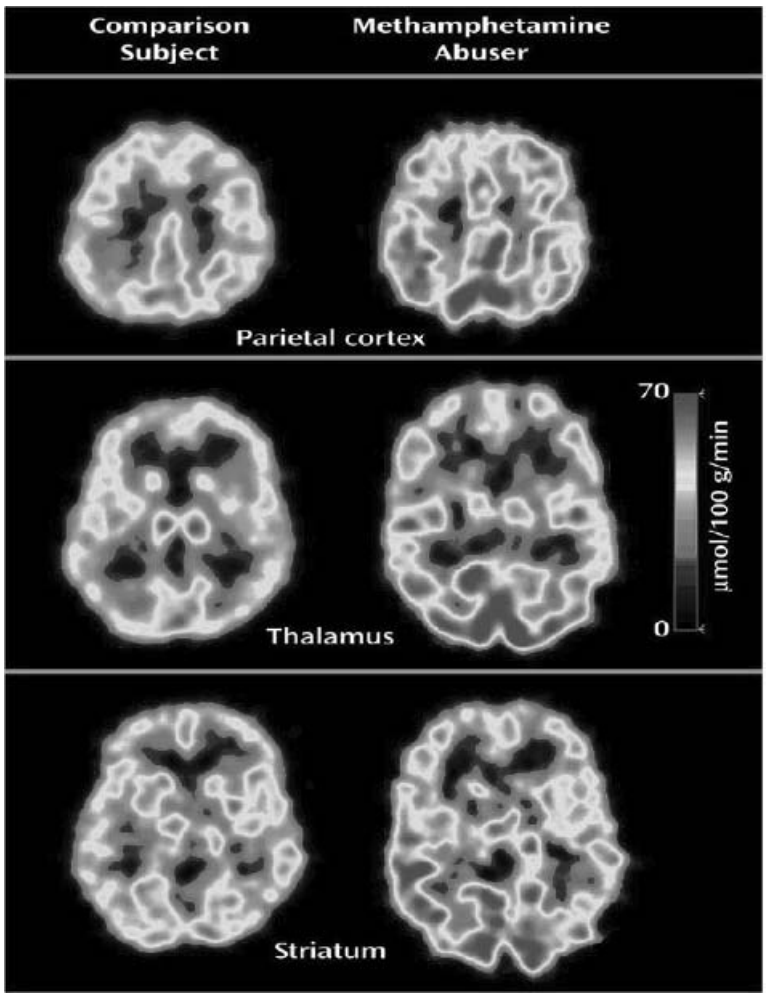

Figure XVI-8.5 Representative comparison of brain metabolism in a normal control and a methamphetamine abuser. Note the increased metabolism in the parietal cortex but decreased metabolism in the thalamus and striatum. Reproduced from Volkow *et al.* (2001b) (See Colour Plate XVI-8.5)

patients, but this study included a control group in double-blind, placebo-controlled study design. The data suggested that there was a decrease in brain perfusion in the frontal and parietal cortex of the methadone-dependent group after saline administration compared with the control group; however, metabolism was increased in the thalamus. Relative to the effects in the normal subjects, withdrawal in the addicts was associated with a decrease in global metabolism and a decrease in rCBF in the right temporal cortex. However, the changes reported were quite small. Rose *et al.* (1996), also using the SPECT technique, examined ten chronic heroin users at 1 week and 3 weeks of withdrawal. The initial scan indicated perfusion deficits in the frontal, temporal and parietal cortices. Two weeks later, improvement in perfusion was noted, suggesting that the deficits were transitory. Hypoperfusion during withdrawal has also been noted by Gerra *et al.* (1998) and Danos *et al.* (1998), although in the former, the hypoperfusion appeared to be related to comorbid behavioural abnormalities, e.g. depression.

Given the availability of numerous opiate ligands suitable for imaging, e.g. ^{11}C-carfentanil and ^{18}F-cyclofoxy, it is somewhat surprising that the question of whether opiate abuse affects opiate receptor binding has not been addressed fully. Post-mortem data (Gabilondo *et al.*, 1994; Schimdt *et al.*, 2000; 2001) indicate that μ and δ opiate receptors are not affected by chronic drug intoxication, similar to earlier preclinical results (Hitzemann *et al.*, 1974). In contrast to these results, Zubieta *et al.* (2000) found substantial increases in μ opiate receptor binding in a small group of dependent subjects maintained on buprenorphine; particularly marked increases were noted in the inferofrontal cortex and anterior cingulate regions. In contrast to these data, Kling *et al.* (2000) found that the binding of the μ and κ opiate receptor ligand, ^{18}F-cyclofoxy, was reduced in a group of dependent subjects maintained on methadone. The measurements were made 22 hours after the last dose of methadone, and the decrements in binding were correlated with the residual plasma methadone levels.

Worsley *et al.* (2000) found that D_2 dopamine receptor density was decreased in a post-mortem sample of heroin abusers compared with controls. Wang *et al.* (1997) noted that receptor availability (PET and ^{11}C-raclopride) was lower in heroin-dependent subjects; further, these authors noted naloxone-precipitated withdrawal did not change binding availability.

Heroin addicts are the fourth group of substance abusers that have a decrease in D_2 dopamine receptor density, the others being alcohol, cocaine and methamphetamine abusers, suggesting the possibility of a common underlying pathology.

CANNABINOIDS

Despite an impressive increase over the past decade in our understanding of the mechanisms of cannabinoid action (e.g. see Chaperon and Thiebot, 1999), including the characterization of the CB1 and CB2 receptors and a number of endogenous ligands for these receptors, there has been relatively little research on cannabinoid abuse from the clinical imaging perspective. Volkow *et al.* (1991b) investigated the effects of acute intravenous administration of 2 mg of Δ^9-tetrahydrocannabinol (THC) on brain metabolism (PET–FGD) in eight normal controls. When compared with baseline measures, the effects of THC were quite variable: three subjects showed a decrease in global metabolism, three showed an increase, and two showed no change. However, all subjects showed an increase in cerebellar metabolism, which was correlated with the subjective sense of THC intoxication and plasma THC. The authors concluded that the cerebellar effect corresponds well with the known high density of THC receptors in the cerebellum. This same group followed with a study in normal controls and chronic marijuana abusers (Volkow *et al.*, 1996c). At baseline, cerebellar metabolism was lower in the abusers compared with the controls. THC increased cerebellar metabolism in both groups but increased metabolism in the orbitofrontal cortex, prefrontal cortex and basal ganglia only in the marijuana abusers. The authors concluded that the activation of the orbitofrontal cortex and basal ganglia by THC in the abusers could be associated with the drive and compulsion to self-administer THC. Amen and Waugh (1998) measured CBF (SPECT–^{99m}Tc-HMPAO) in a group of 30 heavy marijuana users with ADHD and ten attention deficit hyperactivity disorder (ADHD) controls. Both groups showed a perfusion deficit in the prefrontal cortex; however, only the marijuana group showed a marked decrease in activity in the right and left temporal lobes.

3,4-METHYLENE-DIOXYMETHAMPHETAMINE

Although 3,4-methylene-dioxymethamphetamine (MDMA; ecstasy) was first synthesized in the 1960s, its popularity as a recreational drug is relatively recent. Animal models of MDMA neurotoxicity have suggested that in doses similar to those that are abused, the drug produces a loss of serotonergic neurons. Data from non-human primates (baboons) have confirmed that NMDA markedly reduces the density of 5-HT transporters, and that in some regions, these effects can persist for over a year (Scheffel *et al.*, 1998). McCann *et al.* (1998a), using PET and the 5-HT transporter ligand ^{11}C-McN-5652, compared 14 previous users of MDMA with 15 controls. Decreases in transporter binding correlated with the extent of previous drug use (see also McCann *et al.*, 2001 and Ricaurte *et al.*, 2001). Semple *et al.* (1999) found that while MDMA use led to significant decreases in 5-HT transporter binding, there was no change in DAT binding. Reneman *et al.* (2001a, 2001b) have observed that the decrease in 5-HT transporter density is greater in more recent than more abstinent users, and that women may be more be susceptible to the loss of transporters than men. One study has suggested that in response to the apparent loss of 5-HT neurons, MDMA users show an upregulation of cortical 5-HT_{2A} receptors, which appeared to be associated with memory impairments. Several studies have used PET or SPECT to examine rCBF or brain metabolism in MDMA users (Obrocki *et al.*, 1999; Chang *et al.*, 2000; Gamma *et al.*, 2001; Buchert *et al.*, 2001). Some abnormalities in rCBF and metabolism have been observed; however, at present the data do not suggest the same extent or persistence of abnormalities as those seen in cocaine or methamphetamine abusers.

NICOTINE

Arguably, the use and abuse of nicotine-based products, largely cigarettes, has the greatest effect on society from the perspective of both cost and health risk. Each year, about 3 million people die from smoking-associated illnesses (Wald and Hackshaw, 1996). Despite the importance of the problem, neuroimaging studies of nicotine abuse are few. Fowler *et al.* (1996, 1998, 2000) have found that people who smoke cigarettes have a marked inhibition of both brain MAO-A and MAO-B. The two forms of MAO were visualized using ^{11}C-clorgyline and ^{11}C-deprenyl, respectively (Figure XVI-8.6). The smoking-associated decrease in enzyme activity for both forms of the enzyme was about 40–50%. The mechanisms by which cigarette smoking inhibits MAO activity are not clear but apparently are not associated with nicotine *per se* (Fowler *et al.*, 1998). Further, smoking a single cigarette does not decrease MAO activity (Fowler *et al.*, 1999). Thus, it is assumed that the inhibition of enzyme activity is caused by some material in the smoke that accumulates with chronic drug administration. While these data are of interest in their own right, they have particular importance when we consider that nearly all drug abusers also

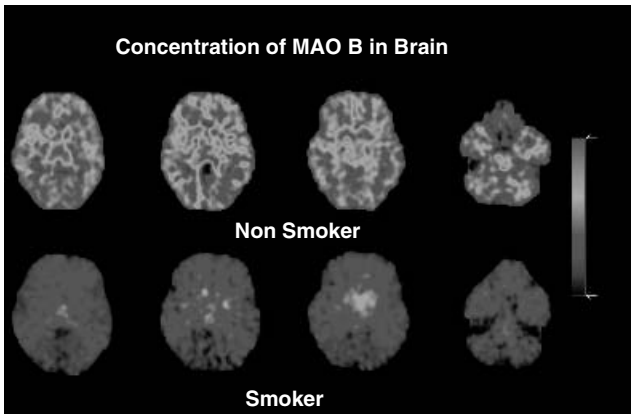

Figure XVI-8.6 Representative comparison of ^{11}C-deprenyl uptake into the brain of a nonsmoker and a smoker. Data are taken to indicate reduced MAO-B activity in the smoker. Adapted from Fowler *et al.* (1996) (See Colour Plate XVI-8.6)

smoke cigarettes. In most imaging studies of substance abusers, smoking is either not controlled for or is controlled for only poorly. These data illustrate that in addition to the substantial effects of nicotine, the smoke contains elements that also affect systems that regulate neurotransmitters.

Domino *et al.* (2000) examined the effects of nicotine on brain metabolism (PET–FDG) in a group of smokers that were abstinent overnight before the study. Three minutes before the scan, a dose of 1–2 mg of nicotine or placebo was delivered by nasal spray. The normalized metabolic data revealed several regions that were activated by nicotine; these included the left inferior frontal gyrus, left posterior cingulate and right thalamus. The authors noted that the increase in the thalamus was consistent with the high density of nACh receptors in that area; however, many areas with a known high density of receptors were not activated, suggesting that the effects of nicotine are more complex than simply receptor activation. Zubieta *et al.* (2001) used a similar experimental design to examine the effect of nicotine on CBF (^{15}O-labelled water–PET). The authors noted decreases in rCBF in the anterior temporal cortex and in the right amygdala. However, nicotine increased rCBF in the right anterior thalamus. Thus, there is agreement between the metabolic and flow data.

CONCLUSIONS

PET and SPECT neuroimaging have both confirmed existing hypotheses and extended our understanding of substance abuse. The most salient points may be summarized:

Individuals who abuse alcohol, cocaine, methamphetamine and heroin all show modest reductions in striatal D_2 dopamine receptor density. The initial interpretation of these results was that of receptor downregulation in response to enhanced dopamine release. However, neuroimaging revealed that the reduction in receptor density is persistent (at least for cocaine addicts), suggesting that lower receptor density may be a risk factor for substance abuse. Of interest is the observation that the subjective response of normal controls to intravenous methylphenidate is associated with D_2 receptor density; those with low receptor density had a 'pleasant' drug response, while those with a higher receptor density had an 'unpleasant' drug response.

Substance abuse is associated with persistent changes (generally decrements) in brain metabolism and rCBF. Although the mechanisms responsible for these deficits are probably drug dependent, there are likely to be common consequences, including cognitive deficits and an extended period of recovery and readaptation of the relevant neural networks.

A common theme that has emerged is the involvement of the striatal-thalamo-orbitofrontal circuit and the development of abnormalities in this circuit that contribute to increased craving and decreased impulse control.

While there has been substantial work on alcohol and cocaine abuse, there is a need for substantially more work on methamphetamine, opiate, marijuana, MDMA and nicotine abuse. Further, there is very little understanding of the interactions between multiple drugs of abuse. However, the preliminary data suggest that the interactions may be substantial.

Neuroimaging of substance abusers is not limited to PET and SPECT. As noted in the introduction, MRI- and MRS-based techniques are used widely and are becoming increasingly popular. The use of these techniques in the study of alcoholism has been reviewed (Mann *et al.*, 2001).

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XVI-9

Genetic Epidemiology of Substance-Use Disorders

Kathleen R. Merikangas

INTRODUCTION

The recent announcement that the human genome has now been sequenced has generated widespread excitement about our ability to understand and treat human disease. Many genes have been identified for disorders caused by a single gene with high absolute and relative risk, such as Huntington's disease (Gusella *et al.*, 1983), muscular dystrophy (Kunkel *et al.*, 1986) and cystic fibrosis (Beaudet *et al.*, 1986). However, the application of knowledge derived from the Human Genome Project will be less immediately apparent for complex phenotypes, characterized by high population prevalence, non-Mendelian modes of transmission, and indistinct boundaries between affected and non-affected (Merikangas, in press).

Substance-use disorders are genetically complex disorders that are automatically characterized by gene–environment interaction because exposure to an exogenous substance is necessary for their expression. The tools of epidemiology are suited ideally to examine phenotypes that result from interactions between individual vulnerability factors and environmental exposure.

Figure XVI-9.1 shows the traditional epidemiological triangle of the joint influences of the host, agent and environment in inducing human disease. The methods of epidemiology have been developed to identify the aetiology of particular diseases through simultaneous investigation of each of the three domains depicted in the triangle. This is an excellent model for studying the aetiology of substance abuse: there is a clear *agent*, a drug; numerous characteristics have been demonstrated consistently to confer increased susceptibility of the *host* or individual to drug use, continued use and dependence; and many of the *environmental factors* involved in exposure to drugs have also been identified. The chief goal of the subdiscipline of genetic epidemiology is identification of the genetic and environmental factors underlying human diseases.

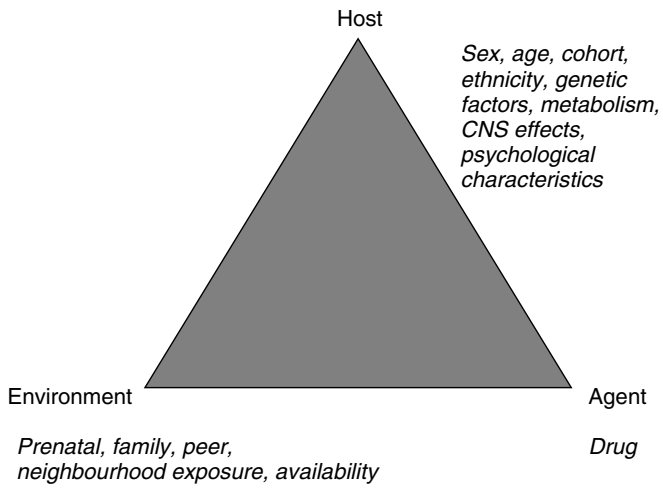

Figure XVI-9.1 Epidemiology of drug abuse

This chapter will: (1) review sources of complexity in identifying genes for complex disorders, (2) review the evidence regarding familial and genetic risk factors for the development of substance-use disorders, (3) review sources of complexity in the aetiology of substance-use disorders, and (4) describe the tools of the discipline of genetic epidemiology that may elucidate the role of genes in the development of substance-use disorders.

CHALLENGES TO IDENTIFICATION OF GENES FOR COMPLEX DISORDERS

Despite advances in characterizing human genotypes, application of this knowledge to human diseases is still limited by the complexity of the process through which genes exert their influence. At present, knowledge of a particular gene does not permit prediction of the phenotype, nor does knowledge of a phenotype permit inferences regarding the genotype, particularly for human behavioural disorders.

A lack of one-to-one correspondence between the genotype and phenotype is clearly the rule rather than the exception for most human disorders. Phenomena such as *penetrance* (i.e. probability of phenotypic expression among individuals with susceptibility gene), *variable expressivity* (i.e. degree to which susceptible individuals express components of genotype), *gene–environment interaction* (i.e. expression of genotype only in the presence of particular environmental exposures), *pleiotropy* (i.e. capacity of gene to manifest simultaneously several different phenotypes) and *genetic heterogeneity* (i.e. different genes leading to indistinguishable phenotypes) have been demonstrated for several human disorders for which susceptibility genes have been identified.

Marfan syndrome is a classic example of pleiotropy. The genetic mutation causing Marfan syndrome has now been identified as fibrillin I, on chromosome 15 (Child, 1997). The diagnostic criteria for Marfan syndrome based on clinical signs require one of the following five conditions: lens dislocation, aortic dilation or dissection, dural ecstasia, skeletal features, plus one other affected system (De Paepe *et al.*, 1996). This single mutation in the genes involved in the production of connective tissue leads to manifestations across multiple organ systems.

Breast cancer provides an illustration of genetic heterogeneity, another major source of genetic complexity. Although family study research is beginning to examine differences in breast cancer among those with and without specific vulnerability genes, the basic breast

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cancer phenotype has not been adequately differentiated. Patterns of comorbidity with other cancers and sex differences have been valuable in discriminating different genetic forms of breast cancer (King *et al.*, 1993). Whereas families with predominantly affected females, or those with both breast and ovarian cancer are more likely to have the BRCA1 mutation (Miki *et al.*, 1994), families with male breast cancer primarily arise from the BRCA2 mutation (Ford *et al.*, 1994). Thus, sex differences in recurrence risk and comorbidity across cancer types may be used to identify more homogeneous forms of cancer.

Gene–environment interaction characterizes a broad range of human diseases such as cancer and birth defects. The classic examples of gene–environment interaction are the inborn errors of metabolism, such as phenylketonuria, that manifest only when susceptible individuals are exposed to a particular protein or exogenous substance. Glucose-6-phosphate-dehydrogenase (G6PD) deficiency, an X-linked disorder caused by mutation on the long arm of the X chromosome, is another illustration of gene–environment interaction. The expression of this disorder becomes manifest as haemolytic anaemia only when the susceptible individual is exposed to certain drugs or fava beans (Cavalli-Sforza and Bodmer, 1971). Birth defects have also been found to result from gene–environment interaction. For example, Yang *et al.* (1998) found that dietary factors mediate the development of familial adenomatous polyposis among mice with genetic susceptibility. Whittemore (1999) presents several examples of gene–environment interaction underlying human cancers.

Not only is the expression of genes modified by the environment, but also there is now substantial evidence to indicate that numerous environmental factors may actually alter the genotype, as is characteristic of many forms of cancer. Francis and colleagues (1999) have shown that maternal behaviour mediates stress reactivity in adulthood and is associated with future maternal behaviour among offspring. Genes may also be involved in the response or resistance to purely environmental agents such as diet, stress, exercise, drugs and nutritional deficiencies (Omenn and Motulsky, 1987). The methods of genetic epidemiology are designed specifically to identify gene–environment interactions (Ottman, 1995; Yang and Khoury, 1997).

Alzheimer's disease (AD) provides an excellent model of a disease with both genetic heterogeneity and gene–environment interaction. There are some major autosomal dominant genes for early onset familial AD with extremely high relative and absolute risk (Slooter and van Duijn, 1997). However, such genes are very rare in the population and have little public health significance. In contrast, there is increasing evidence that the apolipoprotein E (Apo-E) E4 allele is associated with both late-onset familial AD and the more common sporadic AD (Corder *et al.*, 1993; Mayeux *et al.*, 1998; Tsai *et al.*, 1994). Among families at high risk for late-onset AD, disease risk has been shown to increase with the number of E4 alleles (Mayeux *et al.*, 1998). Finally, environmental risks such as head injuries and anti-inflammatory agents have been shown to interact with the apo-E genotypes to protect against or potentiate development of AD (Slooter and van Duijn, 1997).

EVIDENCE FOR ROLE OF GENETIC FACTORS IN THE AETIOLOGY OF SUBSTANCE-USE DISORDERS

Family Studies

The familial aggregation of alcoholism has been well established (for comprehensive reviews of alcoholism see McGue, 1994; Merikangas, 1990). Although there has been less systematic research on the familial aggregation of drug-use disorders, there is increasing empirical research demonstrating that drug-use disorders are also familial (Merikangas, 2000). The results of numerous family history studies, uncontrolled family studies (Croughan, 1985; Gfroerer, 1987; Hill *et al.*, 1977; Meller *et al.*, 1988; Mirin *et al.*, 1988, 1991; Rounsaville *et al.*, 1991) and controlled studies of first-degree relatives of substance abusers (Bierut *et al.*, 1998; Merikangas *et al.*, 1998b) demonstrate consistently that the rates of substance-use disorders are elevated among relatives of drug abusers. An eight-fold increased risk of drug disorders was found among relatives of probands with drug disorders compared with relatives of psychiatric and normal controls (Merikangas *et al.*, 1998b).

High-Risk Studies

Studies of offspring of parents with substance abuse are a subset of family studies that provide information on the order of onset and patterns of transitions across drug categories, as well as on premorbid risk factors for the development of substance abuse. Although there have been several high-risk studies of children of alcoholics (Sher, 1996; Chassin *et al.*, 1991; Hill and Hruska, 1992; Johnson, 1989; Merikangas *et al.*, 1998a; Reich *et al.*, 1993; Schuckit and Smith, 1996), there have been very few controlled studies of offspring of drug abusers. These studies have yielded consistent findings regarding an increased risk of substance-use disorders among offspring of parents with substance abuse or dependence when compared to those of non-substance abusers (Martin *et al.*, 1994; Merikangas, 1998b; Moss *et al.*, 1994).

High-risk studies are particularly informative for prevention efforts as they aid in the identification of premorbid vulnerability factors that serve as sources of identification for children at risk for particular disorders. Aside from genetic factors, there are numerous other mechanisms through which parents may convey an increased risk of substance abuse to their offspring, including serving as negative role models for the use/abuse of drugs and using drugs as a coping mechanism (Brook *et al.*, 1986). Moreover, adolescents with a family history of substance abuse are more likely to associate with deviant peers than those without familial loading (Kandel and Andrews, 1987).

Likewise, nearly all of the high-risk studies reveal that different risk factors may be involved in the different stages of development of substance-use disorders. Whereas individual characteristics and peer influences strongly influence exposure and initial patterns of use of alcohol and drugs, family history and psychopathology play a more salient role in the transition to problematic alcohol use and dependence (Cadoret *et al.*, 1986).

Twin Studies

There have been numerous twin studies that provide evidence that the strong degree of familial clustering of substance-use disorders can be attributed in part to genetic factors. Studies have examined drug use, abuse and dependence in general (e.g. Grove *et al.*, 1990; Jang *et al.*, 1995; Kendler *et al.*, 1999, 2000; Pickens *et al.*, 1991; Tsuang *et al.*, 1998) as well as a diverse range of specific drugs including nicotine, caffeine, tranquillizers, sedatives, cannabis, cocaine, stimulants, hallucinogens and opiates (e.g. Claridge *et al.*, 1978; Gurling *et al.*, 1985; Heath, 1993; Heath and Madden, 1995; Heath *et al.*, 1993; Kendler and Prescott, 1998a; Kendler *et al.*, 1999, 2000; Pedersen, 1981; True *et al.*, 1997, 1999; Tsuang *et al.*, 1998). As described below, the results of these studies have been highly consistent in demonstrating the role of genes in the aetiology of substance abuse and/or dependence. However, the extent of genetic influence differs according to the definition employed, and the age, gender and source of the sample. Although genetic factors are clearly important in the development of substance-use disorders, environmental factors shared between twin pairs have also been

shown to have substantial impact on the development of substance-use disorders (Tsuang *et al.*, 1998).

One of the strongest sources of evidence regarding the role of genetic factors in the aetiology of substance abuse derives from monozygotic twins reared apart. Grove *et al.* (1990) examined the concordance for alcoholism, drug abuse and antisocial personality disorder among monozygotic twin pairs separated at birth. The heritability estimate of drug abuse was 0.45, far exceeding that of alcoholism (0.11).

Adoption Studies

The classic adoption studies of Cadoret and Colleagues (Cadoret, 1992; Cadoret *et al.*, 1986, 1996) have also been highly informative in elucidating the role of genetic factors in the development of drug use and abuse. Although data on biologic parents are often limited with respect to specific patterns of drug use and abuse, their studies provide the strongest evidence to date that genetic factors play an important role in the liability to drug abuse.

Linkage and Association Studies

Although the research reviewed above demonstrates the importance of genetic factors in the development of substance-use disorders, it only provides evidence for the aggregate influences of genes rather than identifying specific genes and the putative mechanisms through which they exert their influence. The two major classes of genes that have been postulated to convey susceptibility to drug-use disorders include those involved in drug metabolism, and those involved in brain reward systems. There has been increasing interest in the genetic control of drug sensitization and tolerance (Phillips *et al.*, 1997). Associations between genes involved in drug metabolism have been evaluated among probands with opioid dependence, cannabis dependence and nicotine dependence as well as alcohol and/or drug dependence in general (Bergen *et al.*, 1997; Comings *et al.*, 1999b; Kranzler and Leibowitz, 1998). Reviews of candidate genes for substance-use disorders are presented by Crabbe and Phillips (1998), Rossing (1998), Enoch and Goldman (1999) and Uhl (1998).

Genes involved in brain reward systems have been the most frequently studied candidate genes for substance-use disorders. There are numerous reports of a possible association of D_2 dopamine receptor gene polymorphisms and genetic susceptibility to either drug dependence in general or to specific drugs, such as opiates or nicotine (Amadeo *et al.*, 1993; Arinami *et al.*, 1993; Blum *et al.*, 1990, 1991, 1993; Comings *et al.*, 1991; Lerman *et al.*, 1999; O'Hara *et al.*, 1993; Parsian *et al.*, 1991; Smith *et al.*, 1992; Sabol *et al.*, 1999). In contrast to these reports, multiple investigators have found no differences in allele frequencies between abusers and controls (Berrettini and Persico, 1996; Bolos *et al.*, 1990; Cook *et al.*, 1995; Gelernter *et al.*, 1991; Goldman *et al.*, 1992; Schwab *et al.*, 1995; Suarez *et al.*, 1994; Turner *et al.*, 1992). Other candidate genes involving the dopamine receptor or transporter systems have also been examined: DRD3 (Duaux *et al.*, 1998), DRD4 (Bergen *et al.*, 1997; Comings *et al.*, 1999a; Duaux *et al.*, 1998) and DRD5 (Vanyukov *et al.*, 1998). Finally, a variety of other candidate systems have been reported in substance-dependent probands, including the cannabinoid receptor gene (Comings *et al.*, 1997) and the Proenkephalin gene (Comings *et al.*, 1999b). Other systems underlying brain reward will also be important candidates for future studies. For example, the role of substance P in the rewarding properties of morphine has recently been reported (Murtra *et al.*, 2000).

There are numerous methodologic issues that are critical to the interpretation of the significance of the findings of this research. First, the wide variation in the ethnic distributions of these systems, particularly with respect to the dopamine metabolism genes, has rarely been considered in association studies (Barr and Kidd, 1993). This problem of ethnically based allele frequency differences is minimized through application of association studies using parental genotypes and/or unaffected sibling genotypes as controls in the analyses (Falk and Rubenstein, 1987; Kidd, 1993; Risch and Merikangas, 1996). Second, many of the findings implicate genes that do not appear to have functional relevance (Paterson *et al.*, 1999). Third, purported replications have resulted from very different phenotypic definitions (i.e. frequency of use versus ability to quit). Thus, although the genetic epidemiologic evidence (from twin, family and adoption studies reviewed above) is consistent with a heritable component to substance dependence, to date there has been limited success in identifying human susceptibility genes that have been replicated across samples and centres.

Significant progress in animal studies has provided a knowledge base that will ultimately be critical to the identification of human genes for drug dependence. Research using inbred strains, selected lines and recombinant inbred strains provides critical information about the relevance of a particular gene product that may be involved in the pathogenesis of substance-use disorders. Information derived from congenic strains, transgenic mice, knockout and inducible knockout mice could lead to better medical intervention for drug addiction. Reviews of this research are presented by Crabbe and Phillips (1998), Pich and Epping-Jordan (1998), Crabbe *et al.*, 1999. However, the application of information gleaned from animal research is still problematic because few animal models are adequate representations of human patterns of drug use and or dependence. Moreover, these models fail to account for the pervasive human tendency to use multiple drugs both concomitantly as well as historically. Nevertheless, such studies are extremely valuable in providing information regarding the genetic systems underlying metabolism and the effects of specific substances.

SOURCES IN COMPLEXITY OF GENETICS OF SUBSTANCE-USE DISORDERS

Table XVI-9.1 presents some of the key issues that lead to complexity in examining the role of genetic factors in the aetiology of substance-use disorders. Each of these issues is described below.

What is the Phenotype?

Substance-use disorders emerge from a series of complex processes that are difficult to capture using the currently available measurement tools. Different risk factors are associated with different levels of progression of drug use. One of the major impediments to summarizing the results of family and twin studies is the use of different definitions and components of drug-use behaviour. For example, twin studies of smoking have examined diverse components of smoking, including use, frequency, quantity, age at onset,

Table XVI-9.1 Factors related to the complexity of genetics of substance-use disorders

- Definition of the phenotype
- Sex differences
- Age
- Cohort and generation effects
- Environmental exposure
- Spouse concordance
- Polysubstance use/dependence
- Comorbidity with psychopathology

regular use, continued use, current use, current frequency, dependence, severity and ability to quit (Heath and Madden, 1995). Many studies have employed a few items regarding drug use on self-reported questionnaires, whereas others used structured diagnostic interviews that collected full diagnostic criteria for substance abuse and dependence. Van den Bree *et al.* (1998a, b) present a summary of the results of twin studies of drug abuse according to a wide range of definitions. They found that the heritability was greater for clinical diagnoses of abuse/dependence than for use.

In the first twin study using diagnostic criteria for drug abuse and dependence, Pickens *et al.* (1991) reported that approximately 35% of the variance in substance-use disorders was attributable to genetic factors. Moreover, the heritability for drug dependence was significantly greater than that for abuse. Additional analyses of these data by McGue *et al.* (1994) revealed far greater heritability of drug-use disorders among males with early age of onset compared to either those with later age of onset or females. Likewise, Tsuang *et al.* (1998) found that the contribution of genetic factors was greater for frequent use or abuse than for non-problematic use of drugs.

The aggregate twin study data are remarkably consistent in demonstrating that genetic factors play a far greater role in the aetiology of more severe patterns of drug use, particularly that which meets diagnostic criteria for abuse or dependence, than initial use or early stages of use, which appear to be more strongly determined by environmental influences. With respect to specific substances, there is a growing body of literature on the role of genetic factors for tobacco, cannabis and cocaine use and dependence.

Evidence regarding the role of genes in tobacco use has been derived almost exclusively from twin studies. Summaries of twin studies of smoking provided by Heath *et al.* (1993), Heath and Madden (1995), True *et al.* (1999) and Kendler *et al.* (1999) reveal that the proportion of variance in regular tobacco use attributable to genetic factors (i.e. heritability) is about 50%, whereas shared environmental factors explain an additional 25%. Studies of twins reared apart have yielded inconclusive findings. Kaprio *et al.* (1984) found no significant difference between monozygotic (MZ) and dizygotic (DZ) twins reared apart (proband-wise concordance rates of 0.67 and 0.68, respectively), whereas for twins reared together, the concordance rates were greater among MZ twins (0.91) than for DZ twins (0.67). However, these findings differed dramatically among male and female twin pairs, and by definitions of smoking. In a more recent and larger study, Kendler *et al.* (2000) found weak evidence for a genetic effect for lifetime cigarette use in males, but not in females.

Data on female twins from the Virginia Twin Study (Kendler and Prescott, 1998b) revealed that genes had a moderate influence on cannabis use among the female twins, but far stronger impact on heavy use and abuse of cocaine (Kendler and Prescott, 1998a). Likewise, the heritability of cocaine abuse and dependence was greater (MZ and DZ proband-wise concordance rates of 47% and 8%, and 35% and 0%, respectively) than that of cocaine use (proband-wise concordance of 54% and 42%) (Kendler and Prescott, 1998b). With respect to other substances, their results indicated that opiate and sedative use were solely attributable to common genes, whereas hallucinogen and stimulant use resulted from both family environment and genes (Kendler *et al.*, 1999). A recent innovative analysis of the Vietnam Era Twin Registry examined the role of genetic influences in transitions from use to abuse of a range of substances, as well as transitions across particular substances. The rate of transition from use to regular use was greatest for heroin, whereas cocaine had the highest conditional probability for the transition from regular use to dependence.

The adoption study paradigm has been applied to study aggregate drug abuse and its association with antisocial personality. The results of adoption studies also confirm the stronger impact of genes on more severe patterns of substance use. Cadoret *et al.* (1992) found that exposure to a sibling or peer with deviant behaviour appears to contribute to drug use, but not to abuse. None of the adoption studies have thus far been able to detect a gene–environment interaction in drug initiation or in the transition from use to abuse (Cadoret, 1992).

Sex Differences

Substance-use disorders are more common in males than in females. Based on the rates from Epidemiologic Catchment Area Study, men are five times as likely to have an alcohol-use disorder and two to three times more likely to have a drug-use disorder as compared to women (Anthony and Helzer, 1995). Likewise, the National Comorbidity Survey (Kessler *et al.*, 1997) found that substance-use disorders were more prevalent among men than women, with a greater difference for alcohol dependence than drug dependence.

Gender differences in the contribution of familial and genetic factors have also been reported. The results of the Yale Family Study of comorbidity of substance-use disorders and psychopathology demonstrated differential familial transmission of alcohol abuse and dependence among male and female relatives. Whereas female relatives of probands with alcohol dependence had elevated rates of both alcohol abuse and dependence, male relatives of affected probands only exhibited an elevation in rates of alcohol dependence (Merikangas *et al.*, 1998b).

With respect to twin studies, there is little evidence regarding gender differences because of the lack of large-scale twin studies of drug-use disorders in samples with both male and female twin pairs. Pickens *et al.* (1991) found that although both male and female MZ twin pairs had a 1.5-fold increased risk of drug abuse compared to DZ pairs, the concordance for males could be attributed to both shared genes and environmental factors, whereas for females the majority of variance was attributable to the unique environmental experiences of individual twins. Likewise, Van den Bree *et al.* (1998b) found that genetic influences on drug abuse/dependence were generally stronger for males than for female twins. Although the twin studies of Kendler *et al.* are the largest to date, the analyses of the male and female twin samples have been conducted independently. Independent analyses of male and female twin series yielded some sex differences in heritability; there was less genetic influence on cocaine abuse and dependence in males (Kendler *et al.*, 2000) than in females (Kendler and Prescott, 1998a). However, the small numbers of pairs from these population-based samples diminish their value in evaluating the role of genetic and environmental factors in dependence of any one class of drugs.

Age

Twin studies have also begun to examine the role of genetic factors in the development of substance-use disorders in prospective samples of children and adolescents. These studies may inform our understanding of the influence of age and developmental level in the aetiology of substance-use disorders. Since exposure to drugs generally occurs during early adolescence, the above-cited data derived from adult samples is necessarily susceptible to the biases of all retrospective studies. Whereas a study of adolescent twins from the Netherlands (Boomsma *et al.*, 1994) yielded evidence for nearly equal impact of genetic and social influences on smoking among adolescent twins, Maes *et al.* (1999) reported substantial heritability for both lifetime (82%) and current (84%) tobacco use in the Virginia Twin Study of Adolescent Behavioural Development. In contrast, in the latter study, use of other drugs such as cannabis was more strongly attributable to shared environmental factors than

to genes. In the Netherlands sample, the heritability of alcohol use increased with age, whereas shared environmental factors had stronger impact in early adolescence (Boomsma *et al.*, 1994). Follow-up of these samples will elucidate the joint influence of genetic and environmental factors on persistence and dependence as these cohorts pass through the risk period for the development of tobacco dependence.

Recent twin studies of adolescents have also examined the heritability of cannabis use in adolescent twins (Miles *et al.*, 2001). Both genetic and environmental factors were found to contribute equally to cannabis use in both male and female twin pairs.

Cohort and Generation Effects

One key source of complexity in studying the familial transmission of drug abuse is the dramatic changes in patterns of drug use in the general population, as well as within specific subgroups. Rapid shifts in the availability and cultural and geographic patterns of specific drugs impede traditional inspection of vertical patterns of concordance for drug use and dependence. Whereas alcohol has been readily available during the past several decades, and cannabis has been somewhat stable as well, crack cocaine and ecstasy have only been widely available during the past decade. Ironically, nicotine use has been decreasing rapidly in more recent cohorts. These differences in availability and cultural norms make it difficult to discriminate exposed but unaffected relatives from those who were never exposed to a particular drug. Within-generation comparisons are therefore more likely to control for exposure to specific substances. However, siblings are often responsible for the initiation of other siblings to drugs, as well. This could lead to an overestimation in the heritability of drug use.

Evaluation of the family and twin study data by generation of the relative with respect to the proband (i.e. parent, sibling, offspring) reveal that both drug use (Gfroerer, 1987) and abuse/dependence (Bierut *et al.*, 1998; Merikangas *et al.*, 1992, 1998b) are particularly elevated among siblings of drug abusers when compared to parents and offspring. There is also a direct relationship between parental drug use (Gfroerer, 1987) and abuse (Luthar *et al.*, 1992; Merikangas *et al.*, 1992) and use and abuse in offspring.

Environmental Influences

With respect to parental drug use, few genetic epidemiologic studies have considered the effect of foetal exposure to substances on the susceptibility to drug-use disorders. Differences between exposed and unexposed youth could either be a product of the exposure or of genetic or familial factors associated with maternal drug use. For example, Yates *et al.* (1998) reported that foetal alcohol exposure may be associated with an increased risk for later nicotine, alcohol and drug dependence, beyond the effects of genetic factors associated with maternal drug abuse. Although twin studies can distinguish between environmental influences that are common to twins from those that are unique, they do not provide information on specific environmental exposures without being specifically designed to identify sources of discordance.

Spouse Concordance

The tendency for spouses to be concordant for substance use is another issue that must be integrated in evaluation of genetic evidence. Several studies have shown high levels of spouse concordance for drug use (Merikangas *et al.*, 1992; Price *et al.*, 1982). For example, Merikangas *et al.* (1992) reported that more than 90% of opioid-dependent proband's spouses had a history of opioid dependence themselves. Furthermore, these investigators showed that there is a strong association between rates of drug abuse in adult siblings of opioid abusers and the number of parents with substance abuse. It is therefore critical that spouse concordance be incorporated in genetic analyses of substance abuse.

Polysubstance Use

Another important factor in examining the role of genetic factors in drug-use disorders is the tendency for substance abusers to abuse multiple substances, both simultaneously as well as longitudinally (Merikangas *et al.*, 1998b; Mirin *et al.*, 1991). One twin study of substance use revealed a moderate degree of heritability for the frequency of use and the tendency to use numerous illicit substances ($h^2 = 0.32$) (Jang *et al.*, 1995).

Surprisingly, there is little evidence for the cross-transmission of drug disorders and alcoholism (Merikangas *et al.*, 1998b). The results of several uncontrolled family studies suggest that alcohol and drug dependence aggregate independently in families (Hill *et al.*, 1977; Luthar *et al.*, 1992; Meller *et al.*, 1988; Mirin *et al.*, 1991; Rounsaville *et al.*, 1991). Likewise, a controlled family study of both drug abuse and alcoholism found that alcoholism did not modify the association between drug disorders in probands and relatives and vice versa (Merikangas *et al.*, 1998b). In contrast, the results of a large twin study of males revealed common genetic vulnerability to nicotine and alcohol dependence (True *et al.*, 1999).

The *specificity* of familial aggregation of particular types of substance dependence has been examined in both family and twin studies (Bierut *et al.*, 1998; Merikangas *et al.*, 1998b; Tsuang *et al.*, 1998). The results of these studies yield remarkably similar trends towards specificity of aetiologic factors for particular drugs. Data from the Yale Family Study of Comorbidity of Substance Abuse and Psychopathology examined the specificity of familial aggregation of the predominant drug of abuse among adult relatives of probands classified likewise. The results revealed a remarkable degree of specificity for familial aggregation of opiates, cannabis and alcohol, and to a lesser extent for cocaine (Merikangas *et al.*, 1998b); there was an 8-fold increase in opioid dependence among relatives of opioid-dependent probands, and a 2-fold increase in cocaine dependence among relatives of probands with cocaine dependence (Table XVI-9.2). In a study of siblings of alcohol-dependent probands, Bierut *et al.* (1998) found an approximately 1.8-fold increase in rates of nicotine, cocaine and cannabis dependence, respectively, among relatives of probands with each of these types of dependence. Patterns of concordance for specific drugs in twins from the Vietnam Era Twin Registry (Tsuang *et al.*, 1998) also revealed a significant degree of specificity. In summary, the aggregate findings of the twin and family studies provide evidence for common familial and genetic factors underlying substance-use disorders in general, as well as substantial components that are unique for specific drugs.

Table XVI-9.2 Yale family study of comorbidity of drug-use disorders: specificity of familial aggregation of predominant drug-use disorder

	Relatives (%)			
Probands	Opioid	Cocaine	Cannabis	Alcohol
Opioid	10	4	6	20
Cocaine	2	8	5	22
Cannabis	6	2	13	20
Alcohol	2	3	4	29
Controls	1	1	2	11

Source: Merikangas *et al.*, 1998a.

Comorbidity with Psychopathology

The comorbidity between substance use and psychiatric disorders has been well documented by numerous studies. Ross *et al.* (1988) found that 78% of their treatment sample of patients with substance-use disorders met DSM-III criteria for a lifetime comorbid psychiatric disorder. Specific non-drug related disorders suffered by substance abusers include affective illness, anxiety disorders, antisocial personality disorder, and attention deficit disorder (Khantzian, 1983; Khantzian and Treece, 1985; Kleber and Gold, 1982; Mirin *et al.*, 1984, 1988; Quitkin *et al.*, 1972; Rounsaville *et al.*, 1982; Weiss *et al.*, 1985).

The adoption studies of Cadoret and Colleagues (Cadoret, 1992; Cadoret *et al.*, 1986, 1996) also examined links between antisocial personality in biological parents and drug abuse in offspring. Their findings identify two major biological/genetic pathways to the development of drug abuse in adoptees: one which is driven by substance abuse in the biological parent and is limited to drug abuse and dependence in the adoptee, and another which appears to be an expression of underlying aggressivity and related to criminality in the biological parent (Cadoret *et al.*, 1995, 1996). A study of separated twins confirmed these findings by demonstrating that a large proportion of the heritability of substance abuse in adulthood can be attributed to shared genetic factors that underlie the development of behaviour problems in childhood (Grove *et al.*, 1990).

Several family studies of drug-dependent probands have also examined the effects of comorbid disorders on familial aggregation of both substance-use disorders and other psychiatric disorders. Most of the family studies have found increased rates of all major disorders among relatives of substance abusers; however, most have been uncontrolled, and few have accounted for comorbid disorders within the probands (Croughan, 1985; Mirin *et al.*, 1991; Rounsaville *et al.*, 1991). The results of the family studies have demonstrated consistently that there is independent familial aggregation of antisocial personality disorder and drug-use disorders (Rounsaville *et al.*, 1991). A controlled family study designed specifically to study the co-aggregation of substance-use disorders and psychopathology also demonstrated independence of familial aggregation of most disorders, including affective, anxiety, antisocial personality and other substance-use disorders (Merikangas and Stevens, 1998).

Twin studies may provide information on the extent to which familial correlations between disorders result from shared genetic or familial factors. For example, Lin *et al.* (1996) compared the familial versus non-familial links between major depression with alcohol-use disorders and drug-use disorders. They concluded that whereas comorbidity between major depression and alcohol-use disorders resulted from common familial factors, comorbidity with drug-use disorders was attributable to non-familial factors. The most widely investigated disorder associated with the familial association with substance abuse is antisocial personality disorder. The results of family studies concur that the two disorders aggregate independently in families, despite the high magnitude of comorbidity at the individual level (Merikangas *et al.*, 1998c). Scherrer *et al.* (1996) and True *et al.* (1999) found that antisocial personality disorder and cannabis abuse were not genetically related.

The sources of complexity described above will require substantial research integrating numerous disciplinary approaches. Rapid advances in human neuroscience, pharmacology, pharmacogenetics and preclinical research should be integrated into the genetic epidemiologic research summarized herein. Below is a description of the future directions in the field of genetic epidemiology that are likely to advance our understanding of the role of both genetic and environmental factors and their interaction in producing substance-use disorders.

GENETIC EPIDEMIOLOGY: MOVING INTO THE COMMUNITY

> Although experimental species are of great value for the initial identification and functional analysis of complex disease genes, final evidence for the involvement of these genes in human diseases must come from extensive epidemiological studies, preferably in different populations.
>
> (Peltonen and McKusick, 2001)

In a recent editorial in *Science*, these two prominent geneticists conclude that the best strategy for gene identification will ultimately be large epidemiologic studies from diverse populations. The importance of epidemiology in the future of genetics has also been described by others such as Risch (2000) and Khoury and Yang (1998), who predict that population-based association studies will assume increasing importance in studying the role of genetic risk factors in human complex diseases. The concept of *heritability*, a purely statistical phenomenon, will be replaced by the concepts of relative risk to individuals and attributable risk in populations. Population-based epidemiological studies will be necessary to calculate the attributable, relative and absolute risk of genetic risk factors identified in family-based linkage and association studies. Moreover, they anticipate that population-based case-control studies will have an increasing role in the genome-wide search for susceptibility genes for complex human disorders.

The chief goal of the subdiscipline of genetic epidemiology is identification of the genetic and environmental factors underlying human diseases. There is increasing interest in regenerating the field of genetic epidemiology, which is distinguished from its parent disciplines in three specific ways: (1) its focus on population-based research, (2) its goal of detecting the joint effects of genes and environment, and (3) the incorporation of underlying biology of a disease into conceptual models (Thomas, 2000). Unfortunately, the field of genetic epidemiology has become almost exclusively focused on statistical methods for identification of genes, as witnessed by recent trends in the journal *Genetic Epidemiology*. As such, epidemiology has been detached from the field; in fact, the majority of scientists who perceive themselves as genetic epidemiologists have never had any formal training in epidemiology, since they often equate epidemiology solely with community-based sampling.

The major applications of genetic epidemiology in order to advance our understanding of mental disorders are: (1) establishment of *population-based registries* of mental disorders that will be increasingly valuable in validating the numerous genetic tests that will emerge from advances in human genetic research and the Human Genome Project (Yang *et al.*, 2000), (2) identification of *more homogenous subtypes* of mental disorders through family and high-risk research investigating both biologic and contextual factors, (3) investigation of familial patterns among affected and unaffected probands to *estimate strength and mode of genetic transmission*, (4) *quantification of risk* at the levels of the individual and population (i.e. absolute risk, relative risk, attributable risk), and (5) development of a richer conceptualization of *environmental factors* that may be important mediators of expression of genetic risk for mental disorders through integration of the tools of genetic epidemiology, behavioural neuroscience, developmental psychology and neuroscience.

SUMMARY

This review has summarized the results of family, twin, adoption and high-risk studies that concur in concluding that both drug use and abuse are highly familial. In fact, a family history of alcohol or

substance-use disorder is the most potent and consistent risk factor for the development of substance-use disorders in offspring. The results of twin and adoption studies demonstrate that genetic factors underlie a substantial component of the familial clustering of drug-use disorders. However, in contrast to twin studies of numerous other disorders, the common family environment appears to have a significant impact on the development of drug-use disorders. Drug dependence is far more heritable than either drug use or abuse, and genetic factors appear to be more important in the transmission of problematic drug use rather than drug use *per se*. Comparisons of twin studies of youth with those of adults suggest that heritability is far greater among adults with established patterns of substance use than during its early stages of development. With respect to comorbidity, the results of family studies suggest that drug-use disorders aggregate independently from the affective, anxiety, behaviour, and other substance-use disorders. However, there may be independent pathways to the development of substance-use disorders through psychopathology.

Additional genetic epidemiological research is critical for elucidating the role of genetic and environmental factors in the transmission of substance abuse, validating phenotypic definitions of substance use/abuse, and identifying sources of heterogeneity in the aetiology of substance abuse, particularly with respect to the role of comorbid psychiatric disorders and polysubstance abuse.

The Human Genome Project is expected to facilitate identification of genes and, more importantly, understanding of their role in human diseases, with the ultimate goal of developing successful approaches to prevention and treatment (Collins *et al.*, 1988). This may be relevant to the field of substance abuse in several ways. First, application of genetic case-control studies may identify malleable environmental risk factors that may inform prevention efforts. Second, genetic research on drug metabolism may assist in the development of drugs that block craving or rewarding effects of drugs. Third, closer collaboration between basic research and the fields involved in the research described in this review will enhance our ability to understand the pathophysiology of drug abuse and hence to develop more informed prevention and cessation programmes.

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XVI-10

Gender Issues in Substance Abuse

W.R. Yates

INTRODUCTION

Both men and women share a significant risk for the development of substance abuse. Although men and women share many common features in vulnerability, natural history and outcome, gender-specific issues play an important role in the clinical features of substance abuse (Valliant, 1983). An example of a unique gender issue is the diagnosis, treatment and adverse fetal effects of substance abuse in pregnant women. Another unique gender-specific clinical issue would be the effect of specific illicit substances on spermatogenesis.

This chapter will focus on some of the important clinical issues related to gender in substance abuse. Although the level of scientific knowledge in women trails that in men for a variety of reasons, this review will focus on well-designed studies where gender-specific information is available. Since alcoholism is the most widely studied of the substances of abuse, this chapter will have a predominance of information related to alcohol. This reflects only the state of knowledge and should not be interpreted as minimizing potential gender issues for nicotine or illicit substances of abuse.

EPIDEMIOLOGY: PREVALENCE RATES

Environmental Catchment Area Study (US)

The ECA study conducted in the early 1980s was the largest population-based estimate of the prevalence of psychiatric illness in the USA (Regier *et al.*, 1990). Gender comparisons for alcohol and drug diagnoses confirmed a male predominance in both categories. The 6-month prevalence rate for alcohol abuse or dependence ranged from 8.2% to 10.4% for men compared to 1.0% to 1.9% for women. For drug abuse or dependence, the 6-month prevalence rates ranged from 2.5% to 3.0% for men and 1.2% to 1.6% for women.

National Comorbidity Study (NCS) (US)

The NCS examined a population-based sample of adults between 15 and 54 in the USA (Kessler *et al.*, 1997). Lifetime and 12-month prevalence rates for drug abuse and dependence (DSM-IIIR) were compared. Additionally, data regarding drug use was collected for gender comparisons. A significant cohort effect for both genders was noted in this study — cohorts of both men and women born later had higher rates of drug use, and higher rates of drug dependence diagnosis. Twelve-month prevalence rates for alcohol abuse among men was 3.4% (SE 0.4%) and among women was 1.6% (SE 0.2%), the alcohol dependence rate among men was 10.7% (SE 0.9%) and among women was 3.7% (SE 0.4%), the rate for drug abuse was 1.3% (SE 0.2%) for men and 0.3% (SE 0.1%) among women; for drug dependence the rate among men was 2.3% (SE 0.4%) and for women was 1.3% (SE 0.8%). This study confirms others noting the rate of alcohol diagnoses is 3–6 times higher in men than women and the rate of drug diagnoses is 1.5–3 times higher.

EPIDEMIOLOGY: RISK FACTORS

Genetic

Some early studies of the role of genetic influences on the risk of developing substance abuse suggested genetic factors were more important for men than for women. However, later more sophisticated studies using large samples have shown strong genetic influences for alcohol abuse and dependence in both males and females. In a twin study of over 9000 male and female twins, genetic influences were felt to account for between 55% and 66% of the liability for alcohol abuse and dependence in women and for between 51% and 56% of the liability for men. Women and men appear to share some genetic sources of risk, although there do appear to be some gender-specific genetic risks also in play.

Depression prevalence rates are higher in women and alcohol abuse/dependence prevalence rates are higher in men. This sex-specific prevalence pattern produced speculation that a common genetic vulnerability could be expressed as depression in women and as alcohol abuse or depression in men. However, a recent twin study focusing on this issue did not supported this hypothesis (Prescott *et al.*, 2000). This study found that depression and alcoholism appear to have some common genetic and environmental risks. However, these risks appear sex-specific and the factors that predispose to depression in women do not appear to be the same factors increasing the risk for alcoholism in men.

ENVIRONMENTAL FACTORS

Psychiatric Comorbidity

Antisocial personality disorder appears to have a strong relationship with the development of alcohol and drug use problems in both men and women. Since antisocial personality disorder includes conduct disorder symptoms before age 15, this psychiatric illness is one likely to predate significant alcohol or substance abuse diagnoses. A family study of alcoholism examined the risk of alcohol dependence based on latent class analysis of antisocial personality symptom data (Bucholz *et al.*, 2000). Conduct disorder symptom severity strongly correlated with risk for alcohol dependence in both men and women. This study supports identification of conduct

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disorder symptoms in both boys and girls and may identify a high-risk group appropriate for substance abuse/dependence prevention interventions.

The ECA study documented a strong association between alcoholism and antisocial personality for both men and women. Prevalence ratios for psychiatric comorbidity were calculated for a variety of psychiatric disorders. The prevalence ratio is the prevalence of a psychiatric condition in those with alcoholism versus those without. For women, the prevalence ratio for antisocial personality disorder was 29.6, more than twice the prevalence ratio for men at 12.0. Women and men with alcoholism demonstrated comparable prevalence ratios for a variety of other DSM-III disorders in the ECA. The prevalence ratios for women were: mania 9.3, drug abuse/dependence 8.8, schizophrenia 5.6, panic disorder 4.4, major depression 2.7, dysthymia 2.2 and obsessive compulsive disorder 2.1.

Similar associations between drug abuse/dependence and psychiatric illness were noted in the ECA study. Antisocial personality disorder had a prevalence ratio of 26.6 for women with drug abuse or dependence compared to 10.6 for men. Prevalence ratios for other psychiatric illnesses were comparable between men and women. For women, the prevalence ratios for psychiatric comorbidity were mania 11.1, alcohol abuse/dependence 9.0, schizophrenia 6.4, major depression 3.6, obsessive compulsive disorder 3.5, dysthymia 3.1 and panic disorder 2.9.

These associations underscore the need for psychiatric assessment and treatment for substance abuse treatment populations for both men and women. Additionally, the ECA findings support assessment and treatment of substance abuse problems for women in forensic and correctional settings.

NATURAL HISTORY

Differences in drinking patterns appear consistently in cross-cultural study of the effects of gender (Wilsnack *et al.*, 2000). The rates of current drinking versus abstinence from alcohol are similar between men and women. However, men drink more frequently, drink larger quantities of alcohol, have more heavy drinking days and suffer more total number of adverse consequences from drinking. However, when quantity of alcohol consumed corrected for body weight is controlled, the difference in severity of drinking consequences between male and female drinkers often disappears.

Table XVI-10.1 summarizes some of the clinical features of alcoholism for women compared to men. In studies of clinical populations, women report a later onset of regular and heavy drinking. However, despite this later onset of drinking, women drinkers report a shorter period of time from heavy drinking until the first alcohol problem. This phenomenon has been described as telescoping and has been reported for drugs other than alcohol.

Figure XVI-10.1 demonstrates this telescoping phenomenon in a series of men and women referred from a medical service for alcoholic cirrhosis. This clinical study examined 100 patients with a clinical or biopsy-proven diagnosis of alcoholic cirrhosis. Alcohol histories showed that women with cirrhosis reported a later age of onset for first episode of intoxication and regular drinking. Age of first alcohol problem was reported earlier in women compared to men. The period of time from regular alcohol use until first alcohol problem was shorter in women (12.6 years vs. 18.0 years).

The ECA study documented a trend for fewer women to report alcohol abuse or dependence symptoms than men. Additionally, women with alcohol symptoms tended to have fewer numbers of symptoms compared to men. Ninety per cent of women in the ECA study reported no alcohol symptoms, compared to only 40% of men. Only 3% of women reported three or more alcohol symptoms, compared to approximately 9% of men in the study.

Table XVI-10.1 Clinical features of alcoholism in women compared to men

Women with alcohol problems demonstrate:
Later age of onset of regular and heavy drinking
Shortened time from onset of drinking to problem drinking
Greater rates of drinking at home alone
Variability in drinking related to menstrual cycle (greater use in premenstrual period)
Greater likelihood to use alcohol to regulate emotions
Higher rates of eating disorder comorbidity
Higher rates of history of physical and sexual abuse
Higher risk for development of alcoholic cirrhosis
Higher risk for fibrosis and cirrhosis from hepatitis C virus
Greater disruption in family life function
Greater rates of divorce and separation
Higher rates of referral to treatment by physicians due to medical problem
Greater rates of liver disease corrected for alcohol unit exposure
Higher short-term treatment response rates
Lower long-term treatment response rates
Higher correlation of outcome with outcome of drinking spouse

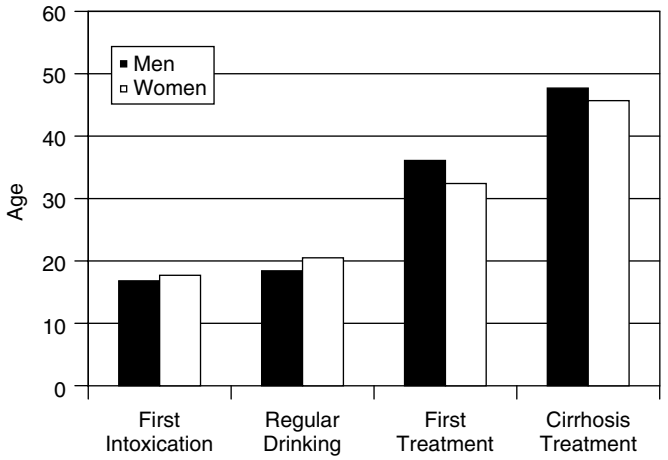

Figure XVI-10.1 A series of men and women with alcoholic cirrhosis were interviewed on the chronology of their alcoholism. This figure demonstrates a later onset of drinking but earlier treatment for alcoholism and cirrhosis

COMPLICATIONS

Alcohol

Many of the medical consequences of chronic alcohol appear very similar between men and women. However, women appear to be more sensitive to the effects of alcohol on the liver. Rates of alcoholic cirrhosis are higher for women than for men with similar drinking histories corrected for body weight. Women significantly increase their risk for cirrhosis by consuming 7–13 beverages per week. To assume a similar increase in risk for men requires 14–27 beverages per week. This gender difference is recognized by a variety of guidelines that have been published for safe drinking limits based on gender.

Table XVI-10.2 Gender-specific recommended daily and weekly standard drink units guidelines for moderate drinking

	Daily		Weekly	
Agency/Author	Men	Women	Men	Women
AHC	6	4	28	14
UKRC			21	14
NIAAA			7	7
Sanchez-Craig	4	3	16	12

AHC, Australian Health Council; UKRC, United Kingdom Royal College; NIAAA, National Institute on Alcoholism and Alcohol Abuse. Sanchez-Craig *et al.* (1995).

Table XVI-10.2 summarizes some of the major published guidelines for moderate drinking for men and women. These guidelines have taken several factors in to account. Since blood alcohol concentrations are proportional to body weight, it would be expected that drinking guidelines for men would exceed those for women by 20–30%. However, due to the specific hepatic sensitivity of alcohol for women, most guidelines recommend a 30–50% reduced quantity for women. The guidelines vary by significant amounts, pointing to the need for further study of this issue. The Australian Medical Council supports the most liberal definition of moderate drinking, supporting a limit of 28 drinks per week for men and 14 drinks per week in women. The National Institute for Alcoholism and Alcohol Abuse in the USA supports the most stringent guidelines, with a recommendation for a seven drinks per week limit for both men and women.

Most guideline recommendations have developed by expert consensus after comprehensive literature review. Sanchez-Craig *et al.* (1995) completed one of the few empirical studies of the quantity of drinking and specific problems for men and women. This study support gender-specific guidelines. Based on an analysis of positive and negative predictive value, sensitivity and specificity, the Sanchez-Craig *et al.*, study found 16 weekly drinks for men and 12 week drinks for women to be the best cut-off.

Several of the drinking guideline recommendations endorse daily drinking limits as well as weekly consumption. Although weekly consumption frequently best approximates the potential medical complications of drinking, daily drinking quantities approximates the highest blood alcohol concentrations associated with behavioural complications from drinking. Daily drinking limits approximate the quantities of alcohol required to reach the legal limits of intoxication. In the Sanchez-Craig study, four drinks for men and three drinks for women were recommended as daily limits.

Hepatitis C is a common viral illness that effects the outcome of alcoholic liver disease. Alcohol use in conjunction with hepatitis C increases the risk and rate for the development of fibrosis and cirrhosis. Some studies suggest that women are particularly sensitive to the effects of alcohol on progression of hepatitis C infection (Logueriicio *et al.*, 2000). Additionally, it appears that women may be more sensitive to the toxic effects of ethanol on peripheral nerve fibres.

Menstrual Cycle Factors

Women face a unique clinical feature: the effect of menstrual cycle on substance use and substance effects. Premenstrual symptoms and premenstrual dysphoric disorder may influence the risk of substance use. A study of 2912 women serving in the military examined the relationship between premenstrual symptoms and substance use (Kritz-Silverstein *et al.*, 1999). Premenstrual symptoms and menstrual irregularities correlated with current cigarette smoking. Current smokers were more likely to report problems with severe menstrual cramps and bleeding irregularities. Alcohol consumption did not correlate with menstrual problems.

Pregnancy-Related Morbidity

Female alcohol and drug use during pregnancy represents a gender-specific public health challenge. A US survey of 4 million women who gave birth found that 757 000 women drank alcohol during pregnancy and 820 000 women smoked cigarettes. Other drug use was reported by an additional 221 000 women. Marijuana was the leading drug, being used by 119 000 women, with 45 000 women reporting cocaine use. Fetal effects of drug use relate to the dosing and timing factors. Heavy daily use is likely to represent the largest risk. Heavy use during the first trimester of pregnancy may increase the risk of developmental abnormalities. Heavy use during the last trimester of pregnancy may result in a neonatal substance withdrawal syndrome.

One effect of pregnancy-related alcohol or drug use may be an increased risk for adult substance abuse in the exposed fetus. A study of adoptees examined the role of fetal alcohol exposure on later development of the adult symptoms of nicotine, alcohol and drug dependence (Yates *et al.*, 1998). In this study, maternal alcohol consumption resulted in a lower birth weight (−377 g). Possible genetic confounding effects were controlled in this study by comparing mothers with and without an alcohol dependence diagnosis who did and did not consume alcohol during the index pregnancy.

Alcohol exposure during pregnancy in this study produced a unique added risk for adult symptoms of nicotine, alcohol and drug dependence. This increases the importance of public health efforts to identify and reduce alcohol consumption during the course of pregnancy.

The effect of substances other than alcohol on the developing fetus is less clear. Early studies of the effects of prenatal cocaine exposure on the fetus suggested significant teratogenic and developmental effects. However, a recent review (Frank *et al.*, 2001) failed to find specific effects of cocaine on physical growth, cognition, language skills or motor skills. Prenatal cocaine may have subtle effects on later attentiveness and emotional expressivity. Studies of the prenatal effects of specific drugs of abuse are hampered by the need to control for confounding variables such as prenatal exposure to alcohol and nicotine. Confounding effects of environmental deficits following birth are also important.

MORBIDITY AND MORTALITY

Accidental Mortality

Women are less likely than men to drink and drive. This may be related to lower tendencies to engage in a variety of risk-taking behaviours. Additionally, women are less likely to view driving after the consumption of one or two drinks of alcohol as acceptable (17% vs. 27%). However, women appear to be involved in higher percentages of fatal alcohol-related motor vehicle accidents over the last few decades. The proportion of alcohol-related fatal accidents for women increased from 12% in 1980 to 16% in 1996 in the USA.

Medical

Medical complications related to alcohol consumption may be influenced by unique metabolic factors in women. Women appear to achieve higher blood levels of alcohol when equivalent quantities of alcohol are consumed. However, women eliminate alcohol from the blood at a faster rate than men. This effect may be due to women demonstrating a higher liver volume per unit lean body mass (Kwo *et al.*, 1998).

Although women have lower rates of heavy drinking, alcohol abuse and alcohol dependence than men, their rates of medical complications frequently approach that for men. Women have high rates of alcohol-related cardiomyopathy (Urbano-Marquez *et al.*, 1995) and alcohol-related liver disease (alcoholic hepatitis and alcoholic cirrhosis).

PSYCHIATRIC COMPLICATIONS

Psychiatric Comorbidity

Multinational studies of the epidemiology of psychiatric illness confirm higher rates of mood and anxiety disorders for women. For clinical substance abuse treatment populations, this finding holds true. Women in treatment demonstrate higher rates of major depression, dysthymia, panic disorder and generalized anxiety disorder. Longitudinal studies of treatment populations note that women are much more likely to have a primary (chronologically first) mood or anxiety disorder. This finding underscores the importance of treatment for a primary mood or anxiety disorder in conjunction with substance abuse treatment. For men, depression or anxiety symptoms commonly emerge after a period of heavy and chronic substance use. Abstinence from alcohol or other substances frequently induces a remission of mood or anxiety symptoms in men. Women are more likely to continue to exhibit a significant mood or anxiety disorder following abstinence from alcohol or drugs. There is also some evidence that women with substance abuse histories are more vulnerable to prescription sedative/hypnotic misuse, abuse and dependence. Clinicians should carefully screen for prior substance abuse histories in those being considered for sedative/hypnotic use. For women, a strong family history of alcoholism should also be considered a risk factor for prescription drug problems.

This comorbidity suggests women are more likely to use alcohol and drugs to self-treat a mood or anxiety disorder. Alcohol, benzodiazepines and other sedative/hypnotics reduce anxiety symptoms in the short term. However, with physiological withdrawal, underlying anxiety symptoms re-emerge. This re-emergence or rebound anxiety problem can facilitate continued self-administration.

Suicide

A large study of over 18 000 drinkers examined the relationship of alcohol to suicidal ideation in men and women (Grant and Hasin, 1999). Men and women with suicidal ideation shared many features including higher rates of unmarried marital status, higher rates of major depression, higher rates of alcohol dependence and higher rates of recent physical injury. However, in a comparative logistic regression model analysis, only women demonstrated an additional risk of suicidal ideation for drug use or a drug use disorder in the past year. This study supports additional vigilance for suicidal ideation among women with alcohol and drug problems.

OUTCOME: TREATMENT RESPONSE

Psychological Treatments

Few studies comprehensively study the effect of psychological treatment response for women. Studies that have examined the effect of gender have tended to find few differences in treatment outcome. Alterman *et al.* (2000) followed 145 men and 149 women receiving a variety of treatment interventions. Patients in this study received treatment through fee-for-service programmes or a managed care model. Outcomes compared by treatment and gender failed to find significant differences on seven Addiction Severity Index Scores.

Although few treatment studies suggest a specific gender response, clinical differences in gender groups may contribute to specific treatment needs. Women in treatment report high rates of previous physical and sexual abuse (Rice *et al.*, 2001). Additionally, women in treatment for substance abuse report significant eating disorder comorbidity (Sinha and O'Malley, 2000). Whether treatment focusing on abuse or eating disorder comorbidity improves outcome is unknown.

Women in treatment are more likely to have a spouse who also abuses substances (Westermeyer and Boedicker, 2000). Their substance abuse outcome is more closely linked to the outcome of substance abuse for their spouses. Studies that have found trends in alcoholism outcome for women tend to note good short-term response rates, often higher than males in treatment with similar levels of substance abuse severity. However, longer-term studies often fail to support this advantage. Several studies have demonstrated that long-term outcome for substance abuse may be worse for men and women. There is some evidence to support gender-specific effects on relapse risk for alcoholism. Some studies suggest women may have a high risk of relapse in response to either a positive or negative emotional event.

Brief interventions have been developed to reduce problem drinking in several target populations including heavy drinkers without a substance use diagnosis. A brief intervention study in a group of pregnant women found that brief intervention improved rates of abstinence among women abstinent prior to the study (Chang *et al.*, 1999). However, those with significant prenatal alcohol consumption did not reduce drinking during pregnancy with a brief intervention approach.

OUTCOME: PSYCHOPHARMACOLOGICAL TREATMENTS

Pharmacological treatment options continue to increase for a variety of substance disorders. Disulfiram, naltrexone and acamprosate are agents that appear to be effective in reducing the rates of relapse for alcohol abuse and alcohol dependence (Garbutt *et al.*, 1999; Myrick *et al.*, 2001). Nicotine replacement and bupropion appear to significantly increase abstinence rates among smokers. Few studies have compared the gender-specific effects of pharmacological treatments for substance abuse. It appears that from the few studies available pharmacological treatments are equally effective for both men and women.

CONCLUSION

Gender issues influence the prevalence, risk factors, natural history and outcome for substance use disorders. Limited empirical studies of the effect of gender on clinical issues in substance abuse hamper understanding of the substance use disorders. Clinicians need to be aware of gender-specific issues that can present in clinic practice. Further basic science and clinical research is likely to focus on the specific effect of gender on substance use disorders.

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XVI-11

Therapeutic Armamentarium in Substance-Related Disorders

A. Lingford-Hughes

Pharmacotherapies available for treatment of substance misuse are used for substitution, withdrawal and increasingly for maintaining abstinence. It must be remembered that many patients with drug or alcohol problems also experience concurrent mental health problems that need treating. Pharmacotherapy should be seen as an adjunct to psychosocial treatments delivered in group therapy or on a one-to-one basis. This chapter will describe pharmacotherapeutic approaches used rather than exact protocols or associated psycho-socio-economic benefits. It cannot be exhaustive due to limitations of space, but will aim to acquaint the reader with key themes, concentrating on nicotine, alcohol, opiate and cocaine abuse.

Despite the large numbers of people who suffer from alcohol or drug dependence, compared with many other neuropsychiatric disorders, there is very little research performed in this area. Not only is the dearth of studies of concern, many studies suffer from poor design (e.g. small numbers, lack of placebo, lack of randomization, other therapies (psychological) uncontrolled for) which hinders interpretation or generalization (Moncrieff and Drummond, 1997). This is not to sound gloomy but to emphasize that compared to many neuropsychiatric disorders pharmacotherapeutic approaches for alcohol or drug dependence are in their infancy. As recently remarked by Kranzler (2000) 'consistent interest and high levels of industrial support for medication development internationally are essential for a more rapid development of the field'.

GENERAL PRINCIPLES

Pharmacotherapeutic strategies include blocking the effects of the drug of abuse or substituting it with a similar acting drug but with a longer duration of action, which is then gradually reduced. As the neurobiology of substance misuse is characterized, more novel approaches are likely. McCance (1997) has described properties of ideal pharmacotherapy for cocaine dependence, but the principles can be extrapolated to any substance misuse:

- convenient route of administration: oral, intramuscular, transdermal;
- long acting;
- medically safe with few side effects;
- acceptable to patients presenting for treatment;
- ideally, little abuse liability;
- useful for more than one class of drug since substance abusers tend to be polydrug abusers;
- used in conjunction with behavioural treatments that target the drug abuse and psychosocial problems related to the drug abuse.

NICOTINE

Nicotine is a highly addictive substance, as evidenced by the difficulty in quitting. The health consequences of smoking are legion and are at great cost to both the individual and society. There are several reviews of pharmacotherapy available to help people quit smoking (see Hughes *et al.*, 1999). Nicotine substitutes and antagonists have both been tried, and alternative strategies use drugs active on other neurotransmitter systems, particularly dopamine and noradrenaline.

Substitute therapy is the most commonly used treatment via a nicotine patch, nicotine gum, sublingual tablet, lozenge, nicotine nasal spray or nicotine inhaler. A Cochrane review reported that such substitute therapy increases abstinence rate 1.5- to 2-fold, but the rates remain only around 5–15%, even with psychological/behavioural programmes (Lancaster *et al.*, 2000). A comparison between nicotine gum, patch, inhaler or spray revealed no differences in the abstinence rates at 12 weeks (Hajek *et al.*, 1999). Notably, although the patch was initially the most popular, with gum the least favoured, all products became equally liked (West *et al.*, 2001). Receiving your favoured product did not improve outcome, but women may do better with an inhaler and men with gum.

A nicotine antagonist, mecamylamine, has also been tried since it was hypothesized that it may block the rewarding effect of nicotine and thus reduce the urge to smoke. A Cochrane review found promising results from two small studies of mecamylamine compared to nicotine alone (Lancaster *et al.*, 2000).

Nicotine acts via nicotinic receptors that increase dopamine release in the nucleus accumbens — part of the mesolimbic reward system (Stolerman and Shoaib, 1991). Therefore antagonists of the dopamine D_2 receptor, such as haloperidol, have been used to reduce smoking. These, not surprisingly given their side-effect profile, have not been especially successful.

Bupropion is a weak reuptake blocker of noradrenaline and dopamine. It has been shown in a number of randomized controlled trials to be effective in achieving and maintaining abstinence, even if the first attempt is not successful (Hurt *et al.*, 1997; Jorenby *et al.*, 1999; Gonzales *et al.*, 2001). Bupropion also reduces craving and attenuates withdrawal. While the side effects of bupropion are relatively mild (e.g. dry mouth, insomnia), it has been shown to increase the risk of seizures. Its use is not recommended with epilepsy, or where risk of seizures is increased (e.g. head injury, alcohol abuse) (Ascher *et al.*, 1995). The risk appears less with the slow-release form and more akin with other antidepressants. Bupropion can also be used in combination with nicotine substitute therapy.

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ALCOHOL

Alcohol is a widely abused legal drug. As with nicotine, the effects on an individual and society are immense. Pharmacotherapy is currently used during detoxification, and its use in maintaining abstinence is increasingly accepted. Misuse of alcohol commonly occurs with psychiatric problems (depression, anxiety) and treatment of co-morbid conditions is often a challenge.

Alcohol Withdrawal

A commonly accepted regimen prescribed to prevent complications of alcohol withdrawal includes a benzodiazepine (chlordiazepoxide or diazepam), and vitamins, particularly vitamin B_1 or thiamine (see Williams and McBride, 1998). Chlordiazepoxide has advantages over chlormethiazole, since it is safer if alcohol is drunk and has a lower addictive potential. Chlormethiazole cannot be recommended for outpatient alcohol detoxification but does play an important role in inpatient settings, particularly its intravenous preparation. It is derived from the thiazole moiety of the vitamin B_1 molecule and its exact pharmacology is not clear, but it does enhance GABAergic function. The reader is directed to standard texts regarding suitable protocols for management of alcohol withdrawal. A reducing benzodiazepine regimen typically over 5–10 days is commonly used for uncomplicated withdrawal. Another method requiring more monitoring involves giving the patient diazepam, 10–20 mg every 1–2 h, to result in light sedation. Most patients require 100 mg or less (Salloum *et al.*, 1995).

Although withdrawal for most patients is uncomplicated, recurrent detoxification is associated with 'kindling' whereby the risk of seizures increases (Ballenger and Post, 1978). Benzodiazepines used in detoxification are active on the GABAergic system whose function has been reduced by alcohol. However, increased NMDA glutamatergic function is likely to play a key role in alcohol withdrawal (Tsai *et al.*, 1995). Targetting this system may be important since animal studies have suggested that benzodiazepines are not sufficient to prevent the kindling effect or cell damage (Mhatre *et al.*, 2001). The potential role for NMDA antagonists has recently been reviewed but notably some drugs that affect this system are already been studied or used, e.g. acamprosate and amantadine (Bisaga and Popik, 2000).

The question of whether or not to prescribe anticonvulsants continues to arise either to prevent seizures in those that are at high risk or for those who have already suffered from a seizure. If anticonvulsants are started at the same time as detoxification, then therapeutic levels will not be established until after the period of high risk (24–48 h post alcohol). However, there is no empirical evidence to support the use of anticonvulsants, even if they are started a week prior to detoxification. Carbamazepine itself has been proposed as an alternative to benzodiazepines for alcohol withdrawal and appears to be effective (Williams and McBride, 1998). Anticonvulsants may offer advantages over benzodiazepines in the outpatient treatment of alcohol withdrawal since they lack abuse potential, have minimal interactions with alcohol and are not necessarily contraindicated for patients with alcoholic liver disease.

In alcohol withdrawal, noradrenergic hyperactivity is likely to be present (Nutt, 1999). In opiate withdrawal, adrenergic α_2 agonists that reduce this activity, such as clonidine or lofexidine, ameliorate the consequences of this so-called noradrenergic storm. These drugs are, however, ineffective either alone or as an adjunct to benzodiazepines in alcohol detoxification (Adinoff, 1994; Keaney *et al.*, 2001).

Prescribing thiamine is crucial in any alcohol detoxification. Its deficiency, caused by poor diet and absorption in alcoholism, can result in Wernicke–Korsakoff syndrome, with its associated high mortality and morbidity. For uncomplicated alcohol withdrawal, oral supplementation is required (100–300 mg per day). Since absorption of oral thiamine is variable and potentially low, however, thiamine must be administered parenterally to any patient with suspected thiamine deficiency to increase brain levels (Cook *et al.*, 1998; Thomson, 2000). The use of parenteral formulations has previously been associated with adverse reactions including anaphylaxis, but the benefit:risk ratio for malnourished at-risk alcoholics is still in favour of administration. Newer preparations are less associated with such a reaction. No placebo-controlled studies have been performed to determine the dose or duration of vitamin B complex supplementation required. A suggested regimen delivers at least 500 mg thiamine, three times a day, for 3–5 days or more until the patient shows no signs of Wernicke–Korsakoff syndrome (Cook *et al.*, 1998). A recent study has shown the benefit of thiamine in improving working memory in alcoholics admitted for an inpatient alcohol detoxification who received intramuscular thiamine (200 mg once per day) (Ambrose *et al.*, 2001).

Maintaining Abstinence

Although the use of pharmacotherapy to maintain abstinence from alcohol is increasing, not everyone benefits from such adjunctive therapy and currently there are no clear predictive guidelines to direct prescribing. Alcoholism and vulnerability to alcoholism involve altered function in many different neurotransmitter systems. Consequently, where studies or trials show limited efficacy, is this a result of this complex neurobiology, or the type of alcoholism, the patients chosen, or other treatments offered/experienced? The pharmacotherapy of alcoholism has recently been extensively reviewed (Litten and Allen, 1998; Garbutt *et al.*, 1999; Swift, 1999a, 1999b; Kranzler, 2000). In addition, any co-morbid disorder requires treatment in its own right.

Disulfiram

Disulfiram inhibits aldehyde dehydrogenase (ALDH), an enzyme that catalyses the oxidation of acetaldehyde to acetic acid. The build-up of acetaldehyde that results when alcohol is drunk produces aversive effects such as nausea, flushing, headache and palpitations. Disulfiram is one of the oldest available treatments and the reader is directed to recent reviews (Wright and Moore, 1990; Hughes and Cook, 1997).

In an initial study, Fuller and Roth (1979) compared disulfiram (1 mg (non-aversive) or 250 mg (typical dose)) with placebo and found that although either dose resulted in higher rates of abstinence, the result was not statistically significantly different from placebo. A later study which included psychological therapy rather than 'counselling' found no advantage of disulfiram (either dose) over placebo, although in those that relapsed, patients taking 250 mg of disulfiram drank less (Fuller *et al.*, 1986). These studies illustrate that it is the fear of the adverse reaction that is a significant part of disulfiram's efficacy. While some patients need in excess of 1 g per day (Brewer, 1984) to produce an aversive reaction, others may experience it after applying products containing alcohol such as perfumes.

Patients must be aware of the risks of taking disulfiram and drinking or absorbing alcohol. Disulfiram is probably most effective over the first few months of treatment and it is commonly prescribed for about 6 months. Patients may want to take it on an 'as required' basis, i.e. prior to a social function. Often disulfiram is seen as a 'last resort' in treatment, and for some patients it is very effective.

It is clear that supervision is key in maximizing the efficacy of disulfiram. Chick *et al.* (1992) reported that 'supervised disulfiram with counselling enhanced treatment outcome'. Since compliance is important, disulfiram implants were developed. Studies have not been promising and it seems that insufficient blood levels of disulfiram were achieved to act as a deterrent (see Hughes and Cook, 1997).

Disulfiram cannot be used in everyone, limiting its availability. Neuropsychiatric complications resulting from disulfiram include psychosis, confusional states, peripheral neuropathy and optic neuritis. A history of psychosis is therefore seen as a contraindication to prescribing disulfiram but nevertheless has been used successfully in the presence of psychosis (Larson *et al.*, 1992). Due to previous fatal cardiac complications resulting from the disulfiram–alcohol interaction, patients with cardiac disease are generally contraindicated. Rarely (1 in 25 000 treated per year) disulfiram can cause liver hypersensitivity that is fatal. Despite these adverse effects, it must be remembered that alcoholism itself has a high rate of morbidity and mortality.

Acamprosate

Acamprosate is an amino acid derivative which primarily acts as an antagonist of the glutamatergic NMDA receptor, though it can also modulate GABAergic function (Daoust *et al.*, 1992; Zeise *et al.*, 1993). Its use stemmed from an observation from animal studies that calcium bis-acetyl homotaurine (acamprosate) reduced self-administration of alcohol (Boismare *et al.*, 1984). It has been used for a number of years in Europe and a multi-centre study has recently been completed in the USA. The reader is directed to a number of reviews (Swift, 1999a, 1999b; Kranzler, 2000).

There have been several randomized placebo-controlled trials performed in Europe that have shown acamprosate to be approximately twice as effective as placebo in maintaining abstinence. The first study showed that acamprosate in addition to monthly outpatient appointments doubled the rate of abstinence to ~60% over a 3-month period (Lhuintre *et al.*, 1985). Paille *et al.* (1995) studied two doses of acamprosate (1.3 g per day or 2 g per day) compared to placebo over a 12-month period and thereafter for 6 months on placebo. This study began to address many critical questions such as what dose of acamprosate should be prescribed, for how long and what happens after acamprosate is stopped. Acamprosate resulted in longer abstinence, improved clinic attendance and γ-glutamyltransferase (GGT) levels at 6 and 12 months. Patients who drank alcohol on the higher dose of acamprosate drank less frequently. Six months after acamprosate withdrawal, abstinence was associated with clinic attendance and the higher dose. The results for the low-dose acamprosate were intermediate between high dose and placebo.

Pelc *et al.* (1997) also reported that a higher dose of acamprosate (1998 mg per day) was more efficacious than a lower dose (1332 mg per day) on a similar range of parameters over 6 months. Sass *et al.* (1996) reported greater abstinence from acamprosate (45%) compared to placebo (25%) for those that remained in a controlled trial over 48 weeks. This benefit was maintained in the follow-up period and has similarly been reported in other studies (Whitworth *et al.*, 1996; Poldrugo, 1997; Tempesta *et al.*, 2000).

A recent a UK multi-centre study conducted over 6 months was not so positive about acamprosate (Chick *et al.*, 2000b). Absolute abstinence was only achieved in 12% of patients taking acamprosate, compared to 11% in the placebo group. The most likely explanation for this poor response was that 5 weeks was permitted between detoxification and starting acamprosate, and about one third of patients had already relapsed. Generally in other studies, acamprosate was begun within 2 weeks of detoxification (range 2 days to 28 days). This suggests that acamprosate should be started as soon as possible after detoxification. There is preliminary evidence that acamprosate might have a neuroprotective effect and therefore some clinicians advocate that it should be started with detoxification.

Acamprosate is a safe drug with few side effects, most commonly diarrhoea and headache. Its use is not restricted by liver dysfunction unless very severe. Acamprosate has also been found to be effective in medical and neurological settings rather than just psychiatric or addiction services (Tempesta *et al.*, 2000). Acamprosate combined with disulfiram has also been found to be more effective in some patients than acamprosate alone (Besson *et al.*, 1998).

Craving receives a lot of attention since it is implicated in causing relapse, and acamprosate has been labelled as 'anti-craving' (Littleton, 1995). The evidence from randomized controlled trials, however, is less than robust. Paille *et al.* (1995) reported that craving, as rated by the clinician, improved for all patients but somewhat more so in the acamprosate group. Chick *et al.* (2000b) reported a reduction in craving with acamprosate in weeks 2 and 4 and 1 month after the end of the study. More studies are required not only to elicit the effect of acamprosate on craving but also to further define this complex phenomenon and its neurobiology.

Given that many patients do not respond to acamprosate, are there any characteristics that predict efficacy? Lesch and Walter (1996) reported that acamprosate is more likely to benefit a 'pure' alcoholic (Lesch types I and II) rather than those with a psychiatric (affective) or organic disorder (Lesch types III and IV). Acamprosate was more likely to be beneficial to stop drinking alcohol to overcome withdrawal or anxiety. In the UK study, there were small numbers of the type I and II patients which may have contributed to the lack of efficacy (Chick *et al.*, 2000b).

In conclusion, acamprosate does help some patients maintain abstinence, an effect that can be sustained after the drug has been stopped. It appears that acamprosate should be started near to detoxification for maximum efficacy and continued for up to 1 year. If patients drink while taking acamprosate, consumption is reduced. All the trials include at the very least psychosocial support; consequently acamprosate alone has not been shown to be effective. It is not clear whether any particular form of psychological treatment is best combined with acamprosate. Patients experiencing symptoms of anxiety may derive more benefit from acamprosate.

Naltrexone

Evidence from animal studies supports the hypothesis that opiates are involved in mediating rewarding effects of alcohol. Opiate antagonists were shown to reduce self-administration of alcohol. In addition, some alcohol-preferring strains of animals have high endogenous opioid activity (see Froehlich and Li, 1994). Consequently, naltrexone, a non-specific opiate antagonist, was first introduced in the USA in 1994. Its use has been more limited in Europe.

Volpicelli *et al.* (1992) reported that naltrexone (50 mg) compared to placebo in a randomized controlled trial reduced craving for alcohol, resulted in fewer drinking days and lower rates of relapse for alcohol-dependent patients aiming for abstinence. Importantly, in those patients who did drink alcohol, naltrexone reduced binge drinking. All patients had been abstinent from alcohol for at least 1 week before receiving naltrexone as an adjunct to standard psychotherapy over 12 weeks. Naltrexone is probably effective by blocking the effects of opiates, released by alcohol, that increase dopaminergic function in the mesolimbic system mediating reward (Volpicelli *et al.*, 1992). It was therefore hypothesized and later shown that naltrexone was efficacious in those patients who are likely to 'sample alcohol', leading to further consumption since less euphoria was experienced (Volpicelli *et al.*, 1995).

A criticism of many trials is that they last a limited time — here only 12 weeks. O'Malley *et al.* (1996) showed that, 6 months after stopping taking naltrexone, improved rates of drinking behaviour were maintained, but do eventually diminish with time. Unsurprisingly, abstinence during the study period was associated with better outcome at 6 months. It is not clear how long naltrexone should be prescribed for maximum efficacy.

Unsurprisingly, compliance is critical to outcome and strategies to improve this will increase naltrexone's efficacy. In a 'more

naturalistic' study Volpicelli *et al.* (1997) reported that the more compliant a patient was in completing the 12-week programme, the better they did. Overall, however, naltrexone only showed modest efficacy compared to placebo. A belief in the efficacy of naltrexone is important but the number and severity of side effects (e.g. nausea) early in treatment predict poorer compliance (Rohsenow *et al.*, 2000). O'Malley *et al.* (2000) reported the risk of nausea was greater in younger, female patients with a shorter duration of abstinence and in lighter drinkers. Therefore, increasing the length of abstinence before starting naltrexone and at a lower dose, e.g. 12.5 mg, might improve compliance. It is not clear whether 50 mg is the therapeutic dose for abstinence.

Naltrexone is always given alongside a psychosocial programme, ranging from a daily programme for 1 month to once-weekly psychotherapy. O'Malley *et al.* (1992) examined the interaction of naltrexone with coping skills therapy/relapse prevention or supportive therapy in patients who wanted to remain abstinent. After 12 weeks of treatment, both psychotherapies were equally effective. Cumulative abstinence rates were, however, better in those who received supportive therapy, but in those who began drinking coping skills reduced the risk of a full relapse. Anton *et al.* (1999) found that naltrexone combined with cognitive-behavioural therapy over a 12-week period resulted in a modest improvement compared to placebo. It is therefore not clear whether there is an optimum psychosocial intervention, though those receiving less intensive support appear more likely to relapse.

A multi-centre double-blind randomized placebo-controlled trial has been performed in the UK (Chick *et al.*, 2000a). Naltrexone was not shown to be superior to placebo in a number of drinking variables, including 'time to first drink'. Compliance was an issue, but even in those who completed the study the 'time to first heavy drinking episode' was no different for naltrexone compared to the placebo group. Nonetheless, the total amount of alcohol consumed was less for those who were compliant with naltrexone, corroborated by greater improvements in liver function tests and scores in the obsessive-compulsive drinking scale.

In the studies so far described, naltrexone has been used in patients with alcohol dependence to help them remain abstinent, having stopped drinking. Sinclair (2001), by contrast, suggests that naltrexone can be started while drinking alcohol. He argues that the primary mechanism of this drug involves 'extinction of drinking behaviour' since naltrexone will block the rewarding effects of alcohol. While Sinclair reports beneficial outcomes, this has yet to be replicated.

Another opioid antagonist, nalmefene, has recently been studied as a treatment for alcohol dependence. Compared to placebo, nalmefene (>10 mg per day) has been shown to reduce relapse rates (Mason *et al.*, 1999).

Lastly, since naltrexone and acamprosate act via different mechanisms, their combination has been tried to see if abstinence rates were improved. Stromberg *et al.* (2001) reported in rats that the combination was no more effective than monotherapy. Equivalent studies in man are awaited.

Drugs Active on Serotonergic Neurotransmission

Animal models suggest that the serotonergic system plays a key role in alcohol-related behaviours and vulnerability to alcohol-seeking behaviour (see McBride and Li, 1998). Consequently, drugs that are active on the serotonergic system have been investigated as a treatment for alcoholism. In addition, common co-morbid disorders such as depression and anxiety are also effectively treated with specific serotonergic reuptake inhibitors (SSRIs).

In heavy or 'problem' drinkers (e.g. drinking at least 380 g alcohol per week), various SSRIs (zimeldine, fluoxetine, citalopram), often at higher doses than those used in depression (e.g. fluoxetine, 60 mg per day), have been shown to moderately (10–17%) reduce alcohol consumption (Naranjo *et al.*, 1990; see Kranzler, 2000). The subjects were recruited since they were concerned about their drinking, treated for 1 month, but treatment goals were not clear. The reduction in consumption was due to either an increase in the number of abstinent days (citalopram) or decrease in amount drunk per session (fluoxetine). Although this suggests that SSRIs can improve problem drinking, use of these drugs for this purpose cannot be recommended.

In non-depressed alcohol-dependent patients, there have been a number of trials investigating the effects of SSRIs, with conflicting results. While fluoxetine (20 mg per day) in detoxified alcoholics has been shown to increase abstinence rates, other studies conducted in inpatient 'severe' alcoholics reported no reduction in relapse rates (Janiri *et al.*, 1996; Kabel and Pety, 1996). Fluoxetine (40 mg per day) has been shown to reduce the number of drinks consumed but only in those with a family history of alcoholism (Gerra *et al.*, 1992). Gorelick and Paredes (1992) reported a reduction in alcohol consumption by alcoholics treated with fluoxetine ($\leq$80 mg per day) for 28 days, which, however, only lasted 1 week. Fluoxetine ($\leq$60 mg per day) has been shown to confer no advantage over placebo when combined with relapse prevention over 12 weeks (Kranzler *et al.*, 1995).

Citalopram has also been extensively studied. Naranjo *et al.* (1992) reported that citalopram decreased 'desirability, liking and consumption' of alcohol in alcohol-dependent patients after 1 week's treatment. However, the diagnosis of dependency relied on amount and frequency of alcohol drunk. A placebo-controlled trial of citalopram (40 mg per day) combined with brief psychosocial intervention over 12 weeks replicated the initial beneficial effect, but by 12 weeks there was no advantage over placebo (Naranjo *et al.*, 1995). By contrast, Tiihonen *et al.* (1996) reported that citalopram doubled the rate of abstinence at 12 weeks (19%) in outpatient alcoholics aiming for long-term reduction or abstinence. This was associated with improved GGT levels. Citalopram and fluvoxamine have been compared to placebo as an adjunct to cognitive-behavioural therapy over a 16-week period in alcohol-dependent patients (Angelone *et al.*, 1998). The rates of abstinence were doubled in both drug groups ($\sim$60%) compared to placebo (30%), but once patients had relapsed no benefit from continuing the SSRI was apparent. Citalopram, but not fluvoxamine, reduced craving.

What might contribute to these mixed results? In the majority of clinical studies, both men and women are studied. Naranjo *et al.* (2000) has reported that men benefited more from citalopram (40 mg per day) than women. Kranzler reanalysed the data from the study where fluoxetine was no better than placebo (Kranzler *et al.*, 1995, 1996). The subjects were clustered into type A (low risk or severity, later age of onset) and B (high risk or severity, early age of onset). Interestingly, in type A patients, fluoxetine resulted in no improvement, but in type B patients fluoxetine resulted in poorer outcome. Pettinati *et al.* (2000) reported a similar effect with sertraline (200 mg per day for 14 weeks), with type A alcoholics showing a more favourable outcome from sertraline compared to placebo and sertraline in type B alcoholics showing no effect. These studies raise the possibility that SSRIs may be inappropriate for some alcoholics and that a greater understanding of the neurobiology of alcoholism could have a direct impact on treatment. The serotonergic system has been a focus of research since it was shown that in alcoholism associated with early onset, predominantly males, and impulsive behaviour (type II or B) there is reduced serotonergic function (Heinz *et al.*, 2001). By contrast, increased serotonergic function is proposed to be present in alcoholism associated with anxiety, both genders and later onset (type I or A).

SSRIs have also been used to treat depressed alcoholics. Cornelius *et al.* (1997) reported that fluoxetine (20–40 mg per day) resulted in

improved depression and drinking behaviour compared with placebo over a 12-week period in depressed suicidal alcoholics. Notably in the placebo group there was a correlation between improved depression and drinking that was not seen in the fluoxetine group. When followed up at 1 year, those who had received fluoxetine in the trial continued to show less depression and better drinking behaviour than those who received placebo. The authors suggested that antidepressants in this population should be continued for 1 year. Other studies of fluoxetine in less severely depressed alcoholics are less positive (see Kranzler, 2000).

Sertraline (100 mg per day) has also been shown to improve depression in alcoholics after 6 weeks of treatment who remained abstinent (Roy, 1998). However, a previous history of depression or a positive family history did not result in greater response to sertraline (200 mg per day) in improving drinking behaviour (Pettinati *et al.*, 2001).

More recently nefazodone, a weak SSRI and $5HT_2$ antagonist, has been shown after 12 weeks in depressed alcoholics to result in greater improvement in depression but not in drinking behaviour (Roy-Byrne *et al.*, 2000). In a non-depressed group of alcoholics, nefazodone was similarly shown not to be effective in improving abstinence rates (Kranzler *et al.*, 2000).

Serotonergic receptor antagonists have also been used to treat alcoholism. Although ritanserin, a $5HT_2$ antagonist, moderated alcohol intake in animal models, it has shown no such efficacy in relapse prevention in man (Johnson *et al.*, 1996; Wiesbeck *et al.*, 1999). Ondansetron, a $5HT_3$ antagonist, has also been studied since $5HT_3$ receptors are known to modulate dopaminergic activity in the mesolimbic system. Ondansetron attenuates some of the positive reinforcing effects of alcohol (Johnson *et al.*, 1993). In a placebo-controlled trial in alcohol-dependent patients seeking treatment towards abstinence or reduced drinking, odansetron resulted in improved drinking behaviour, particularly in those drinking fewer than 10 drinks per day (Sellers *et al.*, 1994). Johnson *et al.* (2000b) have reported that ondansetron ($4\,\mu g\,kg^{-1}$ twice a day) reduces alcohol consumption and abstinence rates in early-onset but not late-onset alcoholics. Based on the hypothesis that both ondansetron and naltrexone modulate the dopaminergic reward system differently, the effect of these drugs together was studied. Only early-onset alcoholics were studied and ondansetron plus naltrexone (50 mg) for 8 weeks resulted in reduced alcohol consumption (Johnson *et al.*, 2000a). The authors have put forward hypotheses to explain this effect; however, the results require replication before the implications for clinical practice can be determined.

Buspirone, a partial agonist at the presynaptic $5HT_{1A}$ receptor, is used to treat generalized anxiety, and its efficacy at treating co-morbid anxiety with alcohol dependence has been investigated. Buspirone has been shown to improve both anxiety and alcohol consumption (Tollefson *et al.*, 1992; Kranzler *et al.*, 1994), but not consistently (Malcolm *et al.*, 1992). A meta-analysis revealed that buspirone results in improved treatment retention and anxiety rather than necessarily effecting alcohol consumption itself (Malec *et al.*, 1996). George *et al.* (1999) examined whether the response to buspirone varied in non-anxious early-onset and late-onset alcoholics due to their presumed low and high levels of 5HT. Regardless of medication, late-onset alcoholics took longer to relapse.

Other Antidepressants

Both imipramine and desipramine have been shown to reduce depressive symptomatology in an outpatient group of depressed alcoholics but not necessarily to improve drinking behaviour (Nunes *et al.*, 1996; Mason *et al.*, 1996; McGrath *et al.*, 1996). This also serves to reinforce that depression and alcohol abuse do not necessarily go hand in hand. The side-effect profile of tricyclic antidepressants, particularly when taken with drugs and/or alcohol, and the risk when taken in overdose, prevents recommendation of their widespread use.

Drugs Active on the Dopaminergic System

Since dopamine is critically involved in reinforcement, dopamine antagonists have been used to treat alcohol dependence (see Swift, 1999a, 1999b). While some studies do show that D_2 antagonists such as haloperidol, tiapride and olanzapine reduce drinking and craving, they are not widely used clinically.

Benzodiazepines

The use of benzodiazepines in maintaining abstinence from alcohol is controversial and should not be undertaken without expert advice and monitoring (see Ciraulo and Nace, 2000). Abstinent alcoholics may be at greater risk of benzodiazepine abuse and dependence due to greater rewarding effects. Despite the level of concern, there is evidence to suggest that a history of alcohol abuse/dependence does not necessarily result in greater use of benzodiazepines, particularly for those who are less severely dependent. Characteristics of patients who are most at risk of abusing benzodiazepines include severe dependence, antisocial personality disorder and polysubstance abuse. For some people, maintenance benzodiazepines can be indicated to maximize abstinence and minimize morbidity from alcohol.

OPIATE ADDICTION

From a pharmacotherapeutic perspective, treatment of opiate addiction often involves first substituting their illicit use with a prescribed opiate, followed by a period of stabilization and then detoxification. The reader is directed to a number of sources available describing issues around managing opiate addiction (Department of Health Guidelines (UK); NIH Consensus Conference (USA)). It is clear that our lack of understanding about the neurobiology of opiate addiction and the effects of treatment may be contributing to the lack of efficacy of some treatments.

Methadone

Methadone is the most widely used substitute drug for treatment of opiate addiction. It is a full opioid agonist with a half-life of about 24 h. Before a script is issued, opiate abuse should be confirmed by testing, e.g. urinalysis. The initial starting dose of methadone is calculated from the amount of illicit heroin abused. There is evidence to suggest that 40–60 mg methadone in opiate-naïve people is non-fatal. It would therefore be reasonable to use this as a maximum starting dose, and titrate upwards if needed until withdrawal symptoms are absent (Drummer *et al.*, 1992). There is evidence to suggest that illicit opiate use produces less euphoria in addicts on methadone (Kreek, 2000).

Substitution

Several studies support the use of 'high'-dose methadone to prevent illicit opiate use. Ling *et al.* (1996) showed that 'high'-dose methadone (80 mg per day) over 1 year was superior to low (30 mg per day) dose with improved retention rates, opioid use and opioid craving. Interestingly, buprenorphine (8 mg per day; see later) was no different to low-dose methadone. Other studies have also reported that doses around 80–100 mg are more effective, improving retention in treatment, and also that flexible dosing

and patient participation in dose decisions improved retention (Maddux *et al.*, 1997). Treatment programmes using 'low' doses of methadone may contribute to poor outcomes.

Maintenance

Methadone maintenance as a therapy (MMT) was first proposed in the 1960s and became the basis for many treatment programmes in the USA during the 1970s (Dole and Nyswander, 1965; Courtwright, 1997). The harm minimization strategy that grew in the late 1980s out of a worldwide drive to reduce premature deaths in opiate users from infectious disease such as hepatitis C and AIDS also relied on MMT. Such a strategy has been shown to be effective in improving health, and reducing illicit heroin use, infectious disease transmission, death from overdose and criminality (Ward *et al.*, 1999).

The risks associated with MMT include diversion of methadone to other addicts or accidental consumption by others, e.g. children. These risks can be reduced by daily prescribing or supervised consumption where methadone is taken at either the drug team base or pharmacy.

The Cochrane Collaboration has recently provided reviews of treatment of opioid dependence and protocols, including MMT (Mattick *et al.*, 2001). In the USA, the National Institutes of Health (NIH) convened a Consensus Panel on Effective Medical Treatment of Heroin Addiction (1997). The recommendations included increased access to methadone maintenance treatment programmes and noted that substance abuse counselling and psychosocial therapies enhance retention and successful outcomes in MMT programmes (http://consensus.nih.gov.).

Detoxification

Detoxification from methadone can occur at a variable rate depending on the initial starting dose and time available, but usually reduction takes at least a minimum of 7–10 days, if done with no adjunctive treatment (see below).

Other Substitute Drugs

Availability of alternative substitute drugs offers addicts an important choice of treatment since methadone treatment can be stigmatized, reducing the number of addicts seeking help and its acceptability. In many countries methadone can only be prescribed by specialized services, unlike buprenorphine, increasing its availability. Buprenorphine in some parts of the world, however, has had a significant history of abuse (e.g. Scotland).

LAAM

L-α-Acetyl-methadyl (LAAM) is a full opioid agonist at the μ-opioid receptor, with a longer half-life (2–3 days) than methadone. The metabolites of LAAM, nor-LAAM and dinor-LAAM, are active as parent LAAM. The FDA approved LAAM in 1993 for use in the treatment of opiate addiction. At the time of writing, LAAM is still under consideration in many countries. Recently it has become apparent that LAAM is associated with QT prolongation and severe arrhythmia, which has led to cardiovascular screening and to restrictions of its use.

Compared to methadone, addicts typically require 1.2–1.3 times more LAAM and can take longer to stabilize. Rapid induction in those who need a higher dose can lead to opioid agonist adverse effects and drop-out (Jones *et al.*, 1998). Due to its long half-life, LAAM need be taken only every other day or less to prevent opiate withdrawal, and addicts requiring supervised consumption need attend less frequently than if they were receiving methadone. This may not be an advantage if the patient does require more intensive support and monitoring.

LAAM is equally or more effective than methadone (see Finn and Wilcock, 1997; Rawson *et al.*, 1998; Jones *et al.*, 1998). LAAM has been shown to control opiate craving and block the euphoric effects of additional opiate use. Higher doses appear to be more effective, as with methadone. Eissenberg *et al.* (1997) compared thrice-weekly (Monday/Wednesday/Friday) oral LAAM dose regimens of 25/25/35 mg, 50/50/70 mg, and 100/100/140 mg with non-mandatory counselling. While there was no significant difference in the retention rates, self-reported heroin use and opiate-positive urinalysis, the greatest reductions occurred in patients receiving the highest dose.

Buprenorphine

Buprenorphine is a partial agonist at the μ-opioid receptor and a κ antagonist (Jasinski *et al.*, 1978). It can be used for maintenance treatment and detoxification. Two formulations are available: buprenorphine alone and combined with naloxone (buprenorphine:naloxone, 4 : 1 ratio). Both preparations are taken sublingually, and if taken in this manner the naloxone is not active. However, if the formulation with naloxone is injected the naloxone is effective, thus blocking any effects of buprenorphine and inducing withdrawal of varying intensity. This has been shown to prevent significant intravenous abuse (Robinson *et al.*, 1993). Lastly, the bioavailability of buprenorphine in tablet form is about half of the liquid form which was used in early studies, leading to different recommended doses.

Buprenorphine has particular advantages over methadone, with its longer duration of action, more limited withdrawal syndrome, and less propensity to cause respiratory depression and sedation (Bickel *et al.*, 1988; Fudala *et al.*, 1990). Buprenorphine has a slower rate of onset than heroin, resulting in less of a 'high'. Its longer half-life also results in precipitating less withdrawal. The dose–response relationship of buprenorphine is that of a partial agonist. By definition, partial agonists result in lower levels of response at maximal occupancy compared to full agonists. In addition, when a partial agonist occupies all the receptors, no receptors are available to a full agonist (e.g. heroin) to effect a response. The partial agonist is therefore acting like an antagonist. Consequently buprenorphine will stimulate the μ-opioid receptor, but not maximally (hence less risk of respiratory depression) and also prevent effects of heroin taken 'on-top'. Therefore, while the peak effects from 8 mg are much greater than from 2 mg, differences between 8 mg and higher doses are smaller. The duration of effect from these higher doses is longer, however, due to its storage in fatty tissue and slow off-rate from the μ-opioid receptor. Thus higher doses of buprenorphine result in increased duration but not magnitude of effect.

Substitution and Maintenance

The clinically effective dose range for buprenorphine in treating opiate addiction is 4–32 mg per day. Addicts leaving treatment in the induction phase with buprenorphine has been reported (Fischer *et al.*, 1999). Adequate doses of buprenorphine to prevent withdrawal are therefore important. There have been a number of reviews and meta-analyses which suggest that the efficacy of buprenorphine and methadone is similar (West *et al.*, 2000; Barnett *et al.*, 2001). The doses of buprenorphine ranged from 2 mg daily to 16 mg daily and of methadone from 20 mg to 120 mg daily. Patients who had previous methadone treatment preferred buprenorphine.

Buprenorphine can be given less frequently than daily due to its long pharmacological half-life. Thrice-weekly compared to daily dosing of buprenorphine (total = 56 mg per week) was

shown to result in equivalent retention in treatment but was less effective in reducing illicit opioid use and opiate craving (Perez de los Cobos *et al.*, 2000). A negative relationship was found between plasma levels of buprenorphine and positive urinalysis and opiate craving. Perez *et al.* therefore concluded that a dose of >8 mg daily equivalent of buprenorphine tablets should be used. Schottenfeld *et al.* (2000) reported that there were no differences with respect to retention or reductions in illicit drug use between daily dosing of 16 mg buprenorphine tablets or thrice-weekly as 34/34/44 mg (16 mg daily equivalent). More recently, a thrice-weekly dosing regimen of the buprenorphine:naloxone (8 : 2 mg) combination has been shown to be superior to daily dosing, with improved medication compliance and higher patient satisfaction (Amass *et al.*, 2001).

κ-Opiate receptors are proposed to mediate dysphoria. As a κ antagonist, buprenorphine should induce less dysphoria than other substitute drugs. Improved depression ratings have been reported in patients treated with buprenorphine (Kosten *et al.*, 1990). However, a lifetime history of depression has not been shown to predict better retention in treatment with buprenorphine compared to methadone (Schottenfeld *et al.*, 1998).

Detoxification

The role of buprenorphine in detoxifying patients from opiates has been a subject a Cochrane review (Gowing *et al.*, 2000). Most (4/5) of the studies compared buprenorphine with clonidine ($\leq$0.9 mg per day in divided doses) and in all but one study patients were withdrawing from heroin. It was concluded that buprenorphine showed potential to overcome withdrawal from heroin but that further study was required.

Comparative Studies

Methadone (low 20 mg, high 60–100 mg daily), LAAM (75–115 mg, three times a week) and buprenorphine (16–32 mg, three times a week) have been compared (Johnson, R.E. *et al.*, 2000). While only half the patients completed the 17-week study, high-dose methadone, LAAM and buprenorphine all resulted in longer periods of time in treatment, fewer opioid-positive urines, and lower self-rating of 'severity of drug problems' compared to low-dose methadone. LAAM did, however, result in higher drop-out rates than high-dose methadone, which was attributed to the longer time taken to achieve the targetted maintenance dose.

Injectable Opioid Scripts

Although reducing injecting behaviour is a goal of many treatment programmes, some services and countries have supported injectable formulations (e.g. opiates or amphetamines) for some addicts. Many ethical considerations are raised by such a prescription, particularly around maintaining injecting behaviour that is implicated in drug-related deaths. The reader is directed to a recent debate concerning prescribing of injectable heroin or methadone to addicts (Zador, 2001) and the following comments. Zador (2001) argued that in the UK there is almost no evaluation of this practice and that its availability is more reliant on a personal view of the clinician than on any patient characteristics. However, there is evidence, particularly from Switzerland, that it can be a beneficial strategy for some patients, but at greater financial cost than methadone (Perneger *et al.*, 1998).

Opiate Detoxification

Non-Opiate Protocols

While methadone or buprenorphine can be reduced gradually, non-opioid drugs are equally able to cover detoxification and are increasingly used (Mattick and Hall, 1996). In addition to administering medication to ameliorate withdrawal symptoms such as stomach cramps or insomnia, many symptoms can be treated with α_2-adrenergic receptor agonists such as clonidine or lofexidine. These drugs reduce the hyperactivity of the ascending noradrenergic system projecting from the locus coeruleus seen in opiate withdrawal.

A Cochrane Review of the use of α_2-adrenergic receptor agonists for the management of opioid withdrawal reported on the results of 24 randomized controlled trials. Although the intensity of withdrawal symptoms was greater, the symptoms resolved more quickly with α_2-adrenergic receptor agonists than with methadone (Gowing *et al.*, 2001). Clonidine was associated with adverse effects such as low blood pressure, dizziness, dry mouth and lack of energy. A similar picture for lofexidine was reported except for less hypotension, which responded to a reduction in lofexidine dose. Clonidine should now only be used in an inpatient setting, but lofexidine can be used safely in outpatients. Strang *et al.* (1999) recently reviewed randomized and open controlled trials of lofexidine for opiate detoxification compared with clonidine or methadone. Importantly, lofexidine appeared more acceptable to patients than clonidine but may not be preferred over methadone.

In a series of studies of inpatient, Bearn *et al.* (1996, 1998) showed that lofexidine compared favourably with methadone for detoxification and that an accelerated lofexidine regimen (5 days) compared to a 10-day regimen was associated with a faster resolution of withdrawal responses. The 10-day regimen of lofexidine was superior to methadone. Lastly, adding in naltrexone led to an even more rapid resolution of withdrawal symptoms.

More Rapid Protocols

While most detoxification occurs over days to weeks, faster regimens exist. Rapid and ultra-rapid opioid detoxification has been recently reviewed (O'Connor and Kosten, 1998). Ultra-rapid detoxification is a treatment that has courted controversy with advocates and protagonists since it was first reported by Loimer *et al.* (1989) (Strang *et al.*, 1997; Brewer, 1997; Kleber, 1998). The procedure typically involves using propofol as an anaesthetic or midazolam for heavy sedation and precipitating withdrawal by an opiate antagonist (naltrexone), together with benzodiazepines, an α_2-adrenergic agonist and drugs to treat gastrointestinal symptoms (ondansetron, anti-diarrhoeal) over 4–6 h. There is only limited literature available with variable protocols, but some have concluded that currently the risk/benefit ratio is still on the side of too much risk (Kleber, 1998). Such accelerated detoxification regimens are appealing to patients who want to minimize the length and discomfort of this process. However, detoxification is only the first step to achieve abstinence and patients are unlikely to maintain abstinence without further support.

Maintaining Abstinence

Use of an antagonist to treat addiction has been developed from the classical behavioural concept of 'extinction' since the positive effects (e.g. euphoria) of opioids that drive further use are blocked when the patient is treated with an antagonist. Naltrexone was approved by the FDA in 1985 for treatment of opiate dependence. While its use is not ubiquitous, it is more widely used in some highly motivated patient populations, e.g. medical or criminal, with some success. A recent Cochrane Review reported that the methodology of many studies were poor, permitting limited conclusions about naltrexone, except in the populations above (Kirchmayer *et al.*, 2000).

Concerns have been raised about increased dysphoria or depression with naltrexone since it will block endogenous opioids released

in pleasurable circumstances. A review of naltrexone in opiate addicts reported that depression appeared to improve with naltrexone treatment (Miotto *et al.*, 1997). Nevertheless, there are a number of case reports suggesting an association between naltrexone and depression or suicide, so careful monitoring is required.

PSYCHOSTIMULANTS

All psychostimulants result in increased synaptic levels of noradrenaline, serotonin and dopamine by inhibiting uptake of released neurotransmitter (cocaine) or stimulating release (amphetamine). Enhanced levels of dopamine are thought to be key in mediating positive reinforcement which drives further use of these drugs (Koob, 2000). The exact attribution of specific behavioural consequences to particular dopamine D_1-like (D_1, D_5) or D_2-like (D_2, D_3, D_4) receptors remains to be elucidated. This group of drugs results in increased sympathomimetic activation, leading to behavioural arousal. After experiencing the 'high' or 'rush' from psychostimulants, users experience withdrawal consisting of dysphoria or depression, hypersomnia, hyperphagia and craving for more drug. Treatment has therefore been targetted at reducing positive reinforcement or ameliorating withdrawal symptoms to reduce the drive for further drug use. Pharmacotherapy has been developed particularly for cocaine misuse, since standard psychosocial treatment packages appeared to have limited effect, but the principles can be applied to other psychostimulants. There are no medications currently available to treat cocaine addiction specifically; however, several are showing promise and are in development.

COCAINE

The range of pharmacotherapeutic options for cocaine misuse has been reviewed (Mendelson and Mello, 1996). Many different types of treatment have been investigated in the treatment of cocaine misuse, some with promising results that unfortunately do not necessarily hold up in randomized placebo-controlled trials. These can be broadly grouped into those that alter dopaminergic function, antidepressants, mood stabilizers and drugs to treat other substances of abuse.

Altering Dopaminergic Activity

Cocaine is a potent inhibitor of the dopamine transporter, resulting in increased synaptic levels of dopamine. Increased dopamine levels have been shown to be associated with euphoria and 'high' (Volkow *et al.*, 1999) and hence blockade of this effect may reduce drug use. Dopamine D_2 receptor blockade can be achieved with typical antipsychotic medication such as haloperidol or flupenthixol. Use of such drugs has not been shown to help cocaine addicts achieve abstinence. These drugs would block the 'natural pleasure' system which may lead to unacceptable side effects, since dopaminergic hypofunction has been shown to be associated with dysphoria.

Ecopipam, a D_1/D_5 antagonist, has been shown in animal studies to reduce cocaine self-administration. In cocaine addicts, ecopipam has been shown to attenuate the euphoric and stimulating effects of cocaine and reduced the drive for further use (Romach *et al.*, 1999). However, in inpatient cocaine addicts, ecopipam failed to attenuate either the effects of or craving for cocaine (Nann-Vernotica *et al.*, 2001). Furthermore, in cocaine smokers, ecopipam actually increased smoking, craving and the effects of cocaine (Haney *et al.*, 2001). Currently it seems unlikely that ecopipam or D_1/D_5 antagonists will be useful in treating cocaine misuse.

Since psychostimulants increase dopamine levels, dopamine agonists (acting as a substitute) have been tried to treat cocaine misuse. Although bromocriptine initially showed promise in pilot studies, no benefit over placebo was subsequently shown (Eiler *et al.*, 1995; Handelsman *et al.*, 1997). The results of using amantadine are mixed (Alterman *et al.*, 1992; Handelsman *et al.*, 1995; Kampman *et al.*, 1996). Kampman *et al.* (2000) recently suggested that amantadine might be of particular use in those patients who experience more severe withdrawal from cocaine, hypothesizing that increased levels of dopamine from amantadine ameliorate the hypodopaminergic state seen in psychostimulant withdrawal.

Partial agonists are currently in development for treatment of psychostimulant misuse. BP-897 is a partial agonist at the dopamine D_3 receptor which has been shown in animal studies to reduce cocaine self-administration from cue exposure (Pilla *et al.*, 1999). However, once cocaine had been taken, there was no reduction in the total amount consumed. It is currently in Phase I trials (Preti, 2000).

Since cocaine is a dopamine reuptake inhibitor, drugs that also block this site have been developed as pharmacotherapy for cocaine misuse (Howell and Wilcox, 2001). Although in non-human primates such drugs have been shown to reduce cocaine self-administration, they also have some reinforcing potential. Nevertheless, they are less reinforcing than cocaine and altering the pharmacokinetics of these drugs for a slower onset and longer duration of action may make these drugs therapeutically useful.

Vaccination

Another strategy has been to develop a vaccine to cocaine. This raises a number of ethical issues including loss of privacy, use of the antibodies to identify patients, or mandatory vaccination (Cohen, 1997). Currently vaccines are in development and results in humans are awaited.

Antidepressants

Cocaine withdrawal is associated with a 'crash' consisting of depressive and anxiety symptoms. While generally it does not require treatment, it can drive further use. Desipramine, a tricyclic antidepressant which is predominantly a noradrenergic reuptake blocker, has been extensively studied as a treatment for cocaine dependence. A double-blind placebo-controlled randomized study found no advantage of desipramine, though a low dose was used (40 mg per day) (see Mendelson and Mello, 1996). No advantage of desipramine over placebo was found when combined with relapse prevention or clinical management (Carroll *et al.*, 1994). Desipramine only showed greater effectiveness in the first 6 weeks and thereafter only in patients with a lower level of abuse (1–2.5 g per week), and those with co-morbid depression. This emphasizes the heterogeneity of such patients and the need to develop specific tailored treatments.

Other antidepressants investigated include fluoxetine, which has been shown to acutely reduce subjective pleasurable effects of cocaine and show some potential in treating cocaine dependence. Batki *et al.* (1996) found that fluoxetine (40 mg daily) over a 12-week period resulted in increased length of abstinence and better retention in treatment.

Mood Stabilizers

Carbamazepine has been shown in animal models to block cocaine-induced kindling, a mechanism thought to underlie craving. Several studies have not shown that carbamazepine reduces cocaine use in humans (see Kranzler and Bauer, 1993).

Drugs used for Other Addictions

Disulfiram has been used to treat cocaine misuse alone, but it is also efficacious in reducing cocaine misuse complicating either opiate or alcohol dependence. In addition to inhibiting aldehyde dehydrogenase, disulfiram also blocks a key enzyme in the synthesis of dopamine, dopamine β-hydroxylase. It is not yet clear whether this inhibition plays a role in the efficacy of disulfiram in treating cocaine misuse, or whether reduction in alcohol drinking is associated with less opportunity for or more control over taking cocaine. Carroll *et al.* (1998) reported that disulfiram in co-morbid alcohol and cocaine abuse or dependence resulted in improved treatment retention and longer duration of abstinence from both substances. At 1 year follow-up, the benefits from this 12-week trial were still apparent (Carroll *et al.*, 2000).

In opiate addicts maintained on buprenorphine, cocaine dependence also responded to disulfiram with increased rates of abstinence (George *et al.*, 2000). Similarly in a methadone-maintained population, Petrakis *et al.* (2000) recently showed that addition of disulfiram reduced cocaine use. However, almost one-fifth of patients were unable to tolerate disulfiram and cautious use is required due to risk of hepatoxicity in a population with a high prevalence of viral hepatitis. Another concern is cardiotoxicity of disulfiram given the effects of cocaine on the heart.

Since opioids are known to modulate dopaminergic activity (see above), naltrexone has been used in cocaine misuse. Naltrexone does not appear consistently to acutely alter the subjective effects of cocaine (Kosten *et al.*, 1992; Walsh *et al.*, 1996). In patients also diagnosed with alcohol abuse, naltrexone had no effect on cocaine or alcohol use.

CONCLUSION

Pharmacotherapy will continue to play a central role in the treatment of substance misuse alongside psychosocial interventions. Their use in maintaining abstinence is likely to increase. Currently robust evidence to guide use of pharmacotherapy is often lacking for a specific substance disorder at a particular stage of addiction. Increased knowledge about the neurobiology of substance misuse and the effects of drugs and our treatments is urgently needed to inform future pharmacotherapeutic developments.

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XVII

Schizophrenia and Other Psychotic Disorders

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XVII-1

Animal Models for Schizophrenia

B.A. Ellenbroek and A.R. Cools

INTRODUCTION

'Schizophrenia is arguably the worst disease affecting mankind, even AIDS not excepted' (Nature Editorial, 1988). Nevertheless, and in spite of more than a century of research, the exact aetiology and pathology still elude us (Jablensky, 1997). Moreover, there is still an urgent need for improving treatment, especially for the more negative or defect symptoms. Although there are a number of reasons why our knowledge of schizophrenia is still so limited, one of the main reasons is undoubtedly the lack of adequate animal models (Ellenbroek and Cools, 2000a). Studying the function of the animal brain rather than the human brain has a number of clear advantages. First of all, its accessibility is much higher. Using microdialysis or *in vivo* voltammetry techniques, it is possible to detect changes in very small brain areas (<0.5 mm) in very short periods of time (in minutes with microdialysis, and with voltammetry even in seconds). Moreover, the detecting methods have improved so much that quantities as low as 20–50 fmol of a given neurotransmitter or metabolite can be detected. Another great advantage of animal research is the use of drugs which are not (yet) allowed in humans. These include highly selected receptor agonists or antagonists, but also drugs with a broader working profile. As discussed in a previous paper the reintroduction of clozapine, after the agranulocytosis incident in the 1970s, was no doubt due to the continued use in animals, showing its unique profile (Ellenbroek and Cools, 2000a). However, more important than these advantages is the possibility to alter the brain of animals and directly study changes in behaviour. Whereas in human research one is often limited to studying correlations between activity in neurotransmitters or brain regions and certain behavioural or cognitive parameters, in animal research one can actively enhance or decrease the neurotransmitter activity and directly study the functional consequences. This allows us to directly study the causal relationship between brain and behaviour.

AN INTRODUCTION TO ANIMAL MODELS

Unfortunately, when developing animal models for schizophrenia, it is quickly evident that no animal model will ever be able to completely cover the entire range of symptomatology. Patients with schizophrenia typically suffer from perceptual aberrations such as hallucinations and delusions. By definition, such symptoms cannot be modelled in animals since these rely on an interview with the patient.

The inability to model the entire disease has led to a plethora of animal models, each addressing different aspects of the disease, and each limited in its own right. In general, models for a human disease can be subdivided into two broad categories, depending on the specific goals pursued in the model. When the principal aim is to investigate the pharmacology, and evaluate new pharmacological treatments the models are generally referred to as screening tests. Such models have also been referred to as animal models with predictive validity (Willner, 1984) or animal models with pharmacological isomorphism (Matthysse, 1986). When, however, specific aspects of the disease are being investigated, the models are generally termed simulation models (Willner, 1991; Ellenbroek and Cools, 2000a). These models have also been referred to as animal models with face and construct validity (Willner, 1984) or animal models with cross-species psychological processes (Matthysse, 1986). Although these models generally assess very different aspects of the disease, and will be dealt with separately in the remainder of the chapter, it is important to realize that each of the models can be used for more than one purpose. Thus screening models are not only used for detecting new antipsychotic drugs, but can also be used to investigate the neuronal mechanisms of existing drugs. Likewise simulations models, although predominantly developed to study the neuropathology of the disease, can also be used to develop new treatments.

SCREENING TESTS FOR SCHIZOPHRENIA

Screening tests are primarily developed to test potential new therapeutic drugs. For that purpose, such tests should be quick and simple, and should have unequivocal parameters (Willner, 1991). In general, screening tests are developed following a standard scheme, which is depicted in the flow diagram in Figure XVII-1.1. Generally, the model's starting point is a very effective known clinical compound, generally referred to as the '*gold standard*'. This drug usually has a long clinical tradition and its effects in patients are very well described. Although not absolutely necessary, it would be beneficial if this drug has only limited side effects. In screening tests for schizophrenia the gold standard is generally haloperidol, although chlorpromazine is also often used. Both drugs have been used in clinical practice for well over 40 years now and their clinical effects are very well known. Unfortunately, the drugs have some quite severe side effects, most notably extrapyramidal side effects, which has been a serious limitation in developing a good screening test. As a result of that, in recent years models have been developed with other gold standards such as clozapine. Clozapine is almost completely devoid of extrapyramidal side effects, and it seems effective in therapy-resistant patients.

The second step in animal modelling is devising the right parameter. Theoretically any parameter could be used provided it can be assessed unequivocally. Sertindole, a novel antipsychotic that has been removed from clinical practice because of its ECG side effects, was primarily selected on the basis of a differential influence on dopamine cell firing in the substantia nigra versus the

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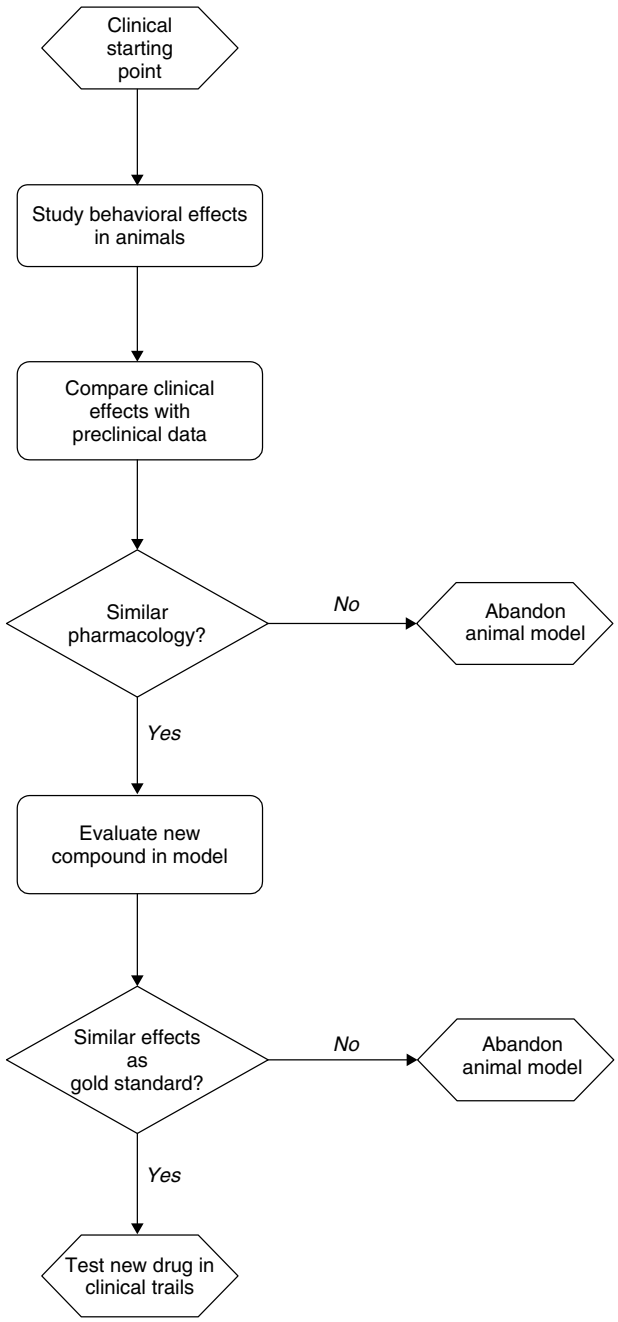

Figure XVII-1.1 A flow chart for developing screening tests for schizophrenia

ventral tegmental area (Skarsfeldt, 1992). Like clozapine, sertindole selectively reduced dopamine cell firing in the ventral tegmental area upon repeated treatment (White and Wang, 1983; Chiodo and Bunney, 1983). Likewise, biochemical data or binding profiles have been used to select novel (potential) antipsychotics. For instance, a number of selective dopamine D_4 receptor antagonists have been developed based on the suggestion that clozapine binds strongly to this receptor (Seeman, 1992). So far, however, these D_4 selective ligands appear to show very little antipsychotic-like efficacy (den Boer and Korf, 2000). Most screening tests are based on behaviour, which offer a number of advantages. Just as the symptoms of schizophrenia, behaviour is the final outcome of a number of alterations in brain activity. Therefore, behavioural studies take into account all the possible integrative and compensatory activity of the central nervous system. Moreover, it takes into account pharmacokinetic aspects, such as the blood–brain barrier penetration of a drug and possible active metabolites. In this respect *in vitro* experiments are severely limited (Willner, 1991). There are quite a number of antipsychotics which produce active metabolites, most notably thioridazine (which leads to mesoridazine) and risperidone (which is rapidly metabolized to 9-OH-risperidone). Although domperidon is a powerful dopamine D_2 antagonist like most other antipsychotics, it fails to be active in any behavioural screening test, since it does not pass the blood–brain barrier. Many different behavioural tests have been developed and the selection is only limited by the fact that the behaviour should be clearly identifiable and altered by the gold standard. They may include spontaneous behaviour, drug-induced behaviour or learned behaviour (Janssen *et al.*, 1965; Arnt and Skarsfeldt, 1998; Ellenbroek, 1993).

The third step in developing a screening test is the most crucial one, which is, unfortunately often underestimated, or only rarely investigated to its full extent. In this step the behaviour under study is compared with the known clinical features. This is the validation step, in which as many criteria as possible are investigated. Scrutinizing the clinical literature has led to a number of criteria based predominantly on pharmacological experiments (Ellenbroek, 1993; Ellenbroek and Cools, 1990). These criteria (see Table XVII-1.1) can be subdivided into general validation criteria (applicable to all screening tests) and specific criteria (applicable only to screening tests for antipsychotics). The general criteria aim to investigate the specificity of the model. Thus in screening tests all antipsychotics should mimic the gold standard (i.e. no false negatives should occur) and none of the non-antipsychotics should mimic the gold standard (no false positives should occur). Moreover, there should be some correlations between the potency in the model and the potency in the clinic. This correlation, however, depends strongly on pharmacokinetic aspects, and since these can differ between species this criterion does not need to be very strictly fulfilled (Ellenbroek and Cools, 2000a). On the other hand, since some drugs (like benzamide sulpiride) do not pass the blood–brain barrier as rapidly as other drugs (like other benzamides such as remoxipride or raclopride), one would expect that much higher doses of the former drugs are needed to obtain similar behavioural effects.

In contrast to the general criteria, the specific criteria have been developed to distinguish between different clinical effects of antipsychotics. As discussed above, in the ideal situation one searches for a gold standard which has virtually no side effects.

Table XVII-1.1 The criteria for validating screening tests for schizophrenia

Criteria	Description
General	
1	No false positives should occur
2	No false negatives should occur
3	A relationship should exist between potency in the model and that in the clinic
Specific	
1	Anticholinergic drugs should not ameliorate the effects of antipsychotics
2	Chronic treatment should not ameliorate the effects of antipsychotics
3	Benzodiazepines should enhance the effects of antipsychotics.

However, such drugs do not exist. Most of the antipsychotics induce extrapyramidal side effects (with the notable exception of clozapine). Therefore, a screening test with haloperidol or chlorpromazine as a gold standard might model the extrapyramidal side effects rather than the therapeutic efficacy, even if it fulfils most of the general criteria. Indeed a number of the classical screening tests, including catalepsy and reversal of apomorphine-induced gnawing, are now generally considered to be models for extrapyramidal side effects rather than for antipsychotic potential (Ellenbroek, 1993). However, careful analysis of the literature has led to the identification of a number of differences in the clinical pharmacology of extrapyramidal side effects and antipsychotic effects (Matthysse, 1981). Most notably, whereas the extrapyramidal side effects are reversed by anticholinergic drugs, the antipsychotic efficacy seems much less affected by this class of drugs. Likewise, with repeated treatment the therapeutic effect generally increases, whereas tolerance develops against the extrapyramidal side effects. The final criterion is based on the findings that benzodiazepines, although not very effective when given alone, have repeatedly been reported to enhance the effects of antipsychotic drugs (Ellenbroek, 1993).

This validation procedure is basically the final step in model building. Once a model fulfils a number of these criteria (or all of them in the ideal situation) it can be used to its full extent, i.e. new therapeutic agents can be tested, and the evaluation of the mechanism involved in the behavioural action of these antipsychotics can be studied. Given the high predictive validity of such models it is assumed that studies into the neuronal mechanism might also help us understand the mechanism of the therapeutic action of antipsychotics.

As mentioned above, a large number of different screening tests have been developed over the last 40 years, basically starting with the seminal papers by Janssen and his colleagues (Janssen *et al.*, 1965, 1966, 1967). Since then, many more tests have been developed. Arnt, in a recent review, clearly showed that of all the models currently available only a few have been extensively validated according to the criteria as delineated in Table XVII-1.1 (Arnt, 2000). These include blockade of the conditioned avoidance response, reversal of amphetamine-induced locomotor activity and the paw test. Nevertheless even these three models are not without limitations. Thus, the conditioned avoidance response, although popular for over 25 years now, has only a limited selectivity, as opiates and benzodiazepines show up as false positives (Ellenbroek, 1993). Moreover the effects of antipsychotics in this test are reversed by anticholinergic drugs (Arnt and Christensen, 1981). With respect to the reversal of dopamine agonist-induced hyperlocomotion, it is important to ensure that the drugs are effective at doses which do not block spontaneous locomotor activity. Moreover, there are quite a number of different dopaminergic drugs that can induce hyperlocomotion, such as amphetamine (Arnt, 1995), methylphenidate (Koek and Colpaert, 1993) and 6,7-ADTN (Arnt, 1983), each having a different pharmacological profile. Moreover, to complicate matters further, Arnt found that there is a difference in the pharmacology between hyperactivity induced by low ($0.5\,mg\,kg^{-1}$) and high ($2.0\,mg\,kg^{-1}$) amphetamine (Arnt, 1995). Thus, whereas haloperidol was about 10 times more effective in reversing the higher dose of amphetamine, clozapine showed about a 10-fold higher selectivity for the low dose of amphetamine (see Figure XVII-1.2a). This selectivity for the low dose of amphetamine seems to be a characteristic of all antipsychotics with limited extrapyramidal side effect liability. The notable exception is remoxipride, which seems to show hardly any selectivity at all.

The paw test was developed many years ago as an animal model based on two rather than one gold standard (namely haloperidol and clozapine). In previous electrophysiological experiments we had found that the dorsal and the ventral striatum play a differential role in regulating forelimb and hindlimb muscular activity (Ellenbroek *et al.*, 1985, 1988). Given the fact that antipsychotics with and without extrapyramidal side effects had been proposed to differentially affect these two structures, we reasoned that measured fore- and hindlimb activity might be a simple method of detecting differences between these antipsychotics (Ellenbroek *et al.*, 1987). Indeed we found that all classical antipsychotics (i.e. those with extrapyramidal side effects) affected both the forelimb and the hindlimb whereas atypical antipsychotics (i.e. those with very little or no extrapyramidal side effects) preferentially affected the hindlimb (see Figure XVII-1.2b). In a large series of experiments we evaluated all criteria and it appears that the paw test fulfils all of the criteria in Table XVII-1.1 to a large degree (Cools *et al.*, 1995;

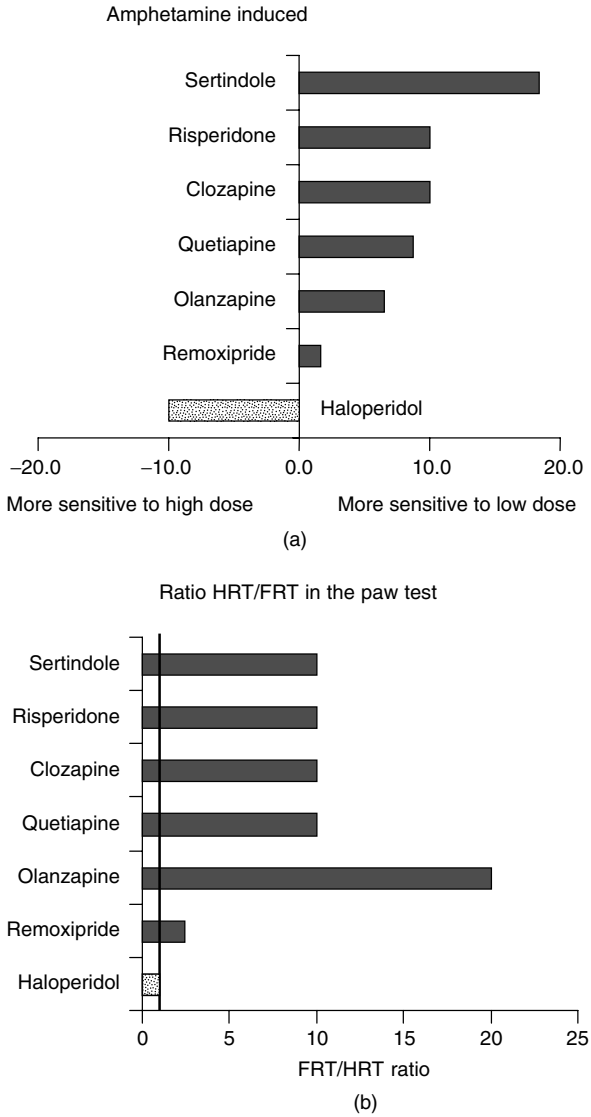

Figure XVII-1.2 (a) The effects of several antipsychotics on amphetamine-induced hyperlocomotion. Haloperidol shows a preference for blocking the high dose of amphetamine ($2\,mg\,kg^{-1}$), whereas all other drugs have a preference for the low dose ($0.5\,mg\,kg^{-1}$). (b) The effects of several antipsychotics on the paw test, represented in the ratio between the minimal effective dose necessary to influence the forelimb and the hindlimb retraction time. Haloperidol is equally effective on both, whereas all other drugs have a preference for increasing the hindlimb retraction time

Ellenbroek and Cools, 1988). Interestingly, as in the amphetamine experiment (see Figure XVII-1.2a), remoxipride also failed to show a clear 'atypical' picture in the paw test.

The paw test has also been used to investigate the mechanism of action of antipsychotics. The data clearly show that even though all antipsychotics effectively increase hindlimb retraction time, they do so via different mechanisms. Thus the haloperidol-induced increase could be reversed by the D_2 agonist quinpirole, whereas the effects of clozapine on hindlimb were sensitive to the D_1 agonist SKF38393 (Ellenbroek *et al.*, 1991). Table XVII-1.2 lists a number of clear differences in the pharmacology between haloperidol and clozapine as observed in the paw test (Ellenbroek *et al.*, 1994; Prinssen *et al.*, 1994a, 1994b). From these data it is evident that there are quite some differences between the two drugs, even in their effects on the hindlimb retraction time. This can also be ascertained when observing the rats: haloperidol-treated rats are rigid with extended toes, whereas clozapine-treated rats show an inability to withdraw the hindlimbs without concomitant rigidity. From the pharmacological studies summarized in Table XVII-1.2, a 'minimal' profile for a clozapine-like antipsychotics was determined, namely a blockade of α_1 adrenoceptors, a blockade of 5-HT_2 and a blockade of D_1 receptors. When selective antagonists of these three compounds are combined, a clozapine-like profile in the paw test was indeed observed. Combinations of any two out of these three, on the other hand, were ineffective (Prinssen *et al.*, 1994a). The differences in the pharmacological profile between the two drugs, especially in relation to hindlimb retraction time, is interesting in view of the finding that clozapine is therapeutically effective in haloperidol-resistant patients.

In spite of the fact that screening tests have been used to detect novel antipsychotics for over 35 years, and have been instrumental in understanding our knowledge on the working mechanism of antipsychotics, they have one inherent disadvantage. Since they are developed according to the analogy model (Figure XVII-1.1), they will produce in the ideal situation only a drug that is similar (or indeed identical) to the gold standard. Although such drugs may have a higher potency, or a longer duration of action, they will most likely work via similar mechanisms, having similar therapeutic and side effects. Whereas there is a need for drugs similar to clozapine, there is an even greater need for drugs with an entirely different mode of action. Only such drugs have the potency of creating a breakthrough in the therapy of schizophrenic patients. As discussed recently, there is a multitude of potential candidates, including glutamate agonists, certain neuropeptides and neurotrophic factors (Stahl and Shayegan, 2000). How such novel therapeutic drugs should be detected, however, is not yet clear, but it seems unlikely that the current generation of screening tests will be very useful.

Table XVII-1.2 The differential effects of selective monoaminergic agonists and antagonists on the effects of haloperidol and clozapine in the paw test

	Haloperidol		Clozapine	
	FRT	HRT	FRT	HRT
D_1 agonist	–	–	–	↓
D_2 agonist	↓	↓	–	–
5-HT_{1A} agonist	↓	↓	↑	↑
5-HT_2 agonist	–	↑	–	↓
5-HT_2 antagonist	↓	–	–	–
α_1 agonist	↓	↓	–	↓
α_1 antagonist	–	↑	–	↓
α_2 agonist	↑	↑	–	↓
α_2 antagonist	–	↓	–	–

↑, effect is significantly increased; ↓, effect is significantly reduced; –, no effect.

SIMULATION MODELS: A GENERAL INTRODUCTION

In contrast to screening tests, simulation models are designed to model specific (when possible all) aspects of the disease. Such models are particularly useful in investigating the neuronal substrate involved in the disease, and develop more rational therapeutic approaches. Indeed, such models might be much more useful in finding a therapeutic breakthrough. Unfortunately, in the case of schizophrenia it has proven very difficult to develop a simulation model. As we will see in the remainder of this chapter, a number of new approaches have recently been developed, which have not yet been extensively investigated and validated, but which might be potentially very relevant.

There are basically two reasons why simulation models have proven to be so difficult in schizophrenia. They are evident once we have studied the flow chart of Figure XVII-1.3, which describes the process of designing a simulation model. As in designing screening tests, simulation models have to have a clinical starting point, and its validation also critically depends upon the interaction between clinical and preclinical research. Depending on the knowledge one has about the disease to be modelled, different approaches can be taken. The best available approach is to mimic the aetiology. Unfortunately, for many diseases, including virtually all diseases involving the central nervous system, the aetiology is unknown. One exception is Huntington's chorea. This severe neurological disease, accompanied by uncontrolled choreatic movements, disturbed speech and deteriorating cognitive capacities, results from a loss of cholinergic and GABAergic cells in the caudate putamen complex. Huntington's disease is one of the few autosomal dominant disorders of the central nervous system which follows a classical Mendelian pattern. Molecular genetic techniques have shown that the aberrant gene is located on the short arm of chromosome 4 and normally contains a small number of CAG repeats in exon 1. When the number of repeats increases above 35 the disease becomes apparent. Moreover, the larger the number of repeats the more severe the disease. Recently, transgenic mice have been created which have CAG repeats in the Huntington's disease gene. It was shown that mice having 48 or 89 CAG repeats manifested progressive behavioural and motor dysfunction (Reddy *et al.*, 1998). Most diseases, however, are presumably due to a combination of genetic and non-genetic factors, most of which are virtually unknown. As we shall see later on, in spite of the fact that the aetiology of schizophrenia is still largely unknown, some of the most interesting and innovative simulation models are based on aetiological theories.

When the aetiological approach fails, because of lack of knowledge in this area, it has been customary to develop models on the basis of the pathology. Again Huntington's disease can be taken as an example. As described above, in Huntington's disease there is a degeneration of acetylcholine and GABA-containing neurones in the caudate–putamen. There have been several approaches to selectively destroy these cells in animals, using for instance local application of quinolinic acid (Beal *et al.*, 1986, 1991; DiFiglia, 1990). More recently, it has been reported that systemic application of 3-nitropropionic acid (an irreversible inhibitor of succinate dehydrogenase) also causes selective degeneration of striatal neurones (Beal, 1994; Borlongan *et al.*, 1995). The pathology approach has also been very successfully applied to Parkinson's disease, a disease in which the dopaminergic cells innervating the basal ganglia undergo degeneration. Using drugs such as 6-hydroxydopamine or

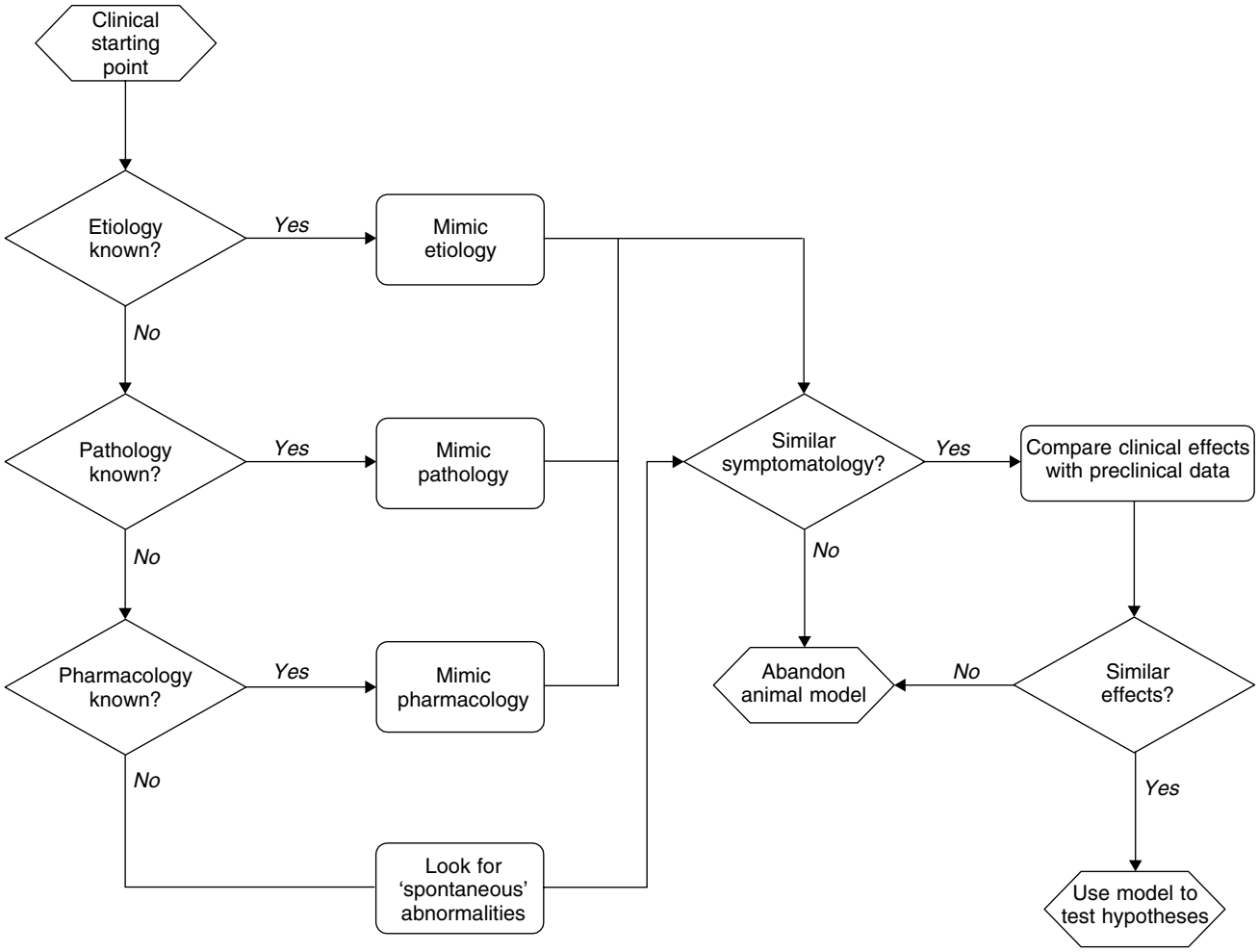

Figure XVII-1.3 A flow chart for developing simulation models for schizophrenia

MPTP a selective lesion of these cells can be achieved (Ungerstedt, 1971; Davis *et al.*, 1979; Andringa and Cools, 2000). In contrast to these neurological disturbances, the pathology of most psychiatric diseases, including schizophrenia, remains largely unknown, thus limiting the possibilities to model this aspect of the disease.

The third approach is based on pharmacological tools. If neither the aetiology nor the pathology is sufficiently known, one can use drugs to induce symptoms characteristic of the disease under study. Although this approach is sometimes used to model neurological diseases (for instance, antipsychotics can induce parkinsonian symptoms; Goldstein *et al.*, 1975), it is most often used in mimicking psychiatric symptoms. For instance, reserpine and yohimbine can induce symptoms of depression (Willner, 1984; Sanghvi *et al.*, 1969). Likewise certain β-carbolines, such as FG7142, can induce severe symptoms of anxiety in man (Dorow *et al.*, 1983) and CCK-4 can induce panic attacks in normal healthy volunteers and increase the existing symptomatology in patients (Bradwejn *et al.*, 1991; Bradwejn *et al.*, 1990; Koszycki *et al.*, 1991).

A final approach to develop simulation models could be termed the 'serendipity approach'. In this non-systematic approach one looks for 'spontaneous' abnormalities that may be related to a disease. This can be done by screening a large number of rat or mouse strains for a specific trait, or it may be a serendipitous finding. For instance, it was discovered by coincidence that olfactory bulbectomy in rats leads to disturbances reminiscent of depression (Cairncross *et al.*, 1978). Likewise, the spontaneously hypertensive rat shows some of the abnormalities seen in patients suffering from attention deficit/hyperactivity disorder (Sagvolden, 2000; Sagvolden *et al.*, 1992). Often, such initial screening is followed by selective breeding, such as the Flinders Low Sensitive Line of rats, originally bred for differences in acetylcholine sensitivity and now generally regarded as an interesting model for depression (Overstreet *et al.*, 1998; Tizabi *et al.*, 1999; Zangen *et al.*, 1999). Likewise, the recognition of differences in behaviour in the elevated plus maze has led to selective breeding for high and low levels of anxiety (Liebsch *et al.*, 1998a, 1998b).

Using any of these different approaches can lead to interesting simulation models of the disease. The next step in the simulation modelling approach is to investigate whether one observes similar symptomatology after mimicking either the aetiology, the pathology or the pharmacology. Purely theoretically, one does not necessarily have to observe similarities in symptomatology, given the species differences between animals. It is quite possible that an identical disturbance in a specific part of the brain may lead to different behavioural changes in humans and in rats or monkeys. However, given the relative lack of knowledge of the underlying aetiology and pathology, it would be very difficult to develop a convincing simulation model without some similarity in symptoms. As discussed below, we agree with Sarter and Bruno (Chapter III) that the clinical symptoms which contribute to the face validity of the

model are less important than symptoms reflecting impairments in underlying processes (such as attentional deficits), which contribute more to the construct validity of the model.

With respect to this modelling, it is important to realize that many aspects of psychiatric diseases cannot be assessed in animals. The key abnormalities observed in neurological patients are generally motor disturbances, like akinesia and rigidity in patients with Parkinson's disease, or abnormal clumsy movements in patients with Huntington's chorea. However, in psychiatric patients the symptoms are generally of a perceptual (like hallucinations), emotional (lack of interest, general anxiety) or cognitive (disturbed thinking, delusions) nature. As these symptoms can only be assessed through a psychiatric interview, they are beyond the realm of animal study. This implies that it is impossible to encompass an entire psychiatric disease in an animal model. It also means that one has to search beyond these symptoms for aspects of the disease which can be measured objectively and quantitatively in humans and animals, where possible using the same methods (Ellenbroek and Cools, 2000a). These may include specific behavioural alterations such as social isolation, specific cognitive phenomena (such as various aspects of memory or executive functioning) or more psycho-physiological aspects (such as abnormalities in information processing).

Another disadvantage is the lack of pathognomic parameters for any of the psychiatric disorders. For instance, chronic mild stress is known to reduce sucrose intake in rats (Papp *et al.*, 1991), which is often considered a model for anhedonia. Since anhedonia is a key symptoms of depression, the chronic mild stress model is often regarded as a simulation model for depression (Willner, 1997). However, anhedonia is also a key symptom of schizophrenia (Andreasen and Olsen, 1982). Likewise, a reduction in pre-pulse inhibition (see below) is often regarded as an animal model for schizophrenia. However, many patients, including those suffering from Gilles de la Tourette (Swerdlow, 1998), obsessive compulsive disorder (Swerdlow *et al.*, 1993) or Huntington's disease. (Swerdlow *et al.*, 1995), also show pre-pulse inhibition deficits. This lack of specificity means that a good animal model should not be based on mimicking one specific symptom, but rather as many aspects of the disease as possible (Willner, 1984).

In the case of schizophrenia, a number of different behavioural, cognitive and psycho-physiological parameters may be used in developing a simulation model (Ellenbroek and Cools, 2000a). With respect to the symptoms, only a few can be objectively assessed, and few of them have been investigated in any detail. The symptoms which can be assessed are anhedonia (see above), social withdrawal and stereotyped behaviour (Ellenbroek *et al.*, 2000). However, in recent years, attention has shifted from investigating the clinical symptoms of schizophrenia to investigating more fundamental deficits, such as disturbances in information processing and attention. These include pre-pulse inhibition, P_{50} gating and latent inhibition. Pre-pulse inhibition refers to the reduction in startle amplitude when the startling stimulus is preceded by a weak prepulse (Graham, 1975). This phenomenon has received much attention since it can be measured in humans and animals with virtually identical methods (Braff and Geyer, 1990). Moreover, pre-pulse inhibition is significantly reduced in schizophrenic patients (Braff *et al.*, 1978; Kumari *et al.*, 2000; Parwani *et al.*, 2000). P_{50} gating also refers to a reduction in response to an acoustic stimulus when this stimulus is preceded by a prepulse. However, there are some clear and important differences between the two paradigms. First of all, in pre-pulse inhibition the interval between the two stimuli is usually around 50–100 ms, whereas in P_{50} gating the optimal interval is longer (500–1000 ms). Another difference is that in P_{50} gating the two acoustic stimuli are identical and do not induce a startle response. In pre-pulse inhibition the pre-pulse is much smaller in amplitude than the stimulus eliciting the startle response. A final important difference between the two paradigms is that in pre-pulse inhibition a behavioural response is measured, whereas in P_{50} gating an electrophysiological (EEG) response is measured. In spite of these differences, it has been found that, like pre-pulse inhibition, P_{50} gating is diminished in schizophrenic patients (Adler *et al.*, 1982, 1985). It was shown recently that in humans (Schwarzkopf *et al.*, 1993) and in rats (Ellenbroek *et al.*, 1999) P_{50} and pre-pulse inhibition are two distinct phenomena with differences in neuronal substrate and pharmacology. Another modification of the acoustic startle response is the habituation which occurs upon repeated exposure. Like pre-pulse inhibition, startle habituation is also reduced in schizophrenic patients (Geyer and Braff, 1982; Geyer and Braff, 1987), but it has received much less attention in animal research.

Latent inhibition refers to the detrimental effect of prior stimulus pre-exposure upon the subsequent conditioning of that stimulus (Lubow, 1989). Although there is controversy about the underlying psychological mechanism, it has been suggested that it depends on the ability to learn to ignore irrelevant stimuli. Since schizophrenic patients have clear deficits in this area, it was expected that latent inhibition was disrupted in these patients. Unfortunately, the data in this respect are far from clear. Baruch and colleagues were the first to report that latent inhibition was disrupted in schizophrenia patients (Baruch *et al.*, 1988). However, the effect was only seen in acute patients and was very transient. When the patients were re-tested 2 months later, latent inhibition was normal again. In a replication study a similar transient phenomenon was reported (Gray *et al.*, 1995). However, disruption in latent inhibition is not found in all studies (Swerdlow *et al.*, 1996). Moreover, in a recent paper it was suggested that the deficit in latent inhibition observed in schizophrenia may be secondary to treatment (Williams *et al.*, 1998). Therefore, it seems difficult to confirm that schizophrenia is accompanied by an impairment of latent inhibition. Moreover, there is evidence that the neuronal mechanism involved in latent inhibition depends on the learning paradigm involved (Killcross *et al.*, 1994; Ellenbroek *et al.*, 1997), and since the learning paradigms differ between humans and animals, the relevance of latent inhibition for schizophrenia is at present questionable.

Apart from these information-processing deficits, there are other phenomena which could be assessed in animals with nearly identical techniques, but which have not been studied in great detail yet (at least not in relation to simulation models for schizophrenia). These include certain types of memory processing (more specifically working memory), aspects of executive functioning and planning, and preservation. With respect to the latter, it was found that schizophrenic patients suffer from an increased tendency to persevere when confronted with a two-choice guessing task (Frith and Done, 1983; Lyon *et al.*, 1986). Such guessing tasks can also be modelled in animals (Evenden and Robbins, 1983). However, these more cognitive symptoms of schizophrenia have so far received little attention.

As in the case of the screening models, simulation models also need to be validated on the basis of a specific comparison between known clinical facts and preclinical results. So far, this step has not received much attention, and therefore criteria have not been developed in any great detail. However, most of the criteria used in screening models also can be applied to validating simulation models. Moreover, some specific criteria may be deduced from a more detailed analysis of the disease. For instance, depression occurs about three times more often in women than in men. Therefore, if we are building an animal model for depression it would improve the validity of the model if the disturbances would occur more often in females than in males. Other features which may prove relevant validity criteria may be increased susceptibility to specific drugs, or abnormalities in specific biochemical processes, preferably extending beyond the central nervous system. In this respect it

has been found that the olfactory bulbectomized rat shows abnormalities in the immune and endocrine system reminiscent of those seen in depression (Leonard and Song, 2000; Kelly *et al.*, 2000). It should be realized that the border between symptom similarity and validation criteria is not always easy to draw. One might argue that abnormalities in the endocrine or the immune system add to the symptom similarity of the model. However, in the present paper we have decided to include only behavioural and psycho-physiological features in the symptom similarity. Biochemical or pharmacological similarities are considered validation criteria (see Tables XVII-1.3 and XVII-1.4).

There is evidence that schizophrenia is a neurodevelopmental hypothesis (see below), due to a combination of genetic and early environmental factors. However, the disease does not present itself until around or after puberty. If, in an animal model, the abnormalities also do not develop until after puberty, it would greatly strengthen the validity. Moreover, if the consequences of such an early disturbance were different between different strains of rats, this would further add to the validity of the model. Many studies have shown that schizophrenic patients are much more susceptible to dopaminergic drugs such as amphetamine and apomorphine (Lieberman *et al.*, 1987; Muller *et al.*, 1998), but also to drugs such as ketamine (Lahti *et al.*, 1994). These increased sensitivities can be used as validation criteria for simulation models. There is also a host of data with respect to alterations in the endocrine and immune system in schizophrenia. With respect to the latter, schizophrenic patients have increased basal levels of cortisol (Altamura *et al.*, 1989, 1999), show an increased cortisol response upon a challenge (Lammers *et al.*, 1995) and show a reduced dexamethasone suppression (Saffer *et al.*, 1985; Altamura *et al.*, 1989), suggesting a marked increase in stress sensitivity (Walker and Diforio, 1997). With respect to the immune system, schizophrenic patients are characterized by increased levels of interleukin (IL)-4 and IL-10, but decreased levels of IL-2 and Interferon-γ. This is characteristic of a shift in the TH_1-TH_2 balance in favour of TH_2. Moreover, schizophrenic patients generally have low plasma levels of natural killer cells (Muller *et al.*, 1999). Even though the data are not uncontroversial, they do suggest abnormalities in the immune system, which might explain the negative association between schizophrenia and autoimmune diseases such as rheumatoid arthritis (Vinogradov *et al.*, 1991). A careful scrutiny of the literature will, no doubt, lead to the identification of other criteria which can be used. Table XVII-1.4 summarizes the main aspects which can be used to validate screening models. These may include abnormalities in the neurochemistry in specific brain regions, preferably findings which have been replicated. Obviously, some of these criteria might be impossible to investigate, depending on the model used. In a recent review Weinberger summarizes the neurochemical alterations observed in the hippocampus in schizophrenia (Weinberger, 1999), but if the simulation model is based on a lesion of the hippocampus (see below) most of these subtle biochemical alterations cannot be assessed. Using these criteria, we can now turn to the various approaches used to build simulation models of schizophrenia.

Table XVII-1.3 The criteria for validating simulation models for schizophrenia

Criterion	Description
1	The model should have a genetic basis
2	The model should lead to alterations in the HPA axis activity
3	The disturbances should occur after puberty
4	The model should show immunological alterations similar to schizophrenia
5	The model should show biochemical alterations similar to schizophrenia
6	The model should show pharmacological alterations similar to schizophrenia

SIMULATION MODELS FOR SCHIZOPHRENIA: PHARMACOLOGICAL APPROACHES

The classical way of producing a simulation model for schizophrenia has been the pharmacological approach. It has long been known that drugs which increase dopamine neurotransmission such as amphetamine (Connell, 1958) or drugs which decrease glutamate neurotransmission such as phencyclidine (Greifenstein *et al.*, 1958) can induce psychotic symptoms in normal healthy volunteers and, more importantly, can worsen the existing symptomatology in schizophrenic patients (Lieberman *et al.*, 1987; Lahti *et al.*, 1994). For that reason these two classes of drugs have most often been used to develop simulation models for schizophrenia.

Dopamine agonists, as expected, induce a number of abnormalities in animals also observed in schizophrenic patients. These include reduced pre-pulse inhibition (Mansbach *et al.*, 1988), reduced latent inhibition (Weiner *et al.*, 1984), reduced P_{50} gating (Adler *et al.*, 1986), increased stereotypy and social isolation (Miczek and Gold, 1983; Ellenbroek, 1991; Ellenbroek *et al.*, 1989). With respect to this latter phenomenon it is important to realize that the effects of amphetamine on social behaviour have not been replicated in rat studies (Sams Dodd, 1998), which may be due either to a species difference, or to differences in methodology. Although dopamine agonists are usually grouped together there is evidence that differences exist between, for instance, amphetamine

Table XVII-1.4 Evaluation of the most important simulation models for schizophrenia

	Symptomatology							Validation					
	anhedonia	Soc iso	Stereo	PPI	P_{50}	LI	Startle Hab	1	2	3	4	5	6
DA ag	−	+	+	+	+	+	(−)	+	+	−	+	0	0
Glu antag	−	+	+	+	+	+	+	−	+	−	+	(+)	(+)
Apo-sus	+	0	+	+	−	+	0	+	+	0	+	+	(+)
NVH	0	+	0	+	0	+	0	+	(+)	+	+	0	+
Isol rear	(+)	(+)	0	+	0	−	−	+	(+)	0	+	0	(+)
MD	0	0	0	+	+	+	+	+	+	+	+	0	+

For a description of the validation criteria see Table XVII-1.3. Abbreviations: Soc iso, social isolation; Stereo, stereotyped responding; PPI, pre-pulse inhibition; P_{50}, P_{50} gating; LI, latent inhibition; Startle Hab, startle habituation; DA ag, dopamine agonists; Glu antag, glutamate antagonists; NVH, neonatal ventral hippocampal lesion model; Isol rear, isolation rearing; MD, maternal deprivation; −, the model does not comply; +, the model complies; 0, not known. If the symbol is in parentheses the effects are not completely clear or consistent.

and apomorphine. Thus, in the latent inhibition direct agonists such as apomorphine were found to be ineffective (Feldon *et al.*, 1991), although using the conditioned taste aversion paradigm some disruption was found (Ellenbroek and Cools, 1996). Recently we also found that some differences exist in the pharmacology between amphetamine- and apomorphine-induced disruption (Ellenbroek *et al.*, 2001).

With respect to the validation criteria (Table XVII-1.3), it has been reported that the effects of apomorphine in pre-pulse inhibition are strain dependent (Rigdon, 1990; Kinney *et al.*, 1999; Swerdlow *et al.*, 2000). In agreement with criterion 2, amphetamine increased cortisol release in humans (Grady *et al.*, 1996) and corticosterone in rats (Abdel *et al.*, 1997; Budziszewska *et al.*, 1996). However, the model fares less well with the puberty criterion, as amphetamine already disrupts pre-pulse inhibition in prepubertal rats (Martinez *et al.*, 2000). There is some evidence that amphetamine can induce some abnormalities in the immune system resembling those found in schizophrenia, including a reduction in IL-2 (House *et al.*, 1994) and in natural killer cells (Nunez-Iglesias *et al.*, 1996). As the effects of amphetamine on schizophrenia-like behaviour are usually observed after acute administration, very few studies have looked into biochemical or pharmacological aspects (criteria 5 and 6). For an overview of the model's validity see Table XVII-1.4.

Like dopamine agonists, NMDA antagonists such as phencyclidine and ketamine induce a number of behavioural abnormalities also observed in schizophrenia. These include a disruption of pre-pulse inhibition (Mansbach, 1991; Mansbach and Geyer, 1991; Mansbach and Geyer, 1989) and in P_{50} gating (Adler *et al.*, 1986). Although it was originally reported that phencyclidine does not affect latent inhibition (Weiner and Feldon, 1992), more recent studies did observe deficits (Turgeon *et al.*, 1998, 2000). Phencyclidine has also been reported to induce social isolation and stereotypy both in monkeys (Schlemmer *et al.*, 1978) and rats (Sams Dodd, 1998, 1999). Moreover, in contrast to amphetamine, phencyclidine also reduces startle habituation (Geyer and Braff, 1987). There is some controversy about the effects of NMDA antagonists on P_{50} gating. Adler and colleagues originally described a reduction similar to that observed after amphetamine (Adler *et al.*, 1986). On the other hand, we recently reported that ketamine did not affect P_{50} gating (de Bruin *et al.*, 1999). Although methodological differences cannot be excluded, it seems more likely that the difference depends on the differences between phencyclidine and ketamine, as phencyclidine has an additional dopamine reuptake-inhibiting effect.

The validity of the phencyclidine effects as a simulation model for schizophrenia have not been investigated as extensively as that of amphetamine (see Table XVII-1.4). In a recent paper, phencyclidine, in contrast to apomorphine, appeared equally effective in disrupting pre-pulse inhibition in several different strains (Kinney *et al.*, 1999). Moreover, phencyclidine was already effective in very young animals. (Martinez *et al.*, 2000), thereby violating both criteria 1 and 3. On the other hand, phencyclidine is know to stimulate the HPA axis, leading to strong increases in ACTH and corticosterone (Pechnick *et al.*, 1986, 1989, 1990) and suppresses IL-2 production (Thomas *et al.*, 1993; Dornand *et al.*, 1987). As with the effects of amphetamine, relatively little research has been performed with respect to criteria 5 and 6, though it has been reported that phencyclidine enhances the sensitivity of rats for amphetamine (Turgeon and Case, 2001; Turgeon and Roche, 1999), which would be in agreement with criterion 6. Moreover, (sub)chronic treatment with phencyclidine can lead to pathological changes in the brain, which may have relevance for schizophrenia (Ellison *et al.*, 1999; Martinez *et al.*, 2000). However, a much more detailed analysis will be necessary before criteria 5 and 6 can be properly evaluated.

SIMULATION MODELS FOR SCHIZOPHRENIA: PATHOLOGICAL APPROACHES

Although there has been a tremendous amount of research using both standard post-mortem analysis as well as more modern *in vivo* imaging techniques, the neuropathology of schizophrenia still eludes us. Moreover, the observed disturbances are often so small and diffuse that they cannot be modelled in animal research. For instance, the most replicated neuropathological finding in schizophrenia is a small increase in the size of the lateral ventricles (Harrison, 1999). Likewise, very subtle neuronal displacements have been described in the hippocampus (Kovelman and Scheibel, 1984), the entorhinal cortex (Jakob and Beckmann, 1986), the cingulate cortex (Benes, 1993) and the prefrontal cortex (Lewis, 2000). However, all these pathological findings are too subtle to be mimicked in an animal. Therefore, very few pathological models have been developed. One possible exception is the kainic acid model. In this model rats are injected with kainic acid into the ventricular space. Since kainic acid is an excitotoxic compound, this leads to relatively widespread cell death. However, as originally described by Nadler and colleagues, the hippocampus is much more vulnerable than other structures to the effects of kainic acid (Nadler *et al.*, 1978). According to some, this model has the advantage that a more subtle pathology in the hippocampus can be induced, compared to focal injections of kainic acid directly into the hippocampus (Csernansky and Bardgett, 1998). Kainic acid also induces intense axonal sprouting, which may lead to axonal disarray, similar to that observed in the brain of schizophrenic patients. Moreover, this treatment leads to increased density of D_2 receptors in the nucleus accumbens and reduced density of kainic acid binding sites in the hippocampus (Bardgett *et al.*, 1995; Csernansky and Bardgett, 1998). Similar changes have also been observed in post-mortem brains of schizophrenic patients, and thus add to the validity criterion 4. Unfortunately, this model has not been evaluated behaviourally to any great extent. Neither pre-pulse inhibition, latent inhibition nor startle habituation have been investigated, although the model seems to fulfil a number of the validation criteria described in Table XVII-1.3. Thus kainic acid-treated animals show an increased response to novelty, suggestive of an enhanced response to stress, and the animals are much more sensitive to both dopamine agonists and NMDA antagonists (Bardgett *et al.*, 1997; Csernansky and Bardgett, 1998).

SIMULATION MODELS FOR SCHIZOPHRENIA: AETIOLOGICAL APPROACHES

The most interesting category of animal models comprises those in which one tries to mimic the aetiology of the disease. Even though we still have only a limited knowledge of the aetiology of schizophrenia, there is now ample evidence that schizophrenia is a neurodevelopmental disorder in which genetic and early environmental factors interact (Pilowsky *et al.*, 1993; Weinberger, 1996; Ellenbroek and Cools, 1998). This has led to a large number of animal models in which the long-term consequences of early manipulations are investigated. These include prenatal treatments with the mitosis-inhibiting drug methylazoxymethanol (Talamini *et al.*, 1998, 2000; Ellenbroek *et al.*, 2000). or with corticosterone (Diaz *et al.*, 1995, 1997). A relatively new strategy has been to selectively alter the genes involved in growth and development. A potentially interesting model in this respect is the NCAM-180 knockout mouse. This mouse, lacking one of the neural cell adhesion molecules, shows, among others, enlarged ventricles and reduced pre-pulse inhibition (Wood *et al.*, 1998). However, most of these models have not been investigated to any great extent. Four animals models have been more extensively studied,

namely selective breeding for dopamine hypersensitivity, early hippocampal damage, isolation rearing and maternal deprivation.

Selective breeding for dopamine (hyper)sensitivity was originally performed to study differences in personality (see Chapter XXVI-1). Wistar rats were divided on the basis of their gnawing response to apomorphine as susceptible (APO-SUS, showing a strong gnawing response) or unsusceptible (APO-UNSUS, showing a very weak gnawing response). Using a special pharmacogenetic breeding strategy which prohibits inbreeding (at least in the short run) these APO-SUS and APO-UNSUS rats were selectively bred. Since dopamine hyper(re)activity has been implicated in the pathophysiology of schizophrenia for a very long time, it is not unexpected that APO-SUS rats show a number of schizophrenia-like behavioural deficits, including stereotypy, disrupted pre-pulse inhibition and latent inhibition (Ellenbroek *et al.*, 1995; Cools *et al.*, 1990). So far startle habituation has not been evaluated in these rats, and P_{50} gating seems to be normal (De Bruin *et al.*, in preparation). Interestingly, APO-SUS rats also drink significantly smaller quantities of sucrose than APO-UNSUS rats, suggesting they show a certain degree of anhedonia (Cools and Gingras, 1998). Moreover, the model seems to fulfil a number of the validation criteria described in Table XVII-1.3. Obviously, it has a genetic basis (criterion 1) and the APO-SUS rats are much more sensitive to dopamine agonists such as apomorphine and amphetamine (Cools *et al.*, 1990, 1997). APO-SUS rats are also much more sensitive to stress, showing an increased ACTH and corticosterone response (Rots *et al.*, 1995). In addition, APO-SUS rats show immunological alterations resembling schizophrenia, including reduced numbers of natural killer cells and a relative dominance of T_{H2} cells (Cools and Gingras, 1998; Kavelaars *et al.*, 1997), which may in part explain their resistance in animal models for autoimmune disease such as rheumatoid arthritis (van de Langerijt *et al.*, 1994). Finally there is some evidence that APO-SUS rats show biochemical alterations similar to those observed in schizophrenia, including increased mRNA levels of tyrosine hydroxylase in the substantia nigra and of D_2 receptor binding in the neostriatum (Rots *et al.*, 1996a).

As discussed above, there is a large literature pertaining to the involvement of the hippocampus in the pathology of schizophrenia (Harrison, 1999; Weinberger, 1999). This extensive literature, combined with the neurodevelopmental hypothesis, has led Lipska and Weinberger to investigate the long-term effects of neonatal lesions of the hippocampus (Lipska *et al.*, 1993). In behavioural studies it was subsequently shown that lesioning the hippocampus at postnatal day 7 leads to a reduction in pre-pulse inhibition in adult rats (Lipska *et al.*, 1995), as well as to deficits in latent inhibition (Grecksch *et al.*, 1999) and in social behaviour (Becker *et al.*, 1999; Sams Dodd *et al.*, 1997). This latter effect was also observed in monkeys (Bachevalier *et al.*, 1999). Unfortunately, neither startle habituation nor P_{50} gating have so far been investigated.

The model seems to fulfil a number of the criteria from Table XVII-1.3. Thus the effect seems to depend on the strain of rats used, suggesting an interaction with the genetic background (Lipska and Weinberger, 1995), and most of the effects do not develop until after puberty (Lipska *et al.*, 1993, 1995). In agreement with criterion 6, rats with neonatal hippocampal lesion are more sensitive to amphetamine (Lipska *et al.*, 1993) and to apomorphine (Schroeder *et al.*, 1999). Whether the model fulfils criterion 2 is not completely clear. Although the hippocampally lesioned rats show an increased locomotion to novelty (Lipska *et al.*, 1993), to our knowledge the ACTH and corticosterone response has not been measured. Moreover, immunological parameters have not yet been measured in this model. Nevertheless, the model seems to be one of the most promising simulations to date (see Table XVII-1.4). A distinct disadvantage of the model is that it involves the virtually complete destruction of the hippocampus. Although some reductions in the volume of the hippocampus have been reported in schizophrenia, the effects are much more subtle (Harrison, 1999). On the other hand, in monkeys the neonatal hippocampal lesion leads to significant reduction in *N*-acetyl aspartate in the prefrontal cortex (Bertolino *et al.*, 1997), a finding also reported in schizophrenia (Bertolino *et al.*, 1998).

An environmental simulation model for schizophrenia is the isolation rearing model. In this model rats are isolated upon weaning (typically postnatal day 21). When the isolation period is long enough (usually 8 weeks or more), abnormalities in pre-pulse inhibition are observed (Geyer *et al.*, 1993; Wilkinson *et al.*, 1994; Varty and Higgins, 1995), though there is a discussion about the robustness of the effect (Domeney and Feldon, 1998; Weiss *et al.*, 1999). Unfortunately, most of the other behavioural parameters seem normal, including startle habituation (Varty and Geyer, 1998) and latent inhibition (Wilkinson *et al.*, 1994). Moreover, sucrose consumption is even increased in rats reared in isolation (Hall *et al.*, 1998). On the other hand, in a recent paper a reduced incentive value for sucrose was observed in rats isolated from postnatal day 21 to 28 (Van den Berg *et al.*, 1999). This same group also reported reduced social interaction in rats after juvenile isolation. (Hol *et al.*, 1999), suggesting this model might be more related to the negative than the positive symptoms of schizophrenia. In agreement with criterion 1, the effects of isolation rearing on pre-pulse inhibition appear to depend on the strain of rats being used (Hall *et al.*, 1997; Varty and Geyer, 1998; Weiss *et al.*, 2000). Moreover, the isolated rats are more sensitive to the locomotor-activating properties of dopaminergic drugs (Phillips *et al.*, 1994; Jones *et al.*, 1990). Isolated rats generally show an increase locomotor activity upon a novelty challenge (Wilkinson *et al.*, 1994; Phillips *et al.*, 1994). It is, however unclear whether they also show an increase in HPA axis activity. Moreover little is known about the effects of isolation rearing on immunological parameters.

The last simulation model is based on the long-term consequences of an early severe stressful life event, namely maternal deprivation. When analysing this model, it is important to realize that there are many different forms of maternal deprivation, including repeated short-term separations (1–3 h). However, the model most relevant to schizophrenia consists of a single 24 h period of maternal deprivation, shortly after birth. Depending on the timing of the deprivation, this maternal deprivation leads to a significant reduction in pre-pulse inhibition (Ellenbroek and Cools, 2000b; Ellenbroek *et al.*, 1998), although some people have been unable to replicate this (Lehmann *et al.*, 2000). The reason for this failure to replicate these findings is unclear, but methodological differences may be partly responsible. Maternal deprivation also leads to a reduction in latent inhibition (Ellenbroek and Cools, 1995), startle habituation and P_{50} gating (Ellenbroek *et al.*, in preparation). So far the effects on social behaviour and sucrose consumption have not been investigated. With respect to validation, the model of maternal deprivation seems to be quite successful. The maternal deprivation effect seems to depend on the genetic background. Thus, whereas maternal deprivation leads to a reduction in pre-pulse inhibition in Wistar rats, it has no effect in Fischer 344 or Lewis rats (Ellenbroek and Cools, 2000b). The insensitivity of Lewis rats is particularly interesting, since this strain also seems to be much less sensitive to isolation rearing and neonatal hippocampal lesioning as well as dopamine agonists (Lipska and Weinberger, 1995; Varty and Geyer, 1998). Moreover, maternal deprivation leads to an enhanced sensitivity to amphetamine (Zimmerberg and Shartrand, 1992) and apomorphine (Rots *et al.*, 1996b; Ellenbroek and Cools, 1995). In agreement with criterion 2 there is a large body of evidence that maternal deprivation leads to alterations in the HPA axis (Levine, 1994; van Oers *et al.*, 1998, 1999), although it should be realized that the exact effect depends upon the maternal deprivation paradigm and the time of measurement (Workel *et al.*, 1997). We recently showed that the effects of maternal deprivation on pre-pulse inhibition are only seen after puberty, in agreement with criterion 3.

So far very little work has been done on the effect of maternal deprivation on immunological processes. Moreover, few biochemical alterations after maternal deprivation relevant to schizophrenia have so far been published. Nevertheless, reductions in hippocampal levels of PSA-NCAM (Foley *et al.*, 2000; Riva *et al.*, 2000) and BDNF have been reported, similar to those observed in schizophrenia (Weinberger, 1999). Finally maternally deprived rats show an increase in mRNA for tyrosine hydroxylase in the substantia nigra (Rots *et al.*, 1996b).

EPILOGUE

Developing animal models for schizophrenia has proven to be extremely difficult because there is a great lack of knowledge on the aetiology, pathology and pharmacology of this disease, which is essential for model building. Moreover, most of the symptoms used in the diagnosis of schizophrenia-like hallucinations, delusions and thought disorder are virtually impossible to measure in animals. For that reason most animal models for schizophrenia belong to the class of screening tests and are more relevant for the pharmacology and for our understanding of the mechanisms of action of antipsychotic drugs. Although these models have been very useful in the past, they do not help us in our understanding of the disease itself, nor are they likely to produce a therapeutic breakthrough.

The notion that schizophrenia is likely due to an interaction between early stressful life events and genetic factors has led to a number of novel models which aspire to simulate the disease. In the present chapter we have discussed the process of developing and validating such simulation models and have evaluated the most relevant models known today. The most promising ones seem to be APO-SUS rats, the neonatal hippocampal lesion model and the maternal deprivation model (see Table XVII-1.4). However, it is important to realize that none of the models fulfils all the criteria laid out in Table XVII-1.3. Moreover, most of the models have not been evaluated to any great extent. Nevertheless, models such as maternal deprivation and neonatal hippocampal lesioning have shown that early alterations can have retarded but persistent effects on brain and behaviour. It is to be expected that such models will help us understand how the organism deals with such environmental insults and may also help us understand how the neuropathology of schizophrenia develops. In the end this may even lead to novel therapeutic approaches aimed more at preventing the development of the disease rather than treating the symptoms once the disease has emerged.

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XVII-2

Aminergic Transmitter Systems

P. Falkai

INTRODUCTION

The biogenic amines include the catecholamines dopamine, noradrenaline and adrenaline, and the indoleamine serotonin. All of these monoamines have a claim to be regarded as central neurotransmitters, and dopamine and serotonin seem to play a crucial role in the pathophysiology of schizophrenia. This chapter attempts to summarize the most important findings related to these two transmitters and schizophrenia. Before doing so, it should be pointed out that these monoamines are interconnected closely in their biosynthesis. Their biosynthesis takes place as the stepwise conversion of amino acids in the presence of specific enzymes. The catecholamines are derived from the L-amino acid tyrosine, which is converted into L-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase. A second enzyme, aromatic amino acid decarboxylase (AADC), converts DOPA into dopamine, which may be converted consecutively into noradrenaline and adrenaline, reactions that are catalysed by the enzymes dopamine β-hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT), respectively.

The sequence of reactions by which serotonin is formed involves two steps: the amino acid tryptophan is first converted into 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. Then AADC converts this intermediary amino acid into serotonin (for an overview, see Niewenhuys 1985).

DOPAMINE

The dopamine hypothesis of schizophrenia, which postulates that schizophrenia is related to increased dopamine function, has been a predominant theory on schizophrenia for a long time (for review, see Carlsson *et al.*, 2001), although many attempts have been made to devaluate the central role of dopamine. Besides the compelling evidence that blockade of dopamine D2 receptors generates an antipsychotic response (e.g. Kapur and Seeman, 2001), there are several other lines of evidence supporting the notion that dopamine plays an important role in the pathophysiology of schizophrenia. Some of this evidence is outlined below, stressing newer approaches and pointing out new frontiers, such as dopamine-stabilizing agents, and the role of dopamine within the complex circuitry of the human brain.

Evidence for Dopamine Dysfunction

Indirect Pharmacological Evidence

Concentrations of dopamine and its metabolite homovanillic acid (HVA) in post-mortem brains of schizophrenic patients have consistently yielded patient–control differences, although the areas where the abnormalities are found are not consistent (e.g. Davis *et al.*, 1991).

Receptor studies have also been fairly consistent, with D2 but not D1 receptors increased in the striatum of schizophrenics. Interestingly, when D2 receptors were examined in patients who were not schizophrenic (e.g. Alzheimer's disease and Huntington's disease) but who had previously been treated with neuroleptics, striatal dopamine receptors were found to be increased by only 25% in contrast to the more than 100% increases found in schizophrenics (Seeman *et al.*, 1987).

A large number of studies measuring HVA found an increase in schizophrenia (for overview, see Widerlov, 1988). However, cerebrospinal fluid (CSF) HVA has been found to be decreased in subgroups of schizophrenic patients compared with control groups, such as patients who respond poorly to neuroleptics.

HVA measured in plasma (pHVA) seems be associated with activity of central, striatal dopamine neurons in human subjects (Kahn and Davidson, 1993). When neuroleptic treatment is discontinued in schizophrenic patients, pHVA increases in those patients experiencing a symptomatic worsening (Davidson *et al.*, 1991b). In contrast, neuroleptic treatment decreases pHVA concentrations, particularly in patients who benefit from that treatment (Davidson *et al.*, 1991a).

Direct Evidence for Dopaminergic Dysfunction from Imaging Studies

Imaging of Dopamine Receptors

In vivo examination of D2 receptor density using positron emission tomography (PET) has provided conflicting results (Table XVII-2.1). Using the ligands raclopride and bromospiperone (Farde *et al.*, 1990; Martinot *et al.*, 1989), no difference was detected between schizophrenics and control subjects, while using methylspiperone a difference in D2 receptor density was detectable (Wong *et al.*, 1986). Meanwhile, meta-analyses have proven that a substantial proportion of newer treated patients with schizophrenia have increased numbers of striatal dopamine receptors (Laruelle, 1998; Zakzanis and Hansen, 1998). Initially, there were difficulties in examining extrastriatal dopamine receptors, as there is only a small number in cortical regions, such as the functionally important limbic nuclei. Since 1997, several interesting papers have been published along these lines: one described decreased D2 receptor binding in the anterior cingulated cortex in schizophrenia (Suhara *et al.*, 2002); another described a marked upregulation induced by classical as well as atypical neuroleptics in the temporal cortex (Xiberas *et al.*, 2001b); a further paper described a differential effect of haloperidol (upregulation) and clozapine (downregulation) in striatal and extrastriatal regions (Talvik *et al.*, 2001); and finally, another study demonstrated a differential effect of amisulpride in low and high dosages (Xiberas *et al.*, 2001a). Low plasma

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Table XVII-2.1 The dopaminergic system in schizophrenia: imaging studies (1)

Receptor subtype	Distribution	Selected literature	Detailed findings in schizophrenia	Change in schizophrenia overall
D1	Cortical and striatal	Okubo *et al.* (1997)	Cortical: increased Striatal: decreased	Cortical: increase Striatal: no change
D2/3	Striatal	Laruelle (1998), Zakzanis and Hansen (1998)	Increased in a substantial proportion of patients	Increase
	Extrastriatal (cortical and limbic areas)	Goldsmith *et al.* (1997), Suhara *et al.* (2002)	No clear pattern of receptor density; change in laminar distribution?	No change
D4	Striatal	Seeman *et al.* (1993), Soares and Innis (1999)	6 × increase; no replication	No change

Table XVII-2.2 The dopaminergic system in schizophrenia: imaging studies (2)

Parameter	Distribution/ localization	Selected literature	Detailed findings in schizophrenia	Change in schizophrenia overall
Dopamine release	Synaptic cleft	Breier *et al.* (1997), Abi-Dargham *et al.* (1998)	Enhanced amphetamine-induced displacement of postsynaptic D2 binding via dopamine outflow Presynaptic (vesicle) and postsynaptic (membrane transporter) are involved	Increase
Dopamine synthesis	Terminals of nigrostriatal fibres	Hietala *et al.* (1999), Lindström *et al.* (1998)	Increased striatal uptake of [^{18}F] FDOPA	Increase
Dopamine basal levels	Synaptic cleft	Laruelle *et al.* (1998)	Increased in schizophrenia	Increase

concentrations induced marked extrastriatal binding and low striatal binding, whereas higher plasma concentrations induced marked binding in both extrastriatal and striatal regions. This suggest that there might be a distinct effect of dopamine on striatal and extrastriatal regions, and that currently the right tools are in development to investigate these questions in more detail.

Finally, PET studies examining different antipsychotics found that the threshold for clinical response and extrapyramidal side effects could be separated in terms of different D2 occupancy (Farde *et al.*, 1992; Nordstrom *et al.*, 1993; Kapur *et al.*, 2000a). This has helped us to understand that neuroleptics act via the dopaminergic system.

Imaging of Further Aspects of the Dopaminergic System: Release, Uptake and Basal Levels

In recent years, modern imaging technology has progressed to measuring the release of dopamine precursors and dopamine itself in schizophrenia (Table XVII-2.2). It has been shown that the synthesis of labelled dopamine or fluorodopamine in the brain, determined by means of PET following administration of radiolabelled L-DOPA or fluoro-L-DOPA, is increased in drug-naive schizophrenic patients compared with age-matched controls (Hietala *et al.*, 1994; Dao-Costella *et al.*, 1997). Single photon emission computed tomography (SPECT) and PET studies, using a sophisticated technique to measure the release of dopamine in the basal ganglia *in vivo*, have shown that following an amphetamine challenge, this release is elevated in drug-naive schizophrenic patients compared with age-matched controls, and that this elevation correlates to the induction of positive psychotic symptoms (Laruelle *et al.*, 1996; Breier *et al.*, 1997; Abi-Dargham *et al.*, 1998). In an additional series of experiments, Laruelle (2000), using the SPECT technique with alpha-methyltyrosine as a tool, obtained evidence that the unchallenged release of dopamine is elevated in schizophrenic patients compared with controls. As well as release of dopamine, its uptake and the basal levels are additional interesting markers to investigate the function of the dopaminergic system in schizophrenia (see Table XVII-2.1 for further details).

Taking several shortcomings of these methods into account (for detailed discussion, see Carlsson *et al.*, 2001), generally these data support the notion that a dysfunction of the dopaminergic system is involved in the pathophysiology of schizophrenia, which supports treatment strategies involving this transmitter system. Recent efforts have focused on the D4 and, partly, D3 and D1 receptor subtypes. In general, although antipsychotic effectively is transmitted via D2 receptor blockade, it this no longer an attractive target for improved antipsychotic therapy. This is partly because classical D2 receptor blockade is associated with concomitant hypodopaminergia, supporting the development of negative symptoms and cognitive deficits.

Further Post-Mortem Evidence for Dysfunction of the Dopaminergic System in Schizophrenia

Using post-mortem histological analyses of human brain tissue, investigators have begun to identify subtle structural abnormalities in the prefrontal cortex of schizophrenia subjects; many of these abnormalities may contribute to the cognitive deficits observed in this illness. Relevant to this chapter are reported abnormalities in the structure of mesoprefrontal dopaminergic neurons in schizophrenia subjects. An early morphometric study provided the first suggestion that the structural integrity of mesoprefrontal dopaminergic neurons may be abnormal in schizophrenia subjects (Bogerts *et al.*, 1983). Investigators observed decreased size and number of neuromelanin-containing cell bodies in the ventral tegmental area of schizophrenia subjects. However, additional studies are required to determine to what extent this effect is due to a specific decrease in the size and number of cortically projecting dopamine-containing neurons. Recent studies of dopaminergic neuron structure have focused on the integrity of dopaminergic nerve terminals within the prefrontal cortex. Immunocytochemical analyses of post-mortem tissue have revealed a decreased density of fibres immunoreactive for tyrosine hydroxylase and dopamine transporter protein in the prefrontal cortex of schizophrenia subjects (Akil *et al.*, 1999). In contrast, the density of fibres immunoreactive for the serotonin transporter is unaffected, suggesting that the loss of proteins associated with

dopaminergic terminals may be a specific correlate of the disorder (Gurevich and Joyce, 1997; Akil *et al.*, 1999).

It is not clear whether the reduction in tyrosine hydroxylase and dopamine transporter levels in the prefrontal cortex of schizophrenia subjects is due to altered protein levels in dopaminergic nerve terminals, or altered protein levels in dopaminergic nerve terminals, or altered number/length of dopaminergic terminals. However, the hypothesis that dopaminergic nerve terminal density may actually be decreased is consistent with emerging data that structural abnormalities in the prefrontal cortex of schizophrenia subjects involve a reduction in neuropil volume (Lewis *et al.*, 1999; Selemon and Goldman-Rakic, 1999; Lewis and Gonzalez-Burgos, 2000). Specifically, recent studies indicate that there is an increase in neuronal density in the prefrontal cortex of schizophrenia subjects with no change in the total number of neurons (Pakkenberg, 1993; Akbarian *et al.*, 1995; Daviss and Lewis, 1995; Rajkowska, 1997; Rajkowska *et al.*, 1998, Selemon *et al.*, 1998). One interpretation of the latter findings is that the increased neuronal density is a consequence of loss of surrounding neuropil.

Structural abnormalities other than those involving dopaminergic nerve terminals are also thought to contribute to reduced neuropil and impaired functional capacity of the prefrontal cortex in schizophrenia subjects. For example, results of post-mortem studies indicate that pyramidal cells in the prefrontal cortex of schizophrenia patients exhibit a decreased somal size and a decreased density of dendritic spines (Garey *et al.*, 1998; Rajkowska *et al.*, 1998; Glanz and Lewis, 2000; Pierri *et al.*, 2001). Several markers of γ-aminobutyric acid (GABA)-containing interneurons are also affected in schizophrenia in the absence of detectable changes in cell number (Akbarian *et al.*, 1995; Woo *et al.*, 1998; Pierri *et al.*, 1999; Volk *et al.*, 2000). The decrease in density of GABA membrane transporter immunoreactive axon cartridges on the axon initial segment of pyramidal cells in the prefrontal cortex of schizophrenia subjects (Woo *et al.*, 1998) suggests that alterations in the structure of interneurons also contribute to a reduction in the neuropil.

Beyond D2 Blockade: Dopaminergic Stabilizers in the Treatment of Schizophrenia

A series of compounds capable of stabilizing the dopaminergic system without inducing hypodopaminergia have been developed and are being tested in clinical trials. Some of these new drugs are partial dopamine receptor agonists, e.g. (−)-3-(3-hydroxyphenyl)-*N*-n-propylpiperidine ((−)-3PPP), acting on the D2 family of receptors (Lahti *et al.*, 1998). Others are pure antagonists, again acting on the D2 family of receptors, and can thus readjust elevated dopamine functions. In contrast to the currently used antipsychotic agents, these antagonists do not cause hypodopaminergia; on the contrary, they antagonize subnormal dopamine function. The reason for this aberrant pharmacological profile seems to be that their action on different subpopulations of dopamine receptors differs from that of the currently used drugs. Thus, whereas they exert a strong action on dopaminergic autoreceptors, they have a weaker effect postsynaptically and seem unable to reach a subpopulation of postsynaptic dopamine receptors (Svensson *et al.*, 1986; Sonesson *et al.*, 1994; Hansson *et al.*, 1995).

Connecting Phenotype and Genotype: Catechol-O-Methyltransferase and Schizophrenia

About half of the risk for developing schizophrenia can be attributed to genetic factors; the remaining half is attributed to nongenetic, environmental influences. The search for a genetic basis of schizophrenia has led to a number of informative chromosomal regions where the successibility genes are situated. It seems likely that each gene will explain only a very limited percentage of the attributed risk. Therefore, future research will try to connect these successibility genes with the neurobiological mechanism contributing to the pathophysiology of schizophrenia. An elegant set of data has been published (Egan *et al.*, 2001) supporting the notion of the dysfunction of the dopaminergic system in schizophrenia. The relationship of a common functional polymorphism (Val (108/158) Met) in the catechol-O-methyltransferase (COMT) gene was examined, which accounts for a four-fold variation in enzyme activity and dopamine catabolism, with both prefrontally mediate cognition and cortical physiology. In 175 patients with schizophrenia, 219 unaffected siblings, and 55 controls, COMT genotype was related in allele dosage fashion to performance on the Wisconsin card sorting test of executive cognition and explained 4% of variance ($P = 0.001$) in frequency of perseverative errors. Consistent with other evidence that dopamine enhances prefrontal neuronal function, the load of the low-activity Met allele predicted enhanced cognitive performance. Then the effect of COMT genotype on prefrontal physiology was examined during a working memory task in three separate subgroups assayed with functional magnetic resonance imaging (fMRI). Met allele load consistently predicted a more efficient physiological response in the prefrontal cortex. Finally, in a family-based association analysis of 104 trios, a significant increase in transmission of the Val allele to the schizophrenic offspring was found. These data suggest that the COMT Val allele, because it increases prefrontal dopamine catabolism, impairs prefrontal cognition and physiology, and by this mechanisms slightly increases the risk for schizophrenia.

This is a fine example of connecting the phenotype of dopamine dysfunction with a functional polymorphism. Although the effect on the risk for schizophrenia is small, it shows how to examine if biological markers are relevant for the pathophysiology of schizophrenia.

Conclusions

Increased dopamine function or activity in striatal areas is suggested indirectly by post-mortem studies finding increased levels of dopamine and HVA as well as increased binding affinity to D2 receptors. Neuroleptics consistently decrease pHVA, an effect associated with treatment response. Since neuroleptic treatment is particularly effective in decreasing the positive symptoms of schizophrenia, and pHVA may mostly reflect striatal dopamine activity, increased dopamine activity in the striatum seems to be associated with the positive symptoms of schizophrenia. Furthermore, there is additional indirect evidence that negative symptoms are associated with decreased mesocortical dopamine activity. However, there is direct evidence from recent imaging studies that challenged and unchallenged dopamine release in newer treated schizophrenia. Furthermore, PET studies using receptor ligands have shown convincingly that D2 blockade is mandatory to cause an antipsychotic effect. Classical D2 blockade is, however, accompanied by undesirable hypodopaminergia, leading to negative symptoms, including cognitive dysfunction. Therefore, the development of dopamine-stabilizing agents would improve the pharmacotherapy of schizophrenia considerably.

SEROTONIN

In 1954, lysergic acid diethylamide (LSD) was observed to have hallucinogenic properties, and a comparison was made with certain psychotic symptoms of schizophrenia (e.g. van Praag, 1992). Since LSD is a serotonin (5-hydroxytryptamine, 5-HT) antagonist, these effects were considered to reflect a relative deficit of 5-HT in the central nervous system. The early 5-HT hypothesis was revised such that both a surplus and a deficit of 5-HT could account

Table XVII-2.3 The serotonergic system in schizophrenia: imaging studies

Receptor type	Localization	Selected literature	Detailed findings in schizophrenia	Change in schizophrenia overall
5-$HT_{2A/C}$ [^{18}F-setoperone]	Cortical areas	Trichard *et al.* (1998), Lewis *et al.* (1998)	No significant difference; not associated with age decrease; no correlation with length of illness or symptomatology	No change

for psychoses (van Praag *et al.*, 1992). The revival of interest in 5-HT research is due predominantly to the discovery that certain compounds inhibiting various subtypes of 5-HT receptors may have therapeutic potential in schizophrenia. Of particular interest was the discovery of atypical neuroleptics, with their inhibiting effects on 5-HT receptor subtypes. Below, we present indirect and direct evidence for the involvement of serotonin in the pathophysiology of schizophrenia.

Indirect Methods for Examining Serotonergic Function

5-HT Agonists and Schizophrenia

Considering the widespread use of selective 5-HT uptake inhibitors, the induction of psychotic symptoms is extremely rare. Few paranoid or psychotic reactions have been reported with fluoxetine (Lindenmayer *et al.*, 1990; van Praag *et al.*, 1992). It may, however, be that 5-HT uptake inhibitors do not increase 5-HT activity sufficiently or in a way that is focused enough to elicit psychotic reactions. It is unlikely that simply increasing 5-HT function would induce psychosis.

5-HT Challenge Studies with Schizophrenia

MCPP, a metabolite of trazodone, binds to all 5-HT receptor subtypes, but most potently to 5-HT_{2c} and 5-HT_1 receptors m-Chlorophenylpiperazine (MCPP) challenge studies among schizophrenics have received much attention, but results have been inconsistent. Most studies have found that MCPP caused hormone release as compared with placebo, and several investigators have found that ongoing treatment with clozapine blocked the release of adrenocorticotropic hormone (ACTH), cortisol, prolactin and growth hormone, whereas treatment with haloperidol did not, thus providing evidence that clozapine effectively blocks the hormonal response of the 5-HT system (e.g. Owen *et al.*, 1993).

Post-Mortem and Cerebrospinal Fluid Studies on 5-HT Function

Of the post-mortem studies reviewed by Bleich *et al.* (1991), some found evidence of increased 5-HT concentrations in schizophrenics compared with normals, while others found decreased 5-HT levels or no differences between groups.

Of the CSF 5-hydroxyindoleacetic acid (5-HIAA) studies reviewed by Bleich *et al.* (1991), three found indications of reduced 5-HT metabolism among schizophrenics and six found no differences between groups.

Owing to the similarity between human blood platelets and central nervous system 5-HT synaptosomes, platelets appear to offer a peripheral model of 5-HT function (Bleich *et al.*, 1991). Eight investigators have found elevated platelet or whole-blood 5-HT levels in chronic schizophrenics, one study found lower levels in schizophrenics, and only three studies found no differences between patients and normals.

Direct Methods of Examining Serotonergic Function from Imaging Studies

Although many current atypical antipsychotics show significantly greater 5-HT_{2A} than D2 occupancy, 5-HT_{2A} occupancy may not be a necessary condition for atypicality (Kapur and Seeman, 2001). PET studies using specific 5-HT ligands have helped to shed light on these questions (Table XVII-2.3). In summary, it seems that 5-HT_{2A} blockade is neither necessary (since antipsychotics achieve response without 5-HT_{2A} blockade) nor sufficient for antipsychotic response (Kapur, 1998; Kapur *et al.*, 2000a). In addition, the published imaging data on 5-$HT_{2A/C}$ occupancy revealed no differences between schizophrenics and control subjects (Trichard *et al.*, 1998; Lewis *et al.*, 1998). These observations support recent reports that fananserin (Truffinet *et al.*, 1999) and MDL-100907 (Report, 1999), both with high 5-HT_{2A} occupancy but devoid of D2 occupancy, failed to work as antipsychotics. Does the presence of 5-HT occupancy exert an attenuating effect on the D2–extrapyramidal side effects relationship? Since the mismatch in D2 occupancy alone can explain the differences in extrapyramidal side effects and prolactin effects between typical and atypical antipsychotics (Kapur *et al.*, 1999; Kapur *et al.*, 2000b), it is unclear whether the superiority of atypical antipsychotics in these domains should be accorded to their 5-HT_2 blockade or to a more appropriate dosing regarding their D2 blockade (Kapur and Seeman, 2001).

Conclusions

The indirect methods of examining serotonergic function partly support the notion that this transmitter is involved in the pathophysiology of schizophrenia. Direct evidence from PET studies does not support a substantial influence of the serotonergic system. Whether serotonin has an attenuating function on the development of extrapyramidal side effects in atypical neuroleptics is still unclear.

BEYOND DOPAMINE AND SEROTONIN

In the past decade, research has moved away from a unitary catecholaminergic hypothesis involving only dopamine, serotonin or others like glutamate and GABA. A new direction has emerged focusing on the interaction of a variety of neurotransmitters in complex neurocircuits engaged in the control of major mental functions, such as affect, emotions and cognition, and their involvement in reward-directed and other complex behaviour (Carlsson *et al.*, 2001). Rather than trying to identify a single culprit in psychotogenesis, the emphasis is now moving towards trying to understand these interactions and how imbalances in such circuitry can arise, perhaps on a multifactorial basis. This would argue for a role of not only dopamine and glutamate but also serotonin, noradrenaline and GABA. There is now convincing evidence that a primary disturbance in one neurotransmitter system will inevitably influence several other systems. To try to establish the order in which a

complex chain of events has arisen, and thus to identify the origin, would in general seem unrealistic. A more modest aim would be to try to describe aberrations in the patterns of physiological and biochemical events, and to try to correlate such aberrations to behavioural changes (Carlsson *et al.*, 2001).

As an alternative to the multireceptor hypotheses, it was recently proposed (Kapur and Seeman, 2001) that the atypical antipsychotic effect can be produced by appropriate modulation of the D2 receptor alone; the blockade of other receptors is neither necessary nor sufficient. The authors of this hypothesis propose that fast dissociation from the D2 receptor makes an antipsychotic more accommodating of physiological dopamine transmission, permitting an antipsychotic effect without motor side effects, prolactin elevation or secondary negative symptoms.

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XVII-3

Amino Acid Transmitter Systems

U. Heresco-Levy

INTRODUCTION

Until recently, the study of amino acids systems in the context of schizophrenia has been relatively neglected, although excitatory and inhibitory amino acids are the most prevalent neurotransmitters in the human central nervous system (CNS) (see Chapter V). Currently, accumulating basic science and clinical data indicate that dysfunctions in neurotransmission modulated by the excitatory amino acids (EAA) glutamate (GLU) and its congeners may play a pivotal role in the pathophysiology of schizophrenia. These hypotheses led during the last decade to the evaluation of innovative pharmacological approaches for treatment-resistant schizophrenia patients. Moreover, a possible role of the inhibitory amino acid γ-aminobutyric acid (GABA) in this illness is presently being reassessed in the context of newly proposed interactions between GABA, EAA and other neurotransmitter systems.

EXCITATORY AMINO ACIDS

Most of the excitatory neuronal transmission in the CNS is mediated by the endogenous EAA, GLU, aspartate and homocysteine, GLU systems being the best characterized of the EAA systems. Almost all the neurones in the brain are influenced by GLU, and pre- and postsynaptic GLU receptors are found on neurones throughout the CNS. The action of GLU and its congeners is mediated at three subtypes of ionotropic receptors: amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) and at two distinct families of metabotropic, G protein-coupled receptors (see Chapter V). Due to the involvement of glutamatergic neurotransmission in a wide array of brain functions, the study of EAA systems presently represents one of the most promising research areas in neuroscience. Within this context, a link between glutamatergic neurotransmission and schizophrenia is supported by multiple lines of evidence.

Anatomical and Physiological Considerations

Schizophrenia is a human brain disease characterized by a wide array of mental dysfunctions over a lifelong course. Traditionally viewed as comprising primarily positive and negative symptoms, it is now recognized that cognitive impairments also constitute a key symptom group in this illness. The distribution and functions of glutamatergic systems within the brain strongly suggest that deficits in EAA-mediated neurotransmission may be involved in the pathogenesis of schizophrenia symptomatology. Glutamatergic fibres give rise to the major afferent, intrinsic and efferent pathways through the cortex. Many sensory organs, including the cochlea, olfactory bulb and retina, use GLU as their principal neurotransmitter. Within the brain, thalamocortical fibres, which represent the main input to the cortex, are primarily glutamatergic in nature. Virtually all pyramidal neurones in the cerebral cortex are glutamatergic. The dense, mutually facilitatory GLU-mediated connections between pyramidal cells is a basic requirement for the extensive parallel information-processing operations of the cortex. Pyramidal neurones in the cortex project to both cortical and subcortical structures. The corticostriatal pathway, which originates from the entire neocortex and projects to the ipsilateral candate, the putamen and, to a smaller extent, the nucleus accumbens, may be specifically implicated in the pathophysiology of schizophrenia. Corticostriated fibres impinge on the same intrinsic structural neurones as the dopamergic fibres of the nigrostriatal tract. Corticostriatal and nigrostriatal fibres exert behavioural effects that are genetically antagonistic. In experimental animals, corticostriated ablation produces transient stereotypes similar to those induced by amphetamine, facilitates amphetamine-induced stereotypies and circling, and markedly inhibits haloperidol-induced catalepsy. The effects of corticostriatal ablation are not accompanied by increases in dopamine (DA) metabolite levels or by alterations in DA receptor density. The functional dysregulation of dopaminergic neurotransmission induced by corticostriated ablation may serve as a model for behavioural disorganization in schizophrenia. A similar system, the corticothalamic system, regulates information flow into the cortex. Abnormalities of corticothalamic neurotransmission may contribute to the sensory-gating abnormalities associated with schizophrenia.

A specific model for an involvement of GLU in psychosis and schizophrenia has been suggested, based on extensive animal studies, by Carlsson and Carlsson (1990a, 1990b). According to this hypothesis, GLU acting at the NMDA receptor and DA activity at the D_2 receptor together produce a balanced intracellular signal at postsynaptic neurones in the striatum in order to provide an optimal feedback from basal ganglia and thalamus to cortex. A relative increase in dopaminergic activity and/or a relative decrease in glutamatergic transmission could precipitate psychosis. This formulation, although derived solely from preclinical studies, is highly consistent with the known pharmacology of schizophrenia. Furthermore, it suggests that overactivity of the dopaminergic system observed in schizophrenia may be due to a primary abnormality in glutamatergic neurotransmission or that the hyperdopaminergic state of schizophrenia could be responsible for a disturbance of EAA systems.

Glutamatergic fibres also give rise to the major afferent, intrinsic and efferent pathways of the hippocampal formation, and disruption of the glutamatergic pathways within the hippocampus is associated with severe disturbances in memory formation. The cortex, hippocampus and parahippocampal gyrus are all characterized by a high level of EAA receptors (for review see Ulas and Cotman, 1993). The NMDA subtype of GLU receptors appears to be concentrated primarily in the limbic system, being therefore very important to function in the limbic cortex, anterior cingulate, hippocampus and ultimately other areas of the human brain. NMDA but also

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non-NMDA glutamatergic receptors play an important role in a variety of cardinal normal CNS functions, including memory and learning, synaptic and developmental plasticity, sensory information and coordinated movement patterns (for review see Cotman *et al.*, 1988; Reid and Morris, 1991). Thus, while the involvement of glutamatergic transmission in these complex processes continues to be defined, it is increasingly evident that altered EAA receptor function may be of significance in the pathophysiology of schizophrenia.

Excitotoxicity and Neurodegeneration

In addition to its broad role in human brain functions, glutamatergic neurotransmission may be involved in excitotoxic and chronic neurodegenerative brain disorders (Olney, 1989; Whetsell and Shapira, 1993). Excitotoxicity refers to a paradoxical phenomenon whereby the neuroexcitatory action of GLU and related compounds, an action necessary for normal CNS functioning, becomes transformed into a neuropathological process that can rapidly kill CNS neurones. While stimulation of either NMDA or non-NMDA receptors can lead to excitotoxicity, the final common pathway for cell death appears to be the uncontrolled influx of CA^{2+} through opened unblocked NMDA receptor channels (Rothman and Olney, 1987; Choi *et al.*, 1988).

Either hyper- or hypofunction of EAA transmitter systems may be postulated as a basis for neuropsychiatric disorders. According to the hyperfunction concept, excessive activation of postsynaptic receptors can cause brain damage. However, when endogenous EAA transmitters, due to a hyperfunctional state, induce brain damage, this is essentially an autoexcitotoxic process in which the EAA neural network damages itself; i.e. the cells bearing EAA receptors and EAA receptors themselves are destroyed, rendering the EAA neural system hypofunctional. One mechanism by which GLU hypofunction might occur could be by an autoexcitotoxic process in which subtle brain damage induced by an excitotoxic mechanism in early life would destroy postsynaptic cells that house the GLU receptor system, thereby rendering the GLU neural network functionally deficient. In this context Olney and Farber (1995) have recently described a neurotoxic mechanism model that may be involved in schizophrenia. This pathogenic mechanism, ultimately leading to necrosis, begins with inhibition of NMDA receptors, and results in the final disinhibition of cortical excitatory cholinergic neurones, by way of reduced stimulation of inhibitory GABAergic interneurones secondary to glutamatergic blockade. This complex chain of events could explain how a pathological reduction in glutamatergic stimulation may ultimately result in cell loss. In support of this hypothesis are pharmacological data generated in animal studies using high doses of non-competitive NMDA receptor antagonists. Bridging those data with human pathology will be necessary to verify this plausible hypothesis.

An additional model of EAA-related cell death that may be operant in schizophrenia has been proposed by Coyle and Puttfarcken (1993). According to this model glutamatergic transmission, over a chronic time period, potentiates pathological intracellular primary oxidative processes and hence promotes oxidative cell death. In this formulation, GLU-mediated neurotransmission, especially if mildly elevated, could promote intracellular oxidation and diminish intracellular antioxidative protection. This process could potentiate other primary CNS pathology to result in overt brain illness. These processes should be characterized by cell loss but not by remarkable gliosis. Based on evidence of reduced neuronal density in schizophrenia, this model hypothetically associates oxidative stress mechanisms with the aetiology of schizophrenia.

Neurochemical Abnormalities

Further support for a role of EAA systems in schizophrenia derives from assessments of EAA levels and post-mortem brain neurochemical abnormalities in this illness. Although the first report of reduced GLU concentration in CSF of schizophrenia patients (Kim *et al.*, 1980) was not supported by later studies (Perry, 1982; Gattaz *et al.*, 1985), subsequent reports have indicated a significant reduction in the release of GLU induced by veratridine (Sherman *et al.*, 1991a), kainate and NMDA (Sherman *et al.*, 1991b) in synaptosomal fractions prepared from post-mortem brains of schizophrenics. Moreover, studies documenting altered density of GLU receptors in various regions of post-mortem brain of schizophrenia patients in comparison to normals were reported. The findings include increased [^{3}H]kainate binding in the prefrontal cortex (Nishikawa *et al.*, 1983; Deakin *et al.*, 1989), increased [^{3}H]MK-801 binding in the putamen (Kornhuber *et al.*, 1989) and in the temporal cortex, whereas [^{3}H]AMPA binding changed only minimally in the entire cerebral cortices (Kurumaji *et al.*, 1992). The negative correlation observed between GLU concentration in subcortical areas and receptor binding in terminal cortical areas from post-mortem studies (Toru *et al.*, 1988) and the increase in the number of postsynaptic GLU receptors reported in response to deafferentiation in rat hippocampus (Ulas *et al.*, 1990a, 1990b) support the hypothesis of glutamatergic hypofunction including compensatory upregulation of GLU receptors in some regions of frontal and temporal cortex in schizophrenia.

Recent findings bring further support to this hypothesis. Tsai *et al.* (1995) have reported reduced levels of GLU and aspartate in the hippocampus and frontal cortex in schizophrenia, in comparison to normal tissue. They also have found increased *N*-acetylaspartylglutamate (NAAG) — putative GLU precursor — and reduced activity of *N*-acetyl-α-linked acidic dipeptidase (NAALDase) — the enzyme that converts NAAG to *N*-acetylaspartate (NAA) and GLU in the same tissue. Reduced activity of an important GLU-synthesizing enzyme such as NAALDase could account for regional reduction in GLU-mediated neurotransmission and a secondary upregulation of GLU receptors in the cortex. Furthermore, Ishimaru *et al.* (1994) found that strychnine-insensitive glycine (GLY) binding sites coupled with the NMDA receptor–ionophore complex (see Chapter V) are increased in the cerebral cortex of chronic schizophrenics, not in terms of affinity but in the maximum number of binding sites. Since agonist action at the strychnine-insensitive GLY binding sites is an absolute requirement for the opening of the ion channel at the NMDA receptor–ionophore complex, these findings suggest that the increases in NMDA receptor-associated GLY binding sites, possibly ascribed to the postsynaptic compensation for impaired glutamatergic neurotransmission, might be implicated in the pathophysiology of schizophrenia.

EAA Receptor Regulation by Antipsychotics

Preliminary data indicate that presently available antipsychotics may, directly or indirectly, interact with glutamatergic brain systems. Gattaz *et al.* (1985) demonstrated a lack of change in GLU levels in CSF of neuroleptic-free, paranoid schizophrenia patients, in contrast to significantly elevated (33%) levels of GLU in patients on conventional neuroleptics. Alfredsson and Wiesel (1990) reported that during sulpiride treatment serum GLU levels increased in responders while they decreased in non-responders. Recently, Goff *et al.* (1996) found that patients treated with clozapine had significantly higher serum GLU concentrations than did patients treated with conventional neuroleptics. Moreover, it was shown that switching from conventional neuroleptics to clozapine was associated with increased serum GLU and aspartate concentrations (Evins *et al.*, 1997). In animal studies, acute administration of clozapine, but not haloperidol, increased medial prefrontal cortical GLU and aspartate concentrations (Daly and Moghaddam, 1993; Yamamoto and Cooperman, 1994), suggesting that clozapine may have selective actions on cortical EAA systems.

Several studies suggest that neuroleptics interact with the NMDA receptor–ion channel complex. It has been shown that in rat brain chronic haloperidol treatment upregulates binding of [^{3}H]TCP (an analogue of phencyclidine (PCP) and non-competitive NMDA receptor antagonist) to the PCP receptor located within the NMDA receptor–ion channel (Byrd *et al.*, 1987). The increase in [^{3}H]TCP binding levels appeared to be due to a striking (50%) increase in the maximal density of PCP receptors. This elevated density of PCP receptors after chronic haloperidol treatment contrasts with the observation of Kornhuber *et al.* (1989), suggesting that haloperidol does not interact directly with [^{3}H]MK-801 binding to NMDA receptor-ion channel PCP sites in post-mortem human brain.

Growing evidence suggests that clozapine and possibly other atypical antipsychotics may act in part by increasing activity at the NMDA receptor. Clozapine has been reported to be the most potent of the antipsychotic agents tested in blocking NMDA receptor antagonist-induced neurotoxicity (Farber *et al.*, 1993; Olney and Farber, 1994), stereotypy (Tiedtke *et al.*, 1990), social isolation (Corbett *et al.*, 1995) and deficits in sensorimotor gating of the startle response (Bakshi *et al.*, 1994; Lang *et al.*, 1992). Furthermore, in humans clozapine blunts psychotropic effects induced by the PCP derivative ketamine (Malhotra *et al.*, 1997a). Blocking of NMDA receptor antagonist-induced neurotoxicity (Farber *et al.*, 1996), isolative behaviour (Corbett *et al.*, 1995) and information gating dysfunction (Bakshi and Geyer, 1995) was also produced by olanzapine, suggesting that other atypical agents may share glutamatergic properties in common with clozapine. Taken together, these findings raise the important question as to whether different clinical profiles of conventional and atypical antipsychotics may reflect different mutual interactions of these drugs with both glutamatergic and dopaminergic systems.

Psychotomimetic Effects of NMDA Receptor Antagonists

The most compelling evidence linking glutamatergic receptor function with schizophrenia is provided by the psychotomimetic effects induced by PCP and related agents (e.g. ketamine). These agents were initially developed for use as general anaesthetics. However, in early clinical trials PCP was found to induce psychotomimetic effects in approximately 20% of exposed subjects (Corssen and Domino, 1966). In clinical trials performed four decades ago, the psychotic symptoms induced by PCP closely resembled those of schizophrenia, leading to the hypothesis that PCP-induced psychosis might serve as an effective clinical model for this illness (Luby *et al.*, 1962). During the last decade, ketamine administration has also been found to induce transient positive and negative schizophrenia-like symptoms and cognitive deficits in normal volunteers (Krystal *et al.*, 1994; Malhotra *et al.*, 1996) and exacerbation of psychosis in drug-free (Malhotra *et al.*, 1997b) or remitted (Lahti *et al.*, 1995a) schizophrenia patients. Lahti *et al.* (1995b, 1995c) found that ketamine increases regional cerebral blood flow (rCBF) in anterior cingulate and decreases flow in hippocampus, lingual gyrus and cerebellum of schizophrenia patients and normals. Furthermore, a correlation between BPRS ratings and rCBF in the medial and anterior aspects of the temporal lobe and in the ventral striatum was found. It was suggested (Tamminga, 1998) that schizophrenia patients may show a greater and longer response of anterior cingulate cortex to a fixed ketamine dose, without gross change in receptor number, suggesting the possibility of increased endogenous sensitivity to excitatory signals in this region. PET scans with [^{18}F]deoxyglucose following ketamine administration to normal volunteers identified a large area of activation in the prefrontal cortex, sparing most of the remaining brain (Breier *et al.*, 1998). In addition, conceptual disorganization was related to the degree of activation. These data suggest that the prefrontal cortex, an important region for many cognitive functions that have been shown to be abnormal in schizophrenia, may mediate the behavioural effects of NMDA receptor antagonists. Moreover, using positron emission tomography (PET) raclopride scanning, Breier *et al.* (1998) found that raclopride binding to D_2 receptors is decreased following ketamine administration, suggesting that ketamine induces DA release in the striatum. These findings bring further support to the hypothesis of GLU/DA interaction in the pathogenesis of behavioural syndromes induced by NMDA receptor antagonists.

PCP and ketamine induce their behavioural effects by binding to the PCP site located within the ion channel associated with the NMDA receptor (Javitt and Zukin, 1991). Their binding to this site leads to non-competitive blockade of NMDA receptor-mediated neurotransmission, indicating that endogenous NMDA dysfunction may play a critical role in the pathophysiology of schizophrenia. Moreover, in rodents, systemic injection of PCP and other non-competitive NMDA antagonists induces a characteristic behavioural syndrome, including hyperactivity and stereotypies. This syndrome is sensitive to administration of GLY-site agonists of the NMDA receptor, including GLY, D-serine and D-alanine (Toth and Lajtha, 1986; Contreras, 1990; Tanii *et al.*, 1994; Javitt *et al.*, 1997), but not to the administration of similar doses of a variety of other amino acids (Toth and Lajtha, 1986), further supporting a primary NMDA receptor involvement.

In recent years, it has been shown that non-competitive as well as competitive NMDA receptor antagonists may induce neurodegenerative changes in corticolimbic regions of the rat brain (Olney *et al.*, 1989, 1991; Hargreaves *et al.*, 1993). Furthermore, competitive NMDA antagonists (e.g. CPP, CGS-19755) that block NMDA receptors by acting at the NMDA, rather than PCP, recognition site have been developed. Although the behavioural effects of these agents are less well characterized than those of ketamine and PCP, they also appear to induce PCP-like behavioural effects in humans (Muir and Lees, 1995; Kristensen *et al.*, 1992; Grotta, 1994). These findings are in agreement with the concept of NMDA receptor hypofunction in schizophrenia and led to the hypothesis that this phenomenon may not be PCP receptor specific, but could result from a dysfunctional blockade of the NMDA receptor–ionophore complex (Olney and Farber, 1995).

NMDA RECEPTOR-BASED TREATMENT APPROACHES

At present there is an intense search for pharmacological strategies aimed at modulating glutamatergic neurotransmission in schizophrenia. Within this framework, research has recently focused on assessing the therapeutic potential of enhancing NMDA receptor function. Due to its structural complexity and relative abundance of modulation sites (see Chapter V), the NMDA receptor represents an inherently rich target for the development of innovative pharmacological interventions and corresponding new drugs. Since direct-acting NMDA agonists are potentially excitotoxic, the main NMDA receptor-based treatment approach for schizophrenia that has been clinically investigated during the last decade is the enhancement of NMDA receptor-mediated neurotransmission via administration of compounds having full or partial agonist activity at the strychnine-insensitive GLY recognition site of the receptor. Based on the general theoretical framework of the PCP model of schizophrenia, five compounds, milacemide, GLY, D-serine, D-cycloserine and D-alanine, have been assessed, mainly as adjuvant pharmacotherapy, in samples of treatment-resistant schizophrenia patients.

Milacemide, an amino acetamide derivative that has been extensively studied in humans as a potential antiepileptic and cognition enhancer, increases GLY levels in the CNS by dissociating into GLY-amide and then GLY, after passing through the blood–brain barrier (DeVarbeke *et al.*, 1988). In rats, after milacemide administration, whole-brain GLY levels increase by approximately 30%.

Milacemide, which was eventually withdrawn from use due to hepatotoxicity unrelated to its CNS effects, was found to be capable of reversing discriminative stimulus effects of PCP and of enhancing cognition in various animal and human models, consistent with its ability to potentiate NMDA receptor neurotransmission via metabolism to GLY. However, when several schizophrenia patients were assessed while they were otherwise drug free and in a stable, yet psychotic state, milacemide did not appreciably affect symptomatology (Tamminga *et al.*, 1992). Similarly, Rosse *et al.* (1991) reported a negative study using low-dose milacemide as add-on therapy with neuroleptic-treated schizophrenia patients. The negative results of these studies could be due to the small number of patients studied and/or to the low potency of milacemide. Furthermore it should be taken into account that milacemide affects additional neurotransmitter systems. For example, it increases central serotonin and DA levels by inhibiting the action of monoamine oxidase type A, raising doubts regarding the role of the GLY site as the specific, primary site of action of this drug.

Glycine (GLY) is a small, neutral amino acid constituting 1–5% of dietary protein that in the spinal cord and brain stem acts as an inhibitory neurotransmitter at a strychnine-sensitive site. By contrast, in forebrain, GLY is one of the most potent agonists known to act at the strychnine-insensitive GLY site associated with the NMDA receptor. Its coagonist action at this site positively modulates NMDA receptor function by increasing the frequency of NMDA ionophore opening and by delaying NMDA receptor desensitization (see Chapter V). Permeation of GLY into brain following peripheral administration is the lowest of any naturally occurring amino acid (Oldendorph, 1971) and large peripheral doses of GLY must be administered in order to obtain modest elevations in CNS levels.

To date, the results of the first 10 open label and controlled studies investigating the therapeutic potential of various adjuvant GLY regimens in a total of approximately 140 schizophrenia patients are available (Table XVII-3.1). The earliest study with GLY (Waziri, 1988) was performed before the relationship between GLY and NMDA function was fully appreciated, based on the observation that schizophrenia subjects had lower serine hydroxymethyl transferase (SHMT) activity than controls. Since SHMT activity is the major source of GLY in the brain, it was postulated that schizophrenia may be associated with decreased CNS GLY levels, although this hypothesis has not been confirmed (Korpi *et al.*, 1987a; Perry and Hansen, 1985). In this naturalistic study, four of 11 patients were noted to show 'definite salutary response' as indicated by decreased need for neuroleptics and improved social/vocational functioning, following administration of 5–25 g per day GLY. Subsequently, as interest in NMDA receptor-mediated neurotransmission increased, additional studies were performed with GLY doses of up to ~60 g per day. The first controlled GLY trial (Potkin *et al.*, 1992), which used a GLY dose of 15 g per day, demonstrated significant global improvement following GLY treatment and a trend towards improvement on the BPRS. Using an increased adjuvant GLY dose of approximately 30 g per day, Javitt *et al.* (1994) found a significant 17% mean reduction in negative symptoms, as measured by the PANSS, in a sample of neuroleptics-treated chronic schizophrenia patients. In contrast, in two controlled studies, no therapeutic effects were registered when 30 g per day (Potkin *et al.*, 1999) and 60 g per day (Evins *et al.*, 2000) GLY were added to the drug regimen of clozapine-treated patients. Recently, Heresco-Levy *et al.* (1996, 1999) reported the results of a 6-week double-blind, placebo-controlled, crossover trial in which 0.8 g kg^{-1} per day GLY — the highest GLY dose used to date in schizophrenia — were added to the antipsychotic regimen received by inpatients fulfilling treatment-resistance criteria. A significant 30% mean reduction in total BPRS scores and a 30% mean decline in negative symptoms, as measured by the PANSS, were observed during treatment with GLY, whereas no significant change in symptoms was observed during placebo treatment. Cognitive and depression symptoms also improved significantly, by 16% and 17%, respectively, during treatment with GLY but not placebo. The treatment by time interaction remained significant for negative and cognitive symptoms even following covariation for changes in depression and extrapyramidal symptom scores. GLY treatment led to a significant 3.5-fold increase in serum GLY levels and low pretreatment GLY levels significantly predicted clinical response. These findings provide initial support for the hypotheses that (a) GLY may be effective for the treatment of primary negative symptoms of schizophrenia and (b) patients with consistently low serum GLY levels may be the population of choice for treatment with GLY-site agonists.

D-Serine (DSR) acts as an endogenous full agonist at the NMDA receptor-associated GLY site (for review, see Hashimoto and Oka, 1997). GLY overloading can increase DSR in the brain through the GLY cleavage system; conversely, DSR can be converted sequentially to *l*-serine and GLY by racemase and SHMT. DSR has the advantage of being more permeable than GLY at the blood–brain barrier, thus requiring a reduced amount per dose. To date, two studies assessing DSR as an add-on agent to other antipsychotics in schizophrenia treatment have been reported. In a 6-week placebo-controlled, parallel group trial (Tsai *et al.*, 1998) a 30 mg kg^{-1} per day DSR dose resulted in significant 21%, 17% and 12% mean reductions, respectively, in negative, positive and cognitive symptoms, as measured by the PANSS. The cognitive enhancing effect was supported by improved performance in the Wisconsin Card Sorting Test (WCST). Given the observed effect on positive symptoms registered in this study, it has been suggested (Krystal and D'Souza, 1998; Tsai *et al.*, 1998) that DSR may represent the drug of choice for a GLY-site agonist monotherapy trial in schizophrenia. Nevertheless, in a methodologically identical study (Tsai *et al.*, 1999a), similarly to results registered with GLY treatment, no significant symptom changes were registered when the same DSR dose (i.e. 30 mg kg^{-1} per day) was added to clozapine treatment.

D-Cycloserine (DCS, d-4-amino-3-isoxazolidone, seromycin) is an antituberculosis drug that, unlike GLY, readily crosses the blood–brain barrier and acts as a relatively selective partial agonist at the GLY site for a narrow range of concentrations (Thompson *et al.*, 1992; Watson *et al.*, 1990). Potentially beneficial DCS psychotropic effects were first reported four decades ago, when 500–1000 mg per day DCS was routinely used as part of multidrug antituberculosis regimens. However, administration of 500–3000 mg per day DCS in a naturalistic study to nine drug-free schizophrenia patients resulted mainly in exacerbation of psychotic symptoms, psychomotor agitation, confusion and mood alterations (Simeon *et al.*, 1970).

The discovery of DCS physiological impact at the NMDA receptor led, during the last decade, to renewed interest in DCS-induced psychotropic effects and initial trials have attempted to define the DCS dose range over which adjuvant treatment with this compound may be beneficial in schizophrenia (Table XVII-3.2). DCS regimens of $\leq$30 mg per day were ineffective (Rosse *et al.*, 1996), while dosages of $\geq$100 mg per day led to positive symptom exacerbations (Cascella *et al.*, 1994; van Berckel *et al.*, 1999). In two single-blind dose escalation trials, only a 50 mg per day DCS regimen led to significant improvements in negative symptoms when administered to typical neuroleptics (Goff *et al.*, 1995), but not to clozapine-maintained (Goff *et al.*, 1996) patients. Serum glutamate concentrations at baseline and the change in GLY concentrations significantly correlated with response of negative symptoms. Recently, Goff *et al.* (1999a, 1999b) replicated the findings of their preliminary dose-finding trials in two double-blind, placebo-controlled studies. Addition of 50 mg per day DCS to clozapine led to significant negative symptoms worsening, compared to placebo (Goff *et al.*, 1999b). In contrast, the addition of the same DCS regimen to typical neuroleptics (Goff *et al.*, 1999a)

Table XVII-3.1 Clinical trials with glycine (GLY) in the treatment of schizophrenia

Study	Sample size	Design	Study duration (weeks)	Antipsychotic treatment	Daily GLY dose (g)	Symptoms[a] measures	Outcome
Waziri (1988)	11	Open label, naturalistic	36	Varied	5–25	Not specified	35% improved; neuroleptic treatment reduced or withdrawn
Rosse *et al.* (1989)	8	Open label	≤8	Varied	10.8	BPRS, SANS, CGI	33%: improved BPRS 33%: worsened BPRS
Costa *et al.* (1990)	6	Open label	6	Varied	15	BPRS: >30% improvement	33% improved
Heh *et al.* (1992)	8	Open label	≤5	Drug-free	≤20	BPRS: >30% improvement	No therapeutic effects
Potkin *et al.* (1992)	18	Double-blind, placebo-controlled	6	Varied	15	BPRS: >30% improvement SANS, CGI	Trend towards improvement with GLY (20% improved >30% in BPRS)
Javitt *et al.* (1994)	14	Double-blind, placebo-controlled followed by open label	8 blind 8 open	Varied	30	PANSS	17% improvement in negative symptoms
Leiderman *et al.* (1996)	5	Open label	8	Varied	60	SANS, PANSS	Significant SANS improvement
Potkin *et al.* (1999)	19	Double-blind, placebo-controlled	12	Clozapine	30	BPRS, SANS	No therapeutic effects
Heresco-Levy *et al.* (1996, 1999)	22	Double-blind, placebo-controlled crossover	6	Varied	60	BPRS, PANSS	30% improvement in negative symptoms
Evins *et al.* (2000)	30	Double-blind, placebo-controlled	8	Clozapine	60	BPRS, SANS PANSS	No therapeutic effects

[a]BPRS, Brief Psychiatric Rating Scale; CGI, Clinical Global Impression Scale; SANS, Schedule for the Assessment of Negative Symptoms;
PANSS, Positive and Negative Syndrome Scale (updated with permission from U. Heresco-Levy: *N*-methyl-D-aspartate (NMDA) receptor-based treatment approaches in schizophrenia: the first decade. *International Journal Neuropsychopharmacology* **3**, 243–258, 2000, Cambridge University Press.)

Table XVII-3.2 Clinical trials with D-Cycloserine (DCS) in the treatment of schizophrenia

Study	Sample size	Design	Study duration (weeks)	Antipsychotic treatment	Daily DCS dose (mg)	Symptoms[a] measures	Outcome
Simeon *et al.* (1970)	10	Open label	2–16	Drug-free	500–3000	Non-standardized	Psychosis exacerbation
Cascella *et al.* (1994)	7	Double-blind, placebo-controlled	6	Conventional neuroleptics	250	BPRS, SANS, CGI	Psychosis exacerbation, negative symptoms improved in 2 patients
Goff *et al.* (1995)	9	4 doses of DCS and placebo, single blind	2 per dose	Conventional neuroleptics	5, 15, 50, 250	BPRS, SANS	Negative symptoms and SIRP reaction time improvement with 50 mg per day
Goff *et al.* (1996)	10	4 doses of DCS and placebo, single blind	2 per dose	Clozapine	5, 15, 50, 250	BPRS, SANS	No therapeutic effects
Rosse *et al.* (1996)	13	2 doses of DCS, double blind, placebo-controlled	4 per dose	Molindone	10, 30	BPRS, SANS, CGI	No therapeutic effects
van Berckel *et al.* (1996)	14	5 doses of DCS single blind	0.6 per dose	Drug-free	15, 25, 50, 100, 250	CGI, PANSS	Negative symptoms improvement with 100 mg per day
Heresco-Levy *et al.* (1998)	9	Double-blind, placebo-controlled crossover	6	Varied	50	BPRS, PANSS	General psychopathology improvement negative symptoms improvement — not significant vs. placebo
van Berckel *et al.* (1999)	26	Double-blind, placebo-controlled	8	Conventional neuroleptics	100	CGI, PANSS	Psychosis exacerbation
Goff *et al.* (1999a)	47	Double-blind, placebo-controlled	8	Conventional neuroleptics	50	PANSS, SANS GAS, HAM-D	Negative symptoms improvement on SANS
Goff *et al.* (1999b)	17	Double-blind, placebo-controlled crossover	6	Clozapine	50	GAS. SANS PANSS	Negative symptoms worsening
Heresco-Levy *et al.* (2001)	24	Double-blind, placebo-controlled crossover	6	Conventional neuroleptics olanzapine, risperidone	50	BPRS, PANSS HAM-D	Negative symptoms improvement on PANSS

[a]BPRS, Brief Psychiatric Rating Scale; CGI, Clinical Global Impression Scale; SANS, Schedule for the Assessment of Negative Symptoms;
PANSS, Positive and Negative Syndrome Scale; GAS, Global Assessment Scale; HAM-D, Hamilton Depression Rating Scale; SIRP, Sternberg's Item Recognition Paradigm (updated with permission from U. Heresco-Levy: *N*-Methyl-D-aspartate (NMDA) receptor-based treatment approaches in schizophrenia: the first decade. *International Journal Neuropsychopharmacology*, **3**, 243–258, 2000, Cambridge University Press.)

resulted in a significant mean 23% reduction in negative symptoms as measured by SANS total scores. The rate of reduction in total SANS scores in the DCS group remained significantly greater than for the placebo group after controlling for change in extrapyramidal, positive and depression symptoms. A 50 mg per day DCS adjuvant regimen has been recently assessed also in a sample of treatment-resistant schizophrenia patients receiving conventional neuroleptics, olanzapine and risperidone (Heresco-Levy *et al.*, 2001). In this study, DCS treatment resulted in a mean 15% reduction in negative symptoms and the negative symptoms improvement did not differ between patients treated with conventional neuroleptics versus risperidone and olanzapine. Low pretreatment GLY and serine serum levels significantly predicted clinical response.

Similarly to the conclusions of GLY studies, the results of DCS trials performed to date suggest that DCS adjuvant treatment may be effective against primary negative symptoms of schizophrenia, in particular among patients having low GLY, and possibly serine, serum levels. However, the therapeutic DCS dose range appears to be restricted around a regimen of approximately 50 mg per day. This limitation may be due to the partial agonist characteristics of DCS. As a partial agonist at the GLY site, producing 40–60% activity compared to GLY, DCS acts as an agonist in the presence of low GLY concentrations and as an antagonist in the presence of high concentrations (Emmett *et al.*, 1991). DCS effects are further determined by the relative concentrations of other endogenous ligands possessing varying degrees of agonist activity, such as serine and alanine.

D-Alanine has a lower affinity for the GLY site than GLY or DSR and, although there is evidence to suggest that it is actively transported into the CNS, its precise bioavailability and pharmacokinetics are yet to be determined. In animal models of psychosis, intraventricular administration of D-alanine inhibits methamphetamine and PCP-induced hyperactivity and blocks PCP/MK-801-induced stereotypies and ataxia. Recently the results of the first adjuvant D-alanine trial in schizophrenia have been presented (Tsai *et al.*, 1999b). In this 6-week controlled study, D-alanine treatment resulted in mean 20%, 15% and 12% reductions, respectively, in negative, positive and cognitive symptoms as measured by the PANSS.

Safety Issues and Future Directions

In the studies performed to date, the administration of GLY-site agonists of the NMDA receptor has been well tolerated by schizophrenia patients and no significant side effects or alterations in clinical laboratory parameters have been registered. Nevertheless, the main safety issue concerning the use of this type of agent is neurotoxicity. In order to examine possible GLY-induced neuronal and/or glial pathology, two controlled studies were recently performed in which rats were randomized to receive dietary supplementation with GLY. In the first study rats received 0.8 and 3.2 $g\,kg^{-1}$ per day GLY for 2 weeks (Shoham *et al.*, 1999); in a subsequent study rats received dietary supplementation with 1 and 5 $g\,kg^{-1}$ per day GLY for 1, 3 and 5 months (Shoham *et al.*, 2001). Although these dietary regimens resulted in significant, dose-dependent GLY levels increases, extensive morphological and immunohistochemical examination did not reveal any evidence of neurotoxic damage at any of the treatment intervals studied.

Overall, the results of the first generation of small clinical trials with GLY-site agonists support the NMDA receptor hypofunction hypothesis of schizophrenia and warrant further, larger-scale investigation. The aims of studies presently performed in this field include: (1) the assessment of GLY-site modulators as mono- versus add-on therapy, (2) the comparison of efficacies of full versus partial agonists of the GLY-site, and (3) the assessment of clinical effects of these agents as adjuvants to widely used atypical antipsychotics such as risperidone and olanzapine. This last issue highlights the importance of better understanding the interactions between this group of drugs and the mechanism of action of atypical antipsychotics. Presently, the lack of clinical response to GLY-site agonists in clozapine-treated patients is hypothesized to indicate that activity at the GLY modulatory site may be a possible mechanism contributing to clozapine efficacy.

A related alternate strategy for enhancing NMDA-mediated neurotransmission may be the use of GLY uptake antagonists. The degree to which presynaptically released GLU stimulates NMDA receptor-mediated activity depends upon the tonic GLY levels in the immediate surrounding region. The recent molecular and biochemical characterization of a class of GLY transporter proteins in brain (Liu *et al.*, 1993; Smith *et al.*, 1992) suggests that GLY concentration in micro domains may be regulated by these transporters. Of these, the GLY transporter type 1 (GLYT1) is expressed primarily in glia and neurones of the neocortex and archicortex in association with regions of high NMDA expression (Zafra *et al.*, 1995). GLYT1 may serve to maintain low intrasynaptic GLY level specifically in the local region around NMDA receptors. Inhibition of GLYT1 transporters may thus lead to GLY level elevations in the immediate vicinity of NMDA receptors and augmentation of NMDA receptor-mediated neurotransmission without requiring administration of exogenous GLY. GLY transport antagonists suitable for clinical trials are not yet available. Nevertheless, the GLY derivative glycyldodecylamide (GDA) was found to be approximately 80-fold more potent than GLY in reversing PCP-induced hyperactivity (Toth *et al.*, 1986). Recently, Javitt and Frusciante (1997) have demonstrated that GDA acts as a selective GLY uptake antagonist at concentrations similar to those that would be obtained after peripheral administration. Furthermore, in animal behavioural studies, the potency of several novel GDA-related compounds for inhibiting PCP-induced hyperactivity *in vivo* correlated significantly with their potency in antagonizing GLY transport *in vitro* (Javitt *et al.*, 1999). These findings suggest that GLY transport antagonists may induce effects similar to GLY and may therefore represent an appropriate alternative for targetted drug development.

ADDITIONAL GLUTAMATERGIC DYSFUNCTION MECHANISMS AND TREATMENT APPROACHES

The pharmacological manipulation of neurotransmission mediated at non-NMDA glutamatergic receptors may also represent a potentially beneficial treatment approach for psychotic states and schizophrenia.

Svensson and colleagues revealed that the major mechanism which drives burst firing in ventral tegmental area (VTA) dopaminergic neurones is activation of NMDA receptors, mediating the influence on DA cells from glutamatergic afferents, e.g. from the prefrontal cortex. Accordingly, schizophrenomimetic NMDA receptor antagonists, such as PCP, were found to cause dramatic distortions of central DA neuronal signalling even at very low doses. DA neurones projecting to subcortical areas deployed a monotonous, high-frequency firing, whereas DA neurones from the VTA that provide the DA innervation of the prefrontal cortex showed literal abolition of burst firing, thus rendering this dopaminergic projection incapable of effective phasic signalling (for review, see Svensson, 2000). Significantly, the firing pattern of dopaminergic neurones activated by systemic administration of the potent and selective NMDA receptor antagonist MK-801 was quite similar to the high-frequency, non-bursting firing pattern previously observed after local application of quisqualate or kainate, i.e. AMPA and kainate receptor agonists (for review see Svensson *et al.*, 1997), indicating the possibility that NMDA receptor antagonists might release GLU onto VTA dopaminergic neurones. Accordingly, it was recently reported that effects of NMDA antagonists may also be mediated

through excess activity at AMPA/kainate receptors. It has been shown that the local administration of CNQX, an AMPA/kainate antagonist, blocks the hyperlocomotion and increase in DA release seen in the VTA after systemic administration of MK-801, suggesting that excess stimulation at non-NMDA receptors may mediate these effects (Mathé *et al.*, 1998). Since most antipsychotics and particularly clozapine have been observed to antagonize the behaviourally stimulant action of PCP-like drugs, the effect of two chemically different AMPA receptor antagonists, GYKI 52466 and LY 326325, were investigated in the classical conditioned avoidance response (CAR) paradigm in the rat. Systemic administration of both compounds resulted in significant suppression of CAR without any effect on escape behaviour. Importantly, no catalepsy was observed (Mathé *et al.*, 1999). Taken together, these accumulating data indicate an antipsychotic effect of AMPA receptor antagonists with a low liability for extrapyramidal side effects, suggesting, in principle, an atypical antipsychotic profile. This possibility is further strengthened by additional findings indicating the ability of AMPA receptor antagonists to ameliorate cognitive deficits induced in rat by ketamine (Moghaddam *et al.*, 1997).

Moghaddam (2000) used a novel approach to normalize the glutamatergic hyperactivity produced by PCP by targetting the group II metabotropic GLU receptors (mGLURs). This group of receptors (mGLU2, mGLU3) has been located in forebrain regions, in particular the striatum and frontal cortex, and are thought to regulate GLU release by presynaptic mechanisms. Stimulation of these receptors in rodents, at an agonist dose that had no effect on spontaneous activity, attenuated PCP-induced working memory deficits, hyperlocomotion and stereotypy (Moghaddam and Adams, 1998). This behavioural reversal was accompanied by reduced activation of GLU efflux by PCP, but not by reduced presynaptic dopaminergic hyperactivity in cortical and subcortical regions. These data suggest that targetting the group II mGLURs may represent an additional therapeutic strategy for normalizing glutamatergic dysfunction in psychotic disorders, including schizophrenia.

Additional evidence in favour of a potential role for this group of compounds in psychosis treatment was hypothesized to derive also from the emerging role of GLU mechanisms in generating the psychotropic effects induced by psychedelic hallucinogens such as LSD. These hallucinogens have been found to enhance glutamatergic neurotransmission via their action at $5HT_{2A}$ receptors. In the prefrontal cortex, $5HT_{2A}$ receptor stimulation increases GLU release, as indicated by a marked increase in the frequency of postsynaptic potentials/currents in the apical dendritic region of layer V pyramidal cells. These effects were found to be suppressed by group II/III mGLURs agonists (for review see Aghajanian and Marek, 2000).

Ampakines are a newly developed class of low-molecular-weight drugs that freely cross the blood–brain barrier, increase AMPA receptor gated ionic currents and thereby enhance post-excitatory synaptic transmission in the cortical telencephalon (Staubli *et al.*, 1994a; Rogers *et al.*, 1997). These compounds facilitate long-term potentiation (Staubli *et al.*, 1994a) and enhance learning and memory in rodents (Staubli *et al.*, 1994b) and humans (Ingvar *et al.*, 1997), suggesting that they may improve cognitive dysfunction in patients. Moreover, ampakines substantially reduce methamphetamine-induced behavioural abnormalities in animals and interact positively with typical and atypical antipsychotics in blocking methamphetamine effects (Johnson *et al.*, 1999). These findings have been interpreted as raising the possibility that this family of drugs could be useful in the treatment of schizophrenia (Lynch, 1999).

Lamotrigine (3,5-diamino-6-[2,3-dichlorphenyl]-1,2,4-triazine) is a new anticonvulsant that stabilizes neuronal membranes and attenuates cortical GLU release. This compound is efficacious in the treatment of affective disorders and preliminary reports indicate that it may also be useful for schizoaffective patients (Erfurth *et al.*, 1998). Moreover, Anand *et al.* (2000) recently demonstrated attenuation of neuropsychiatric effects of ketamine with lamotrigine in healthy volunteers. The efficacy of lamotrigine in the treatment of schizophrenia is presently investigated in controlled clinical trials.

INHIBITORY AMINO ACIDS: γ-AMINOBUTYRIC ACID

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, is prevalent throughout the CNS, but at comparatively low concentrations in brain stem and spinal cord. In the cortex, GABA is localized primarily to intrinsic interneurones that participate in local feedback loops and exert a tonic inhibition on cortical pyramidal neurones to prevent uncontrolled excitatory activity. Physiologically, the most common function of inhibitory GABAergic neurons appears to be to focus and refine the firing pattern (nerve impulse generation) of the projection neurones that transfer neural information from one functional unit to another (see Chapter V). Traditionally, a role for GABA in the pathophysiology of schizophrenia has been assessed, leading to some experimental and clinical evidence, in the context of complex interactions between GABAergic and other brain neurotransmitter systems.

GABA Levels and Brain Structural Measures

Attempts to uncover a GABA disturbance in schizophrenia by direct measurements of GABA levels have had mixed results. CSF GABA has been reported not to differ significantly in schizophrenia patients and normal controls in a majority of studies, although some patients may have lower levels (Lichtshtein *et al.*, 1978; van Kammen *et al.*, 1982). Plasma GABA was also found to be similar in male schizophrenia patients and control subjects (Petty and Sherman, 1984). While several post-mortem studies of schizophrenia patients have revealed decreased GABA in some regions of the brain (Perry *et al.*, 1979; Korpi *et al.*, 1987b; Toru *et al.*, 1988), not all studies have observed such decreases. In a recent study, plasma levels of GABA were associated with prefrontal sulcal widening and ventricle–brain ratios, but not global sulcal widening in 62 drug-free schizophrenia patients. CSF GABA measurements were not associated with brain structural measures, but were associated with age and age of illness onset (van Kammen *et al.*, 1998). It was hypothesized that GABA synthesis and transmission may be impaired only in specific pathways, cortex layers or brain regions in schizophrenia subjects and compensated for in others.

GABA/Dopamine Interactions

A GABA disturbance in schizophrenia was first suggested by Roberts (1972), who hypothesized that low levels of GABA in the brain may lead to a state of disinhibition which, in turn, could produce the symptoms seen in schizophrenia. Subsequently, Roberts (1976) revised this concept into a GABA/DA imbalance hypothesis which postulates inadequate GABA inhibition of DA neurotransmission in this illness. The highest GABA levels in mammalian brain are located in the DA-rich basal ganglia. GABA-containing neurones there are intrinsic to the striatum, form a long feedback-loop path from the striatum to the substantia nigra pars reticulata and globus pallidus (i.e. from postsynaptic to presynaptic neurones), and constitute a major efferent pathway from the substantia nigra to the thalamus, superior colliculus and reticular system. These tracts modify dopaminergic function in these and other sites by decreasing presynaptic DA release. In rats, GABA-mimetic drugs attenuate acute neuroleptic-induced activation of nigrostriatal and mesolimbic DA neurones and retard DA neurone postsynaptic responses to chronic neuroleptic blockage

(Lloyd and Morselli, 1987). Evidence reviewed by Garbutt and van Kammen (1983) suggests that GABA inhibits the firing of DA neurones in the substantia nigra and VTA — the origin of the mesolimbic and mesocortical tracts. The evidence includes spiking in the nucleus accumbens when GABA antagonists were injected into the VTA in an animal model. Additional recent research has shown that GABA mediates the inhibition of cortical DA activity in rat brain (Wassef *et al.*, 1999). When this tonic inhibition exerted by the thalamic mediodorsal nucleus is disrupted, DA activity increases in certain target regions of the cortex (Jones *et al.*, 1988). Moreover, GABA has been observed to produce a dose-dependent decrease in the release of striatal DA. Reid *et al.* (1988) found that the striatal level of homovanillic acid and dihydroxyphenylacetic acid rose in the presence of GABA. It has also been reported that GABA antagonists stimulate DA release that is preloaded into the inner retinal cells of fish in a Ca^{2+}-dependent fashion and not via direct membrane action; the release was shown to be inhibited by excess GABA (Ishita *et al.*, 1988). In one study, valproic acid (a GABAergic compound), when applied to cultured rat cells in concentrations equivalent to therapeutic levels, was observed to limit the repetitive firing of the cells without altering the postsynaptic GABA response (MacDonald and McLean, 1987). The drug enhanced the inhibitory postsynaptic potential in the hippocampal cells of guinea-pigs (CA3 pyramidal cells and granule cells) and suppressed the spontaneous spiking and repetitive discharges evoked by GABA antagonists. The close association of GABA and DA in the lateral septum and nucleus accumbens in rats supports the anatomic plausibility of GABA/DA systems interaction (Onteniente *et al.*, 1987).

The available evidence is nevertheless contradictory regarding the uniform DA downregulation by GABA. DA was found to have a mild stimulatory effect on both the pyramidal cortical neurones and the GABAergic interneurones impinging on them (Penit-Soria *et al.*, 1987). Monteleone *et al.* (1985, 1986) found that sodium valproate failed to enhance basal secretion of growth hormone and did not reduce plasma prolactin secretion in patients with chronic schizophrenia — evidence that seems to contradict the hypothesis of DA downregulation by GABA. Moreover, DA neurones in substantia nigra and VTA may be activated by GABA. Systemic muscimol (a GABA receptor agonist) administration in the rat (Waszczak and Walters, 1979) produced increased DA neurones firing in pars compacta of the substantia nigra. Non-DA neurones in the adjacent pars reticulata were inhibited by systemic muscimol and were found to be more sensitive to iontophoretic GABA inhibition than the pars compacta DA neurones. Similar findings were reported by Grace *et al.* (1980). These studies have suggested the presence of a GABA-sensitive intermediate connection. Depressing the firing of this inhibitory pathway with systemic GABA leads to increased DA cell firing. It appears, therefore, that GABA may actually activate DA neurones in certain areas. In agreement with this concept, GABA has been shown both to increase and decrease DA turnover, depending upon the brain region assayed, the duration of stimulation and whether DA antagonists are administered (Cheramy *et al.*, 1978; Fuxe *et al.*, 1979). Overall, these findings suggest that GABA acts at a number of sites to influence DA neuronal activity. The final outcome may thus represent an integration of several actions and is not limited to monosynaptic inhibition (Garbutt and van Kammen, 1983).

GABA/Glutamate Interactions

GABA and GLU are known to modulate each other's release in rat brain cortex and hippocampus (Bonnano *et al.*, 1993). Recently, Olney and Farber (1995), on the basis of extensive animal investigations, have proposed a model of schizophrenia, implying that GABA and GLU systems may be abnormally integrated in this illness within the cingulate gyrus and possibly other regions such as the hippocampus and amygdala. According to this model, hypofunctional NMDA-sensitive GLU receptors on GABAergic interneurones could be responsible for loss or impairment of inhibitory modulation in schizophrenia. It was emphasized that the NMDA receptor hypofunction might be induced during development by any mechanism that impairs the functional status of NMDA receptors or that impairs or destroys specific GABA neurones through which the function of these receptors is expressed. Accordingly, loss of specific GABAergic neurones would represent an NMDA receptor hypofunction-equivalent condition.

Consistent with this hypothesis, reduction in the density of interneurones (Benes *et al.*, 1991) and upregulation of $GABA_A$ receptor binding activity (Benes *et al.*, 1992) have been demonstrated in the upper cortical laminae of the cingulate region in schizophrenia patients. Furthermore, an additional pathogenesis model, developed from post-mortem studies of the anterior cingulate region of schizophrenia subjects (for review, see Benes, 1993, 1995), shows some striking similarities with the hypothesis of Olney and Farber. According to this model, loss or impairment of GABA cells in layer II may be triggered, at least in part, by excessive glutamatergic inputs that pass through this layer. Moreover, DA projections to the cortex have been found to form direct appositions with GABAergic cells, and a relative increase in the number of such contacts could arise if there is a loss and/or a decrease in the activity of these GABAergic interneurones.

GABA and Synaptic Connectivity

Prefrontal cortex dysfunction in schizophrenia may be related to abnormalities in the synaptic connectivity of this region. Post-mortem studies suggest that such alterations in connectivity may involve GABA neurones. Both the uptake (Simpson *et al.*, 1989; Reynolds *et al.*, 1990) and release (Sherman *et al.*, 1991) of GABA have been reported to be reduced in cortical synaptosomes prepared from schizophrenia subjects. In the prefrontal cortex, the activity of glutamic acid decarboxylase, the synthetic enzyme for GABA, is reduced in subjects with schizophrenia (Bird *et al.*, 1979; Sherman *et al.*, 1991a, 1991b), as is the expression of the mRNA for this enzyme. In addition, ligand binding studies have revealed abnormalities in prefrontal cortex $GABA_A$ receptor in schizophrenia (Benes *et al.*, 1996). Although the expression levels of $GABA_A$ receptor subunit mRNAs are not altered significantly, preliminary studies suggest that expression of the short isoform of the $\gamma 2$ subunit of this receptor may be markedly reduced in the prefrontal cortex in schizophrenia (Huntsman *et al.*, 1998).

The chandelier cells are the GABAergic interneurone subclass hypothesized to be preferentially involved in connectivity dysfunctions in schizophrenia since they (1) receive direct synaptic input from DA axons, (2) exert powerful inhibitory control over the excitatory output of layer III pyramidal neurones, and (3) undergo substantial developmental changes during late adolescence, the typical age of onset of schizophrenia (for review, see Lewis *et al.*, 1999). Consistent with this hypothesis, recent studies have reported that the axon terminals of chandelier neurones, as revealed by immunoreactivity for the GABA membrane transporter, are reduced substantially in the middle layers of the prefrontal cortex of schizophrenia patients. This alteration appears to be selective for the chandelier class of GABA neurones and for the disease process of schizophrenia (Lewis *et al.*, 1999; Woo *et al.*, 1988).

TREATMENT APPROACHES

The available evidence indicates the possibility of a GABAergic deficit in schizophrenia, thus suggesting that activation of GABA receptors may be therapeutically beneficial. Agents that promote

$GABA_A$ neurotransmission prevent NMDA receptor hypofunction-induced neurotoxicity in the rat cerebral cortex (Olney *et al.*, 1991) and attenuate the psychotomimetic effects associated with ketamine use in anaesthesia (Reich and Silway, 1989). However, while more research is clearly needed, the main drawback with GABA hypotheses of schizophrenia is that presently used drugs working directly on GABA systems are not impressive therapeutically in patients suffering from this illness.

Benzodiazepines (BZ) binding to CNS BZ receptors, which are structurally and functionally linked to $GABA_A$ receptors and to chloride ionophores, facilitates GABA neurotransmission and results in decreased neuronal excitability (see Chapter V). Both BZ and GABA receptors are heterogeneous. BZ effects on neuronal excitability apparently mediate their anxiolitic, hypnotic and anti-convulsant action. As treatment for schizophrenia, BZ have been studied extensively, either alone or as adjuvants to antipsychotic drugs. The findings of these studies are overall in agreement with clinical experience and suggest that: (1) response is highly variable and about one-third to one-half of patients improve, (2) actual antipsychotic effects are seen in a small number of patients and only in conjunction with antipsychotic use, and (3) BZ are potentially most useful as adjuvants to neuroleptics in the acute management of psychotic agitation (for review see Wolkowitz and Pickar, 1991; Hollister *et al.*, 1993; Wassef *et al.*, 1999). There is also evidence that supplementing antipsychotic treatment with a benzodiazepine during periods of florid psychosis reduces agitation and that diazepam may be beneficial in treating early-warning signs of symptom exacerbation in schizophrenia (Carpenter *et al.*, 1999). However, efforts to substitute BZ for antipsychotic drugs in treating full psychotic episodes have not proven effective and lower-dose BZ, as adjuncts to antipsychotic medication, have not proven very effective at enhancing therapeutic outcome in partially responsive patients (Wolkowitz *et al.*, 1992).

Regarding the limited efficacy of BZ and other GABA agonists in schizophrenia, Olney and Farber (1995) stressed that since the initial pathological event that may render GABAergic neurones defective in this illness is proposed to occur in prenatal life, the administration of GABA agonist drugs ~20 years later in order to correct this imbalance is not likely to be successful because GABA receptors with which the drug must interact will have been deleted from the brain or will have been reconnected in some aberrant manner.

GABA agonists, including those with preferential affinity for $GABA_A$ receptors (e.g. tetrahydroisoxazolopyridol) or with $GABA_{A+B}$ activity (e.g. SL 76002), have been, overall, disappointing in clinical trials in schizophrenia (for review see Lloyd and Morselli, 1987), as were the studies examining the therapeutic potential of GABA transaminase inhibitors (Casey *et al.*, 1980; Thaker *et al.*, 1983).

Valproic acid enhances central GABAergic tone and its use as adjuvant to antipsychotic drugs has resulted, in a number of studies, in positive treatment outcome (for review, see Wassef *et al.*, 1999). Following a careful risk–benefit assessment, valproate-augmented pharmacotherapy may represent, on a case-by-case basis, a viable treatment option for schizophrenia patients who are resistant to treatment with conventional neuroleptics.

FUTURE TREATMENT DIRECTIONS

The allosteric modulation characteristics of compounds acting at BZ receptors may influence their clinical efficacy. Full allosteric modulators of GABA-gated Cl^- channels have a high incidence of tolerance and dependence liability. Imidazenil is a partial allosteric modulator of $GABA_A$ receptors which binds to BZ recognition sites with high affinity but exerts a moderate allosteric modulation of GABA-gated Cl^- current intensity. Its testing in the treatment of schizophrenia has been proposed since this compound is virtually devoid of tolerance and dependence liability in rodents and non-human primates and its activity is not changed in rats that are tolerant to BZ acting as full allosteric modulators of GABA-gated Cl^- currents (Costa and Guidotti, 2000).

Alternative GABA-based treatment approaches are presently conceptualized. New agents, such as tiagabine, that selectively inhibit uptake of GABA by the neuronal GABA membrane transporter GAT-1 (Borden *et al.*, 1994) may be potentially therapeutic. Moreover, the identification of selective alterations in the chandelier subclass of GABA neurones and related circuitry may also reveal options for novel forms of therapeutic intervention in schizophrenia and other psychotic disorders.

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XVII-4

Peptidergic Transmitter Systems in Schizophrenia

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PEPTIDES AS NEUROTRANSMITTERS

Mental and neuronal functions of mammals depend on the activities of neurones in the central nervous system. These neurones act and transmit information primarily by chemical means. Since the concept of the neurotransmitter was advanced in the 1950s, various substances intrinsically present in the central nervous system were found to be involved in neurotransmission. The concept of a neuromodifier, which does not directly participate in neurotransmission but modifies this function, was also proposed. Neurotransmitter (Panton, 1958) and neuromodifier (Barchas *et al.*, 1978) criteria were precisely defined. However, as research has advanced, it has become difficult to confirm that these criteria are sufficiently fulfilled in many neurotransmitter or neuromodifier candidates, or to clearly distinguish neurotransmitters and neuromodifiers. For these reasons, the idea that all substances involved in chemical transmission should be referred to collectively as neuroregulators has been presented.

Neuroregulator candidates include acetylcholine, amines, amino acids, purine bodies and peptides.

Peptides are macromolecular compounds with a molecular weight of about 10 000 or less in which some amino acids are linked by peptide bonds, consisting of an amino group and an α-carboxyl group. After the discovery of angiotensin in 1945, many peptides intrinsically present in the central nervous system were discovered in the 1970s and 1980s. In 1998 a peptide called orexin was found to be present in the brain, and more than 100 peptides have been discovered to date. These peptides with neurophysiological activities can be called neuropeptides or neuroactive peptides, because they do not strictly fulfil the criteria of neurotransmitters.

Of the neuroregulators, acetylcholine, monoamines and neuroamino acids are called classic transmitters to distinguish them from neuropeptides. Research to data has revealed the coexistence of classic neurotransmitters, classic neurotransmitters and peptides, and peptides in the central nervous system. The presence of both histamine and GABA in the posterior hypothalamus, and of dopamine and GABA in the olfactory bulb (Kosaka *et al.*, 1985), are examples of coexistence of classic neurotransmitters; the presence of acetylcholine and substance P in the tegmental nuclei in the dorsolateral part of the pons (Vincent, 1983b), catecholamine and neuropeptide Y (Everitt *et al.*, 1984), and dopamine and neurotensin in the hypothalamus (Ibata *et al.*, 1983), are examples of coexistence of classic neurotransmitters and peptides. Many other cases of coexistence of peptides have also been noted.

A hypothesis that neuropeptides and classic transmitters always cooperate with each other in neurotransmission has not been established, because not all classic neurotransmitters coexist with neuropeptides. However, as neuropeptides have been shown to be released in large quantities compared with classic neurotransmitters when neurones discharge frequently (Hökelt, 1997), peptide transmitters are considered to be closely related to modification of transmission or the plasticity of synapses, as mentioned later, rather than neurotransmission itself. At any rate, neuropeptides are considered to coexist and compete with so-called classic transmitters or other peptides to enhance the function of neurotransmission. In this sense, the peptidergic neuronal system is not present independently but constitutes part of neurotransmission in the brain.

However, when mental activities and functions of individual neuropeptides in the central nervous system were examined more closely, each of them was found to have characteristic actions. Neuropeptides were first shown to be involved in higher mental activities by De Wied (1964). They demonstrated that learning of aversion was impaired in animals that had undergone resection of the anterior lobe of the pituitary gland, and that this impairment was alleviated by the administration of adrenocorticotrophic hormone (ACTH). Furthermore, they showed that aversion behaviour was impaired in rats that had undergone resection of the posterior lobe of the pituitary gland but that learning of aversion was improved by the administration of vasopressin (De Wied, 1965). From these reports, vasopressin came to be considered to enhance memory. Later, this action of vasopressin was confirmed in the brain as learning of aversion was shown to be promoted by direct infusion of vasopressin into the hippocampus in animal experiments (Kovacs *et al.*, 1986). In addition, anatomical and, primarily, immunohistochemical investigations have revealed the rich presence of peptides in the hippocampus and frontal lobe of the cerebral cortex, leading to speculation of an involvement of peptides in higher mental functions. For example, comparison of the anatomical distribution of substance P between rodents and primates showed that neural networks were much richer in the primates, and that substance P is related to higher mental and neuronal functions (Iritani *et al.*, 1989). From these findings, neuropeptides are considered to play important roles, which are not supplementary to the roles of classic neurotransmitters, in the central nervous system.

NEURONAL DEVELOPMENT AND NEUROPEPTIDES

What are the reasons that neurones select neuropeptides as media for neurotransmission? Although neuropeptides coexist with classic neurotransmitters, this is not always the case, and no pattern has been discovered for their coexistence.

In the process of their development, neurones are considered not only to differentiate according to the genetic programme but also to be affected by various environmental factors. Neurones select a few from a large number of neurotransmitters during the period of development, increase their synthesis as they grow, and begin to accurately transmit neural activities to target cells (Patterson and Nawa, 1993). Recently, functions of neurotrophic factors have come to be studied widely and intensively in connection with neuronal development and differentiation. BDNF (brain-derived neurotrophic factor) and NT-5 (neurotrophin-5) are examples of such

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neurotrophic factors; they act on suppressive GABA neurones of the cerebral cortex and striatum when administered into the cerebral ventricle, and begin to synthesize suppressive neuropeptide Y and somatostatin (Nawa, 1993, Nawa *et al.*, 1994). In fact, the coexistence of GABA neurones and peptides, such as substance P and neuropeptide Y, has been confirmed anatomically. These factors regulate the functional differentiation of neurones, and the production and secretion of transmitters, more dynamically than used to be considered in the process of nerve growth. Neuropeptides are considered to play important roles also in the dynamism of the process of neuronal network formation. Neuropeptides such as somatostatin (Hayashi *et al.*, 1990), substance P (Yamashita *et al.*, 1990) and cholecystokinin (Yamashita, 1992) are known to be expressed in large quantities in the central nervous system during embryonic development and in the period after birth. Recently, the role of neurotrophin in the expression of neuropeptides has been studied extensively (Carnahan and Nawa, 1995). In the process of neuronal network formation, TrkB, which is a specific receptor of BDNF, is expressed in large quantities during the first 4 months after birth, when synapse formation progresses markedly (Hayashi *et al.*, 1999). On the other hand, neuropeptides themselves are thought to have a neurotrophic function in neuronal development (Schwaltz, 1992).

Recently, the hypothesis that schizophrenia is caused by disturbance of neural development has come to be understood widely in relation to functional insufficiency of neurotrophic factors such as BDNF, but knowledge concerning the functions of neurotrophic factors remains incomplete. The hypothesis of neurodevelopmental disorder as a cause of schizophrenia was formed after a large number of reports of morphological abnormalities of the brain of schizophrenic patients appeared from the 1980s as a result of improvements in image analysis; these abnormalities were considered to be due to impaired development of the central nervous system. Recently, Takahashi *et al.* (2000) reported abnormal expression of BDNF in the brain of patients with schizophrenia.

Although morphological abnormalities have been reported in nearly all areas of the brain of schizophrenic patients, the interest of recent morphological studies has been directed primarily to two areas: the medial side of the temporal lobe and limbic system, including the hippocampus (Bogerts *et al.*, 1990), and the frontal lobe (Benes *et al.*, 1991). The features of morphological abnormalities are quantitative changes with statistical significance, and abnormalities of the arrangement or position of cells, which are clarified only by statistical analyses, rather than qualitative abnormalities such as degeneration, and they are explained by disturbance of brain development. Since neurotrophic factors are directly related to neuronal differentiation and the development of the central nervous system (Maisonpierre *et al.*, 1990; Thoenen, 1991), the morphological and anatomical abnormalities of the brain of schizophrenic patients reported to date can be explained by this hypothesis. In addition, biochemical abnormalities as well as morphological abnormalities may be explained in connection with neurotrophic factors. These factors are known to exert marked effects on the expression of neurotransmitters (Wagner and Kostyk, 1990), and a balance is considered to be maintained between neurotransmitters and neurotrophic factors (Kornhuber and Weller, 1994). In fact, BDNF, which is a neurotrophic factor, plays a very important role in the development and differentiation of the dopaminergic neuronal system in the embryonic period (Hyman *et al.*, 1991). This indicates that neurotrophic factors have a role in neuronal construction including biochemical neuronal transmission. Naturally, neurotrophic factors have been shown to affect induction and differentiation of various peptides as well as classic neurotransmitters in the central nervous system (Loudes *et al.*, 2000; Kerekes *et al.*, 2000). Moreover, some studies have demonstrated that these factors act protectively on neurones and are involved in the repair of injured neurones (Hagg and Varon, 1993; Gash *et al.*, 1996), and that they maintain and develop the plasticity of synapses (Lo, 1995). The continuous progression of so-called negative symptoms such as reduced stress tolerance, and impaired learning ability and mental function, can be explained by viewing the functional insufficiency of these factors with the pathological features of schizophrenia. At any rate, findings concerning neurotrophic factors obtained to date have allowed explanation of various phenomena and symptoms of schizophrenia in connection with functional insufficiency of neurotrophic factors. However, clarification of the mechanisms of individual symptoms of schizophrenia, e.g. hallucination and delusion and thought process disorder, along with elucidation of the functional insufficiency of neurotransmitters, is needed.

Here we have shown that neurotrophic factors play important roles in the biological aetiology of schizophrenia and that functions of these factors are closely related to neurotransmitters, which directly mediate mental and neural functions. We will next evaluate pathological changes in neuropeptides, which are a group of neurotransmitters, in schizophrenia.

NEUROPEPTIDES IN SCHIZOPHRENIA

As research on neurotransmitters progressed, it became clear that schizophrenia is caused not by an abnormality of a single neurotransmitter but by a disturbance of balance among several neurotransmitters that interact with each other (Weinberger and Lipska, 1995; Bachus and Kleinman, 1996). Studies of nerve function regulators, including neurotransmitters, in schizophrenia can be generally divided into those of the post-mortem brain, those of cerebrospinal fluid and those of pharmacological effects. In studies of the post-mortem brain, the neuropeptide content and that their receptors is measured, and histological evaluations are made. In studies of cerebrospinal fluid, the neuropeptide content of the cerebrospinal fluid collected from patients is examined. Concerning pharmacological function, the effects of administration of neuropeptides in animal models has been studied.

There are slight differences in the classification of neuropeptides among investigators, but they can be roughly classified as follows:

I. Brain–gut peptides (neurotensin, cholecystokinin, substance P, neuropeptide Y, peptide YY, somatostatin, VIP, etc.)
II. Opioid peptides (endorphins, enkephalins)
III. Hormones released from the hypothalamus (TRH, somatostatin, GRH, CRH, etc.)
IV. Pituitary hormone (vasopressin, oxytocin, etc.)
V. Others (orexin, nociceptin, etc.)

New peptides are continually being discovered in the body; therefore, the conventional classification of neuropeptides has limitations.

The greatest number of reports concerning neuropeptides in schizophrenic patients published during the past 10 years were about brain–gut peptides.

Brain–Gut Peptides

Neurotensin (NT)

Sharma *et al.* (1997) measured the content of NT in cerebrospinal fluid from 29 patients with schizophrenia and 13 patients with schizoaffective disorder. Their psychiatric symptoms were also evaluated by the BPRS (Brief Psychiatric Rating Scale). NT was measured, and symptomatic assessment was made, after a mean drug-free period of 15 or more days. Then, after 4 weeks of neuroleptic medication, NT was measured and symptomatic assessment was made again. In the drug-free state, psychiatric

symptoms were stronger in patients with a lower NT level in cerebrospinal fluid. Also, after 4 weeks' medication, improvements in psychiatric symptoms according to the BPRS, particularly improvements in negative symptoms, were positively correlated with the NT concentration in cerebrospinal fluid. There are already a few reports on the relationship between negative symptoms of schizophrenia and NT in cerebrospinal fluid.

Widerlov *et al.* (1982) measured the NT concentration in cerebrospinal fluid in 21 patients with schizophrenia and 12 normal controls once during a period of no neuroleptic treatment and once during a period of neuroleptic treatment. The NT concentration was markedly lower in nine of the 21 schizophrenic patients during the no-neuroleptic treatment period. No difference was observed in the other schizophrenic patients compared with the normal controls. In the nine schizophrenic patients, NT concentration during the neuroleptic treatment period was increased nearly to the normal control level. All three patients with catatonic schizophrenia and six of the seven patients with disorganized schizophrenia belonged to this group of nine patients. A negative correlation was observed between the symptom of 'slowness of movement' (Comprehensive Psychopathological Rating Scale) and NT concentration.

Similarly, Breslin *et al.* (1994) studied NT concentration in cerebrospinal fluid in 15 patients with schizophrenia and 10 normal controls during a period when neuroleptic medication was off and during a period when it was on. Although no difference was observed in NT concentration between the patients and normal controls, it increased during the neuroleptic-on period in seven patients in whom NT concentration was relatively low during the neuroleptic-off period. The NT concentration in cerebrospinal fluid was related to deficit symptoms.

Nemeroff *et al.* (1989) measured NT concentration in cerebrospinal fluid in patients with schizophrenia, depression, eating disorders and premenstrual syndrome. It was found to be increased in some of the patients with schizophrenia. None of these patients had paranoid-type schizophrenia.

Garver *et al.* (1991) studied the NT concentration in cerebrospinal fluid and its responses to antipsychotic medication in patients who exhibited psychiatric symptoms. They noted that the NT concentration was relatively low, and its response to antipsychotic medication was poor, in a group of female patients. Thinking disorder, hallucination/delusion, behavioural disorganization and impaired functioning were more notable in these patients than in those with a relatively high NT concentration.

From these studies, the presence of a subgroup of schizophrenic patients in whom the NT concentration in cerebrospinal fluid is originally low, is not poorly responsive to treatment, and is closely related to negative symptoms was suggested.

Concerning studies of the post-mortem brain, Wolf *et al.* (1995) quantitatively analysed NT receptors in the entorhinal cortex using autoradiography. Receptors were concentrated specifically in layer II, and they were reduced by 40% in the brains of schizophrenic patients compared with the normal control brain. These sites were in agreement with the sites of cytoarchitectural abnormalities in the brain of schizophrenic patients reported by Arnold *et al.* (1991).

Binder *et al.* (2001) showed that an NT receptor agonist produces similar effects to antipsychotic drugs in an animal model of schizophrenia. Studies to date have accumulated evidence suggesting that NT and the dopaminergic neuronal system influence each other anatomically, neurochemically, electrophysiologically, behaviourally and pharmacologically (Lambert *et al.*, 1995).

Cholecystokinin (CCK)

Mauri *et al.* (1998) measured the CCK concentration in peripheral blood in 30 drug-free schizophrenic patients and 22 normal controls. Simultaneously, the mental state of the patients was evaluated using the BPRS, SAPS (Scale for Assessment of Positive Symptoms) and SANS (Scale for Assessment of Negative Symptoms). The CCK concentration in peripheral blood was measured again in nine patients after 4 weeks' antipsychotic treatment. As a result, a negative correlation was observed between the CCK concentration and SANS score, and the CCK concentration tended to be lower in patients who responded poorly to antipsychotic treatment. However, these are data of the CCK concentration in peripheral blood; how closely they reflect the kinetics of the neurotransmitter in the brain must be evaluated carefully.

Garver *et al.* (1990) measured the CCK concentration in cerebrospinal fluid in 11 drug-free patients with schizophrenia and six normal controls. The CCK concentration was significantly lower in the patients than in normal controls, and in male patients than in female patients. When the responses to antipsychotic medication were examined later, the CCK concentration was significantly lower in the poor-response group than in the good-response group.

Bachus *et al.* (1997) studied the expression of CCK mRNA by *in situ* hybridization histochemistry using the post-mortem brains of seven schizophrenic patients and seven normal controls. The expression of CCK mRNA was reduced in layers III and IV of the entorhinal cortex and subiculum of the schizophrenic patients compared with normal controls. This decrease was considered to be a pathological feature of schizophrenia rather than a result of medication, as judged from the status of medication and the findings from neuroleptic medication in animal experiments.

Virgo *et al.* (1995) studied the expression of CCK mRNA *in situ* using the post-mortem brains of eight schizophrenic patients and eight normal controls. The expression of CCK mRNA was markedly reduced, i.e. by 83% in the frontal lobe and 63% in the temporal lobe, compared with the normal controls. No association was observed between the reduction in mRNA and medication or the duration of illness.

Kerwin *et al.* (1992) studied CCK receptors in the hippocampus of the post-mortem brains of 11 schizophrenic patients and 11 normal controls by autoradiography. Receptors were significantly reduced in the CA1 region of the hippocampus in the schizophrenic patients. Zachrisson *et al.* (1999) found that the expression of mRNA of CCK(B) receptors in the frontal lobe was reduced in patients with schizophrenia. Also, CCK(A) receptors are related to auditory hallucination of schizophrenia (Wei and Hemmings, 1999), and their gene polymorphism has been reported (Tachikawa *et al.*, 2000).

Anatomically, coexistence of dopamine neurones and CCK in the central nervous system has been known, and the findings that the activities of dopaminergic neurones are suppressed by a CCK antagonist (Rasmussen *et al.*, 1991; Rasmussen, 1994), that a CCK antagonist administered to an animal model of schizophrenia produced similar effects to neuroleptics (Feifel and Swerdlow, 1997), and that D_2 receptors and CCK or CCK receptors markedly influence each other (Fuxe *et al.*, 1995), suggest that changes in CCK in schizophrenia are closely related to the dopamine hypothesis.

Substance P (SP)

Rimon *et al.* (1984) measured the concentration of SP in cerebrospinal fluid in 12 patients with schizophrenia, 12 patients with depression and 15 normal subjects. They found that SP concentration was significantly increased in the depression group compared with the normal control and schizophrenia groups. There was no significant finding in the schizophrenia group.

Kaiya *et al.* (1981) measured the immunoreactivity of SP in peripheral blood in a schizophrenia group, non-schizophrenic psychiatric disease group, and normal control group. The immunoreactivity of SP was significantly enhanced in the schizophrenic group

with a 1 year or longer history of medication. When the immunoreactivity was measured again in eight drug-free schizophrenic patients after five months of medication, it was reduced in five but was increased in three, and the results were inconsistent. While the immunoreactivity 120 min after chlorpromazine injection was not changed, it was increased 90 min after electroconvulsive therapy in male patients. Since this study was conducted under the influence of drug therapy, whether the changes in the SP activity are pathological features of schizophrenia or not should be evaluated cautiously.

Toru *et al.* (1988) measured the immunoreactivity of SP at 34 sites in the brain using post-mortem brains of 14 schizophrenic patients and 10 normal controls. The activity was high at 12 sites including the frontal lobe cortex and substantia nigra.

However, some authors reported no significant changes in the SP concentration in cerebrospinal fluid or the post-mortem brain of schizophrenic patients (Heikkila *et al.*, 1990; Iadarola *et al.*, 1991), and no consensus has been reached.

Shirayama *et al.* (2000) administered PCP (an NMDA antagonist, dopamine uptake inhibitor, schizophrenomimetic drug), methamphetamine (MAP) (a dopamine agonist), and MK-810 (an NMDA antagonist) to rats and studied changes in the SP content of the prefrontal cortex and midbrain substantia nigra. As a result, SP in the prefrontal cortex decreased after the administration of PCP and MK-801, and SP in the substantia nigra decreased after the administration of PCP and MAP. Also, when PCP was administered to rats pretreated with haloperidol (a dopamine D_2 receptor antagonist, representative neuroleptic), the SP content in the substantia nigra was not reduced. This indicates that the SP level in the substantia nigra is dopamine dependent. This observation suggests that the SP level in the substantia nigra is closely related to functions of the dopaminergic neuronal system and that the SP level in the prefrontal cortex is closely related to functions of the glutaminergic neuronal system.

As observed above, there have not been consistent findings concerning changes in the SP level in schizophrenic patients, but it is estimated to change in association with the levels of classic neurotransmitters.

Neuropeptide Y (NPY) and Peptide YY (PYY)

NPY and PYY are coded by a common gene and are called the pancreatic peptide family. Widerlov *et al.* (1988) measured the immunoreactivity of NPY and PYY in cerebrospinal fluid of patients with depression, patients with schizophrenia and normal controls. The NPY activity in cerebrospinal fluid was significantly reduced in depressed patients compared with schizophrenic patients or normal controls, and the PYY activity was significantly lower in drug-free schizophrenic patients. These differences suggest that the processes of synthesis, metabolism and degradation of these peptides vary among diseases and that they serve as disease markers.

Peters *et al.* (1990) studied the immunoreactivity of NPY in cerebrospinal fluid in 35 drug-free patients with chronic schizophrenia. The immunoreactivity of NPY was significantly higher in the patients than in normal controls, and it was reduced as the patient grew older and as the duration of illness increased. The authors measured the NPY activity in cerebrospinal fluid before and after suspension of haloperidol administration in 31 patients and demonstrated that the NPY activity was significantly increased by suspension of haloperidol administration. There was no difference in NPY activity between the group in which psychiatric symptoms were exacerbated by suspension of medication and the group in which no exacerbation was observed.

Thus, the results of measurements of NPY and PYY in cerebrospinal fluid were not consistent. Berrettini *et al.* (1987) even reported that diseases cannot be detected from data of the NPY level in cerebrospinal fluid.

Frederiksen *et al.* (1991) measured the NPY and PYY contents in the grey matter of the temporal lobe in post-mortem brains of 14 schizophrenic patients and 21 normal controls and showed that they were significantly reduced in the patients compared to controls.

Sakai *et al.* (1995) chronically administered haloperidol to rats and studied changes in the NPY level in the brain. The NPY concentration was increased in the cerebral cortex but was reduced in the striate body.

Iritani *et al.* (2000) examined NYP-positive fibres in the hippocampus in the post-mortem brains of six schizophrenic patients and three normal controls. They reported morphological abnormalities of NYP-positive fibres in the field of the dentate gyrus not observed in the normal controls, and they linked these findings with the developmental disturbance hypothesis of schizophrenia.

Somatostatin (SOM)

SOM is a hypothalamic hormone and was discovered in 1972. This neuropeptide was subsequently found to be distributed also in the stomach, intestines, and head of the pancreas and is classified as a brain–gut peptide. Its receptors are also distributed widely in the brain, pituitary gland, digestive tract, pancreas and thyroid gland.

Reinikainen *et al.* (1990) measured the immunoreactivity of SOM in cerebrospinal fluid of 11 patients with chronic schizophrenia having cognitive disorders and in eight normal controls. The immunoreactivity was significantly reduced in the schizophrenia group. This decrease was not related to cognitive decline measured by the MMSE (Minimal-Mental Examination), the BPRS, or the dose of neuroleptics. However, an association between cognitive disorders and SOM concentration in cerebrospinal fluid was suggested.

Doran *et al.* (1986) measured the SOM level in cerebrospinal fluid in 44 patients with schizophrenia and 19 patients with depression in connection with the results of the dexamethasone inhibition test. The SOM level in cerebrospinal fluid was lower in the depression group than in the schizophrenia group and in the group that did not respond to the dexamethasone inhibition test regardless of the diagnosis. These results suggest a relationship between the functions of the hypothalamic–pituitary–adrenal axis and the SOM level in cerebrospinal fluid.

Bissette *et al.* (1986) measured the immunoreactivity of SOM in cerebrospinal fluid in 11 patients with schizophrenia, 17 patients with depression, 29 patients with dementia and 10 normal controls. The SOM level in cerebrospinal fluid was lower in all disease groups than in the normal control group. Therefore, impairment of cognitive function was suggested to be related to the SOM level in cerebrospinal fluid. Contrary to these reports, Gerner *et al.* (1985) reported that the SOM level in cerebrospinal fluid was high in some schizophrenic patients. Although reports on the SOM level in cerebrospinal fluid are contradicted and further evaluation is needed, these discrepancies may be due to differences in the selection of patients, selection of normal controls and medication.

Sharma *et al.* (1994) studied changes in the immunoreactivity of SOM in cerebrospinal fluid associated with antipsychotic medication in 14 patients with schizophrenia. It increased significantly in 13 of the 14 patients after medication, with a significant positive correlation between the immunoreactivities of SOM in cerebrospinal fluid during and after treatment. In animal experiments also, the SOM content of the brain was confirmed to be increased by administration of a neuroleptic (haloperidol) (Sakai *et al.*, 1995). However, Beal and Martin (1984) experimentally showed decreases in the immunoreactivity of SOM in the striate body amygdaloid body, and nucleus accumbens after the administration of neuroleptics (pimozide, chlorpromazine, fulphenazine).

Also, Doran *et al.* (1989) studied changes in the SOM and homovanillic acid (HVA) levels in cerebrospinal fluid of 14 patients with schizophrenia associated with fluphenazine treatment. SOM concentration in cerebrospinal fluid decreased significantly after treatment, but HVA increased as SOM decreased. In addition, a positive correlation was observed between SOM and HVA concentrations in cerebrospinal fluid in 46 drug-free patients with schizophrenia. These findings suggest a close relationship between SOM and dopamine, but the mechanism of this relationship remains unknown. The reports by Sharma and Doran disagree concerning changes in the SOM level in cerebrospinal fluid associated with antipsychotic medication. This discrepancy may be due to differences in the drug type, dose, administration period, time of measurement and subjects. At any rate, it appears certain that dopaminergic nerve blockers induce changes in SOM concentration.

Nemeroff *et al.* (1983) reported low SOM levels in the frontal lobe (Brodmann area 12) in the post-mortem brains of schizophrenic patients. Iritani and Satoh (1991) reported that SOM-containing cells and SOM-containing fibres, which are rich and densely distributed in the monkey cerebral neocortex, form a network and suggested that SOM is involved in higher mental functions.

From these reports, SOM is considered to be closely related to the dopaminergic neuronal system and to be an aetiological factor of schizophrenia. Histologically, the frontal lobe cortex is considered to be an area of interest.

Other Brain–Gut Peptides

Rafaelsen and Gjerris (1985) measured the concentration of vasoactive intestinal polypeptide (VIP) in cerebrospinal fluid of patients with schizophrenia, depression and mania. A significant decrease was observed only in the depressed patients.

Roberts *et al.* (1983) measured the VIP level at 10 sites of the brain using the post-mortem brains of 14 schizophrenic patients and 12 normal controls. VIP was increased in the amygdaloid body of patients with schizophrenia (type II). In a similar study of post-mortem brains, a decrease in VIP in the cerebral cortex of schizophrenic patients has been reported (Gabriel *et al.*, 1996), and evaluation at various sites of the brain from functional viewpoints is considered to be needed.

Changes in the calcitonin gene-related peptide (CGRP) level have been evaluated in cerebrospinal fluid of patients with psychiatric disorders. An increase was noted in depression, but no significant change was observed in schizophrenia (Mathe *et al.*, 1994).

Concerning these neuropeptides, their relationships with classic neurotransmitters and their individual physiological activities remain largely unknown, and further accumulation of knowledge is needed before their involvement in the aetiology of schizophrenia can be discussed.

Opioid Peptides (Endorphins, Enkephalins)

Wiegant *et al.* (1988) measured α-, β- and γ-endorphin levels in the hypothalamus of the post-mortem brains of schizophrenic patients and normal controls. The α- and γ-endorphin levels were significantly increased in the schizophrenic patients compared with the normal controls.

Lindström *et al.* (1978, 1986) measured the concentration of fraction I (opioid receptor-active fraction) of endorphin in cerebrospinal fluid of schizophrenic patients. The fraction I level was higher in the schizophrenic patients than in the normal controls, and it was negatively correlated with symptoms such as hallucination and 'indecision' (CPRS: Comprehensive Psychopathological Rating Scale). Among the patients, a high fraction I level was observed significantly more frequently in those with disorganized schizophrenia. In drug-free patients, the increase in fraction I was related to low HVA (a metabolic product of dopamine) level.

Naber *et al.* (1981) measured β-endorphin level in cerebrospinal fluid in patients with psychiatric disorders including schizophrenia, but no significant observation was made in schizophrenia.

Wen *et al.* (1983) measured the met-enkephalin (ME) concentration in cerebrospinal fluid in 18 patients with schizophrenia and 18 patients with neurological disorders such as headache. The ME concentration was significantly lower in the schizophrenic patients.

Opioid peptide levels were studied primarily in serum and cerebrospinal fluid from the late 1970s to the 1980s, but no results that linked them with the aetiology of schizophrenia were obtained. Following the lack of consistent results the involvement of opioids in the treatment of schizophrenia has not been pursued further.

Weatherspoon *et al.* (1996) showed that β-endorphin inhibits the release of dopamine mediated by NMDA receptors in the nucleus accumbens and caudate putamen in guinea-pigs. It seems more reasonable to consider that opioid peptide levels change in association with classic neurotransmitters rather than that they are directly related to the pathology of schizophrenia.

McDaniel *et al.* (1990) reported dose-dependent impairment of spatial memory in rats after infusion of dynorphin into the bilateral dorsal parts of the hippocampus. This impairment was blocked by the administration of naloxone (an opioid antagonist). This suggests that dynorphin specifically impairs spatial learning and memory in the dorsal part of the hippocampus and that this effect is mediated by opioid receptors. Aigner and Mishkin (1988) evaluated the effect of naloxone administration on visual recognition in monkeys and observed that visual recognition was dose-dependently promoted by naloxone administration. Martinez and Graaf (1985) showed that learning was promoted in mice by naloxone administration.

Thus, opioid peptide inhibits learning, but how this is related to the symptoms of schizophrenia is still unclear. However, it seems likely to be related to symptoms of schizophrenia such as cognition impairment and learning impairment.

Hormones Released from the Hypothalamus

Thyrotrophin-Releasing Hormone (TRH)

TRH is a small peptide consisting of three amino acids which was isolated from the porcine hypothalamus in 1966. TRH is present in the central nervous system and peripheral nervous system and is considered to be a neuropeptide involved in neurosecretion, i.e. promotion of thyroid-stimulating hormone (TSH) and prolactin secretion, and neurotransmission. In the central nervous system, it is distributed in the hypothalamus and medulla oblongata, and its receptors are also observed in many areas including the amygdaloid body, hippocampus and pyriform cortex. Pharmacological actions of TRH are diverse. In the psychiatric and neurological fields, they are evaluated in relation to learning and memory (Mellow *et al.*, 1989) and depression (Prange *et al.*, 1972).

Miyamoto *et al.* (1979) administered TRH to rats intraperitoneally or into the nucleus accumbens and observed a significant enhancement of their activity. Sharp *et al.* (1982) incubated sections of rat brain tissues (hypothalamus, nucleus accumbens) with a TRH analogue and demonstrated dose-dependent increases in dopamine release. This hyperactivity is considered to be due to an enhancement of dopamine release from the septal nucleus accumbens not mediated by direct stimulation of dopamine receptors.

Beasley *et al.* (1988) studied responses of TSH to TRH in 19 patients with acute psychiatric disorders, including seven patients with schizophrenia. They considered that a decrease in the responsiveness of TSH was related to the diagnosis of emotional disorders and that good responses to antipsychotic medications are expected. Siris *et al.* (1991) studied similar responsiveness in 25 schizophrenic patients with postpsychotic depressive state

and 34 age- and sex-matched patients with depression. No difference was observed in the responsiveness between the two groups; this conflicts with the results of Beasley *et al.* that responses to antipsychotic medication can be expected in schizophrenic patients showing reduced responses of TSH. In schizophrenia, TRH is evaluated in relation to the dopamine hypothesis as it enhances the dopamine release aside from its responses to TSH.

Corticotrophin-Releasing Factor (CRF)

CRF is a hypothalamic hormone consisting of 41 amino acids. In the central nervous system, CRF neurones are distributed in the cerebral cortex (particularly layers II and III), limbic system and brain stem as well as in the hypothalamus. Concerning mental and neural functions, CRF administered in the central nervous system promotes discharges of noradrenergic neurones in the ceruleus nucleus and thus induces the release of noradrenaline at sites that the ceruleus nucleus projects to, causing an elevation of the level of consciousness (Owens and Nemeroff, 1991). Matsuzaki *et al.* (1989) administered CRF into the cerebral ventricle of rats and observed an enhancement of dopamine metabolism in the frontal lobe, hippocampus and amygdaloid body, and behavioural changes considered to be associated with them. They are evaluated in connection with stress-induced behavioural changes.

Banki *et al.* (1987) measured the immunoreactivity of CRF in cerebrospinal fluid in 23 patients with schizophrenia, 54 patients with depression and 138 normal controls. CRF concentration was markedly increased in the depressed patients compared with the normal controls and patients with other psychiatric disorders. Also, it was slightly but significantly increased in the schizophrenic patients compared with the normal controls.

Forman *et al.* (1994) evaluated CRF concentration in cerebrospinal fluid in 21 males with chronic schizophrenia. When the concentration was measured after haloperidol was replaced by placebo, it increased in 18 of the 21 patients. The concentration was unrelated to the severity of the disease or symptoms such as depression and anxiety.

In 1995, urocortin was discovered as a member of the CRF family. This peptide is widely distributed in the brain and digestive tract and has extremely high affinity to CRF receptors. Although its physiological actions remain largely unknown, it may be related to schizophrenia (Rubinstein, 2000).

Luteinizing Hormone-Releasing Hormone (LHRH), Gonadotrophin-Releasing Hormone (GnRH) and Growth Hormone-Releasing Hormone (GHRH)

Gil-Ad *et al.* (1981) studied the responses of growth hormone (GH) to LHRH and TRH in 10 adolescent males with schizophrenia with a mean age of 15 years and nine age-matched normal controls. Before antipsychotic treatment, a marked increase in GH due to LHRH was observed in eight of the 10 patients and a marked increase in GH due to TRH was observed in four of the six patients. No change was observed in the normal control group. Three months after antipsychotic treatment, no increase in GH was observed in five of the six patients treated with LHRH, but GH increased in three of the four patients treated with TRH.

Brambilla *et al.* (1976) measured the responsiveness of gonadotrophin to GnRH in 15 males with chronic schizophrenia (dissociated hebephrenia) and 15 controls with mental retardation matched for age and duration of hospitalization. In the schizophrenia group, both follicite-stimulating hormone (FSH) and LH increased compared with the control group. Chlorpromazine therapy had no effect.

Nerozzi *et al.* (1990) studied the responses of GH to GHRH in 18 males with schizophrenia (DSM-III) (13 in the acute period, five in the chronic period) and nine normal controls but observed no significant difference. Peabody *et al.* (1990), on the other hand, studied responses of GH to GHRH in seven patients with schizophrenia (RDC; Research Diagnostic Criteria for a selected group of function disorder), three schizoaffective patients, seven patients with depression and nine normal controls. Responses of GH were significantly smaller in the schizophrenic patients than in the normal controls.

Effects of many neuropeptides released from the hypothalamus on neurotransmission remain unclear. Research to date in psychiatric disorders has focused primarily on neuroendocrine responses.

Pituitary Hormones (Vasopressin, Oxytocin)

Glovinsky *et al.* (1994) compared the oxytocin concentration in cerebrospinal fluid between schizophrenic patients and normal controls but observed no difference between the two groups or change due to neuroleptic treatment.

Legross *et al.* (1992) administered apomorphine (a direct dopamine agonist) to nine males with schizophrenia (drug-free for 2 weeks or longer) and nine healthy male controls and measured the serum oxytocin–neurophysin (Oxt-np) and vasopressin–neurophysin (Vas-np) concentrations. In the normal control group, both Oxt-np and Vas-np increased, with a peak 20 min after administration. In the schizophrenia group, the release of Vas-np was reduced, but the release of Oxt-np was increased, compared with the normal control group. An increase in the activities of dopaminergic nerves was suggested to enhance the functions of oxytocin-mediated nerves more notably in schizophrenia.

Feifel and Reza (1999) evaluated the function of oxytocin in rats using prepulse inhibition (PPI) as a research paradigm. Deficit of PPI is considered to be related to the pathology of schizophrenia. Although the administration of discipline (a non-competitive NMDA antagonist) and amphetamine (an indirect dopamine agonist) reduced PPI, this reduction was dose-dependently prevented by subcutaneous administration of oxytocin. These results suggest that oxytocin prevents inhibition of PPI via the dopaminergic or glutaminergic nervous system and that it may have an antipsychotic activity.

Thus, neuropeptides known as pituitary hormones have been studied primarily with regard to pharmacological aspects. However, details of the mechanisms of their relationships with classic neurotransmitters are still unknown.

Some patients with schizophrenia are known to exhibit symptoms of water intoxication, but the causes of these symptoms remain unknown. There have been a few studies on secretion and actions of vasopressin as an antidiuretic hormone in patients exhibiting such symptoms.

Goldman *et al.* (1997) measured the secretion of arginine-vasopressin (AVP) in six schizophrenic patients showing polydipsia and hyponatraemia and eight patients not showing these symptoms. In the hyponatraemic group, AVP secretion was increased with exacerbation of mental symptoms. Delva *et al.* (1990) showed that responses of AVP in the kidney were poor in patients with chronic schizophrenia who develop water intoxication. However, how these observations are related to the essential pathology of schizophrenia is uncertain, and whether there are abnormalities of this peptide related to neurotransmission or not is unknown.

Others (Orexin, Nociceptin)

As a result of the marked advances in molecular biology, information concerning many genes has become available, while their functions remain unknown. In the course of genome research, many genes considered to code for receptors of physiologically active substances have been discovered. Two peptides, i.e. orexin-A and -B, were identified in studies of such orphan receptors (Sakurai

et al., 1998). Orexin is distributed over the entire mammalian cerebrum and has been shown to be involved in diverse mental and neural functions: mice administered orexin in the frontal lobe and limbic system exhibit an enhancement of eating behaviour and spontaneous activities, and promotion of stereotyped behaviour, involuntary actions, drinking behaviour and awakening (Sakurai *et al.*, 1999; Hagan *et al.*, 1999). Also, orexin is closely related to sleeping and awakening, and functional insufficiency of the orexin-mediated nerves was found to be involved in the pathology of narcolepsy (Peyron *et al.*, 2000). No relationship between this peptide with the pathology of schizophrenia has yet been reported. However, as it is distributed richly in the regions that have been concerned principally with the pathogenesis of schizophrenia, and as its diverse actions are related to a wide range of mental and neural functions, it will be a major subject of future research.

Similarly, nociceptin, a neuropeptide, was discovered in research of ligands of orphan receptors (Meunier *et al.*, 1995). Although this nociceptin receptor structurally resembles the opioid receptor, it has no effect on known opioids. Nociceptin and its receptors are present densely in the central nervous system including the hippocampus, and this peptide was suggested by studies using mice defective in nociceptin receptor gene to control plastic changes in the nervous system (Manabe *et al.*, 1998). How this peptide is related to the pathology of schizophrenia must still be clarified, and it must be studied in connection with the nerve development disturbance hypothesis from the viewpoint of the regulatory mechanism of synaptic transmission.

As observed above, new peptides are being discovered and identified one after another as a result of improvements in molecular biological techniques, but their mental and neurological functions remain largely unknown, and their relationships with the pathology of psychiatric disorders must as yet be evaluated.

STUDIES OF NEUROPEPTIDES FROM PHARMACOLOGICAL VIEWPOINTS

Some neuropeptides and their antagonists may serve as antipsychotic drugs, and studies concerning their use as possible drugs have accumulated. NT is the most widely studied among such neuropeptides.

More than 20 years ago, Nemeroff (1980) presented the hypothesis that NT is an endogenous neuroleptic. Since then, many studies concerning the actions of NT as an antipsychotic have been carried out. These studies have been conducted on the basis of the thought that functional insufficiency of the NT-mediated nervous system is an aetiological factor of schizophrenia and that NT receptors interact with dopamine D_2 receptors (Fuxe *et al.*, 1995). Von Euler *et al.* (1991) showed that NT reduces the affinity of dopamine D_2 receptors to dopamine agonists through direct receptor–receptor interaction. Many studies have demonstrated that NT acts as an antipsychotic in the limbic system and, particularly, that it produces effects similar to those of a non-typical antipsychotic such as clozapine (Jolicoeur *et al.*, 1981, 1993; Kasckow and Nemeroff, 1991; Radke *et al.*, 1998).

Several clinical studies have been performed on the basis of the hypothesis that disturbance of endorphin metabolism is an aetiological factor of schizophrenia. This hypothesis assumes that α-endorphins with amphetamine-like activities are produced in excess and that the production of γ-endorphins is reduced. Verhoeven *et al.* (1978) reported the first of these clinical studies. Moderate improvements were observed in drug-free schizophrenic patients by the administration of Des-Tyr^1-γ-endorphins (DTγE). A few authors then reported the clinical efficacy of DTγE (van Ree *et al.*, 1978; Metz *et al.*, 1981) while others reported opposite results (Tamminga *et al.*, 1981; Meltzer *et al.*, 1982). Nowadays, the efficacy of DTγE for the treatment of schizophrenia is thought to be doubtful (Montgomery *et al.*, 1992).

Naloxone (an opiate receptor blocker) therapy was attempted in schizophrenic patients on the basis of the concept that schizophrenia is related to hyperactivity of the opioid-mediated fibres in the central nervous system (McNicholas and Martin (1984). Gunne *et al.* (1977) first administered naloxone clinically to schizophrenic patients and noted alleviation or disappearance of auditory hallucination after treatment. After this report, the effectiveness of naloxone as an antipsychotic was evaluated, but there have been reports that supported (Cohen *et al.*, 1985) and those that refuted (Pickar *et al.*, 1989) its effectiveness.

Ceruletide, a CCK-related peptide, was administered to schizophrenic patients on the basis of the results of animal experiments suggesting that CCK acts on the dopaminergic neuronal system and produces behavioural pharmacological effects similar to those of antipsychotics (Cohen *et al.*, 1982; van Ree *et al.*, 1983; Feifel *et al.*, 1999). Moroji *et al.* (1982) showed that ceruletide has therapeutic effects on both positive and negative symptoms of schizophrenia and that these effects are of long duration. However, there have been reports that denied its effectiveness (Mattes *et al.*, 1985; Tamminga *et al.*, 1986).

From the results of animal experiments using a CCK antagonist (Zetler, 1985), a CCK receptor antagonist was estimated to produce its antipsychotic effect by reducing the enhancement of dopamine metabolism, and proglumide (an unselective CCK antagonist) was used tentatively in schizophrenic patients. However, its effectiveness was not established (Innis *et al.*, 1986; Hicks *et al.*, 1989).

As observed above, the possibility of neuropeptides or their antagonists as treatments for schizophrenia has been evaluated. However, there remain a number of unknown aspects including the essential functions of the peptides, their relationships with classic transmitters and their pharmacological behaviour, and further research is needed concerning their action mechanisms, which provide the basis for this hypothesis. However, as dopamine antagonists are the mainstay of antipsychotic medication today, neuropeptides are closely related to the aetiology and pathology of schizophrenia, and drugs that act via the peptidergic neuronal system are still attractive as possible treatments for the disease.

THE FUTURE OF NEUROPEPTIDES IN PSYCHIATRY

During the past decades, a number of neuropeptides have been discovered in the body and their functions have been studied. These research activities are still continuing. As a result, neuropeptides are known to be involved in diverse brain functions including memory and learning, emotion, senses, appetite, sexual drive and sleep. In addition, new peptides are being discovered as a result of technical improvements in molecular neuroscience. For example, many receptors, ligands of which have not been found, have been cloned recently, and orexin and nociception have been identified in the course of the search for their ligands. It is considered that new neuropeptides will continue to be identified. Detailed functions of neuropeptides have also been clarified as a result of the advent of new technology such as the preparation of knockout mice.

The relationship between the aetiology of schizophrenia and neuropeptides has been discussed in connection with classic neurotransmitters. Classic neurotransmitters such as dopamine and noradrenaline are known to coexist with neuropeptides in the same neurones, and they act under a strong mutual influence. However, despite accumulation of research, the relationship of neuropeptides with the aetiology of schizophrenia remains unknown. Further evaluation by various approaches, such as anatomical, physiological, pharmacological and molecular neurological, is needed. On the

basis of these results, the role of neuropeptides in the pathogenesis of schizophrenia is still unclear.

Most of the anatomical changes in neuropeptides reported to date have been observed in areas such as the medial part of the temporal lobe and frontal lobe. These areas have attracted attention in morphological research of schizophrenia. Akbarian *et al.* (1993a, 1993b) examined the development hypothesis of this disease on the basis of examination of the distribution of cells containing NADPH-d (nicotinamide adenine dinucleotide phosphate) in the brain of schizophrenic patients. Many NADPH-d-containing cells coexist with neuropeptides such as SOM and NPY (Vincent *et al.*, 1983a; Unger and Lange, 1992). Although their interactions must still be clarified, evaluation of neuropeptides from the viewpoint of the development disturbance hypothesis of schizophrenia is necessary.

Recently, the concept that homeostasis of the body is maintained by a network that tightly links the neural, endocrine and immune systems was presented (Blalock, 1994). This is a concept that allows comprehensive understanding of the pathophysiology and aetiology of various diseases including neurological disorders, responses to stress and protective mechanisms of the body. Neuropeptides play important roles as mediators of information transmission in such a biological system. For example, some neuropeptides have been shown to exert marked effects on the immune system, and specific parts of the brain have been found to be closely related to immune functions. Thus, neuropeptides perform as yet unknown functions in the body, and the quest for their roles in the aetiology of mental and neurological disorders should lead to clarification of their pathology.

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XVII-5

Neuroimmunology of Schizophrenia

N. Müller

INTRODUCTION

Immunological alterations in schizophrenia have been described in the international literature since the beginning of the last century (Bruce and Peebles, 1903; Dameshek, 1930; Lehmann-Facius, 1939). However, for several reasons the focus of interest has changed from the immune system since the 1960s. One reason was the introduction of antipsychotics into the therapy of schizophrenia, leading to the dopamine hypothesis as the focus of research activities. Another reason was that the components and functions of the immune system have not been understood very well during those times, while during the last decade major advances in immunological research could be observed.

With regard to clinical symptomatology, acuity of the symptoms, course, treatment response and probably also aetiology, schizophrenia is a heterogeneous disorder. Besides methodological pitfalls, this heterogeneity might contribute to the heterogeneity of the results of biological investigations, which can be found in nearly all fields of biological research in schizophrenia. A widespread heterogeneity can also be observed in the results of immunological studies in schizophrenia (overview: Müller and Ackenheil, 1998).

The objectives of psychoneuroimmunology are to elucidate the reciprocal influences of the nervous system and the immune system and their effects on behaviour and health. This field covers *in vitro* studies of tissue and lymphocytes, investigations of the influence of stress, stress-coping and personality traits on the function of the immune system, also the role of psychological factors during pathogenesis, and the course of tumour disorders and their psychotherapeutic treatment. Additionally, human behaviour studies and animal experiments, e.g. conditioning studies (Ader and Cohen, 1975) or investigations into the dependency of coupling behaviour from the HLA system (Eggert *et al.*, 1990), belong to the broad spectrum of psychoneuroimmunology.

Cytokines mediate the information between cells of the peripheral immune system and the central nervous system (CNS). In part, they are actively transported through the blood–brain barrier, but they are also released from (activated) astrocytes and microglial cells. Interleukin- (IL-)1, IL-2, IL-4, IL-6, IL-10 and tumour necrosis factor α (TNF-α) are the most relevant activating cytokines known to act on the CNS. Cytokines behave like a network since they can activate cells to produce other cytokines. Recent findings show that cytokines are relevant in psychiatric disorders, possibly mainly due to their influence on neurotransmission. From a clinical point of view endogenous psychoses show several parallels to autoimmune disorders. These include onset during early adulthood in many cases, genetic vulnerability, waxing and waning course and, as in depression, the sex ratio.

During the 1950s, several investigations tested the hypothesis that schizophrenia may be an autoimmune disorder. Serum components which showed features of anti-self antibodies directed against brain tissue have been described in schizophrenic patients (Fessel, 1963; Heath and Krupp, 1967; Pandey *et al.*, 1981). With the increasing dominance of the dopamine hypothesis of schizophrenia created by the 2000 Nobel prize winner A. Carlsson (Carlsson and Lindquist, 1963), immunological investigations in psychiatry were disregarded by the scientific community for several years. Later Knight tried to integrate the autoimmune and dopamine hypotheses of schizophrenia by postulating that schizophrenia may be due to dopamine receptor-stimulating auto-antibodies (Knight, 1982). During recent years, several inadequacies of the dopamine hypothesis as to the cause of schizophrenia became evident, e.g. the schizophrenic negative symptomatology or the resistance to antipsychotic therapy with dopamine receptor-blocking substances in about 20–30% of patients. Therefore, other biological models of explanation became of increasing interest. In parallel, the noradrenaline (Schildkraut, 1965; Matussek, 1966) and serotonin hypotheses (Coppen and Swade, 1988) of depression were not sufficient to explain depressive disorders. Nevertheless, there is no doubt about the major role of the catecholaminergic neurotransmission in affective and schizophrenic disorders, and one focus of psychoimmunological research in this field is the influence of immunological processes on catecholaminergic neurotransmission.

The increasing role of psychoneuroimmunology is also due to the rapid development of immunological methods. Thanks to the recent immunological and molecular biological investigation techniques, our knowledge of the function, mechanisms of regulation and interaction of the immune system is growing, as shown in Figure XVII-5.1. A much more accurate description of the interaction and function of the highly differentiated and variable immune system is possible owing to the recent methodological advances.

That immune processes can mediate psychotic phenomena is shown by psychiatric disturbances which occur during different

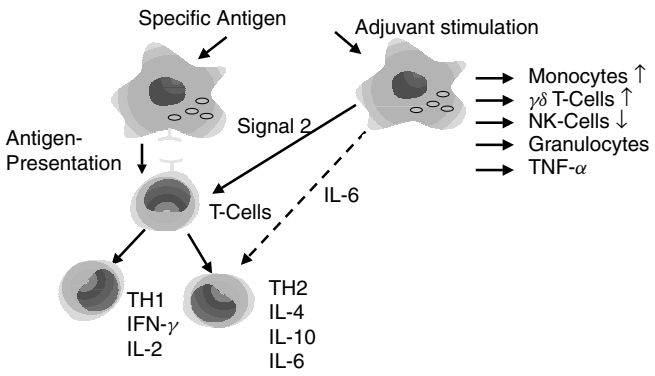

Figure XVII-5.1 Model of immune activation in schizophrenia: predominance of the unspecific immune system and the TH2 answer

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autoimmune disorders, such as lupus erythematosus (Krüger, 1984), scleroderma (Müller *et al.*, 1992), Sjögren's syndrome (Raps *et al.*, 1986), and anti-phospholipid syndrome (Kurtz and Müller, 1994).

Modern immunological methods at first led to the investigation of lymphocyte sub-populations by monoclonal antibodies. Different cell surface markers point to different functions of the lymphocytes. During the 1980s, several researchers studied the cellular immune system in different psychiatric disorders (McAllister *et al.*, 1989; Villemain *et al.*, 1989; Müller *et al.*, 1991, 1993a, 1993b; Sperner-Unterweger *et al.*, 1992; Maes *et al.*, 1995). The findings in the cellular immune system, in particular in schizophrenic and affective psychoses, indicated that there was an immune activation in these disorders, which, in turn, raised the question as to the function of cytokines, a key factor in the activation of the immune system.

THE CYTOKINE NETWORK IN THE CNS

The signalling, differentiation and function of the (peripheral) cellular immune system is mediated and characterized by cytokines. Cytokines activate CNS cells in different ways. First, several cytokines such as IL-1 (Banks and Kastin, 1992), IL-2 (Banks *et al.*, 1993) and TNF-α (Gutierrez *et al.*, 1993) can be transported from the blood into the CNS by active transport mechanisms, as *in vitro* studies have pointed out. Second, glia cells secrete cytokines after activation by an antigenic challenge. Finally, Norris and Benveniste (1993) recently reported that cytokine secretion in the CNS can be stimulated by neurotransmitters. They showed that noradrenaline stimulates the release of IL-6 from astrocytes *in vitro* in a dose-dependent manner, an effect that can be antagonized by blocking the adrenergic receptors. Since IL-6 is closely linked with the function of other cytokines e.g. IL-1, IL-2 and TNF-α, this finding indicates that neurotransmitters can activate the cascade of cytokines (Ransohoff and Benveniste, 1996). This may represent an important psychoneuroimmunological regulative mechanism affecting (auto-)immune disorders, susceptibility to infections and psychiatric disorders. The noradrenaline released during stress (Engel *et al.*, 1980) may act as a cytokine-activating stimulus, which thus activates immune phenomena mediated by the cytokine cascade.

GLIAL CELL DYSFUNCTION IN SCHIZOPHRENIA

Astrocytes form the largest cell population in the CNS, exceeding neurones by more than 10-fold (Benveniste, 1992). Astrocytes are located immediately beside neurones, often enfolding them. This localization underlines the close relationship between astrocytes and neuronal function. About 10% of the glia cells are microglia cells (Benveniste, 1992). Both microglia cells and astrocytes can produce and release cytokines after activation, as shown schematically in Figure XVII-5.2.

Some authors postulate that microglial dysfunction initiated by early CNS viral exposure results in the abnormal neural development and neurotransmitter dysfunction seen in schizophrenia (Munn, 2000). This theory is confirmed by findings of abnormally high microglial activation in a subgroup of schizophrenic patients (Bayer *et al.*, 1999). An increase of the numerical density of HLA-DR-positive microglial cells (HLA-DR is the marker of HLA class II activation in the peripheral blood and of microglial activation in the CNS) especially in the temporal and frontal cortex of chronic schizophrenics, not related to ageing, has been described recently (Radewicz *et al.*, 2000).

THE CONCEPT OF INNATE AND ADAPTIVE IMMUNITY IN HUMANS

The immune system has developed during evolution over millions of years. In order to guarantee successful defence against numerous varying invading, life-threatening micro-organisms like bacteria or viruses, a highly differentiated system consisting of different lines of defence was established. A widespread heterogeneity was the consequence: two functionally different immune systems both representing different types of barriers and each consisting of

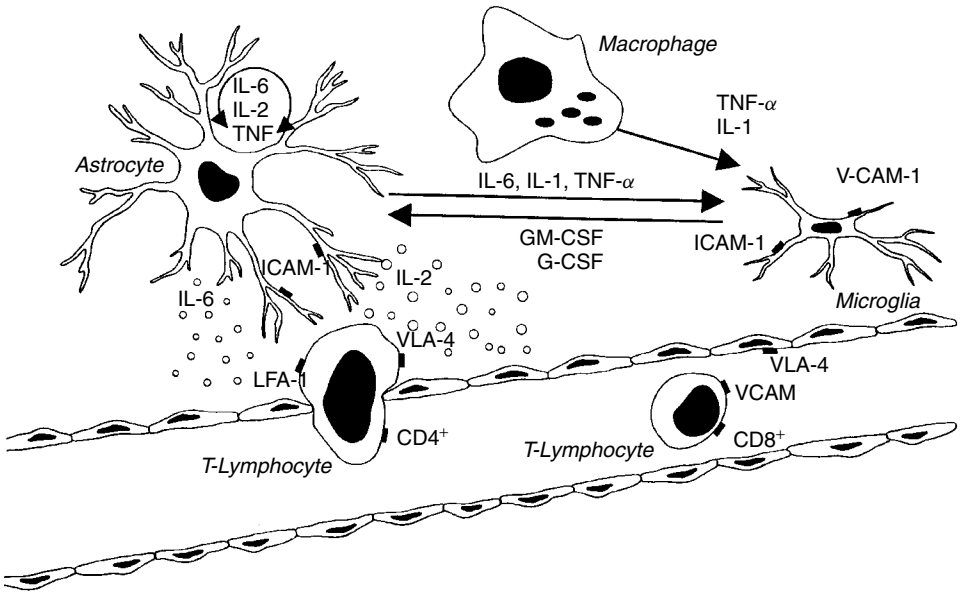

Figure XVII-5.2 Schematic overview on the network of blood lymphocytes, adhesion, glial cells, cytokine production after activation, and the blood-brain-barrier

Table XVII-5.1 Components of the unspecific 'innate' and the more specific cellular 'adaptive' immune systems in humans

Components	Innate	Adaptive
Cellular	Monocytes Macrophages Granulocytes NK cells γ/δ cells	T and B cells
Humoral	Complement, APP, mannose-binding lectin (MBL)	Antibodies

cellular and humoral immune components. The 'innate' immune system is the phylogenetically older, 'primitive' one. Its cellular arm is represented by monocytes/macrophages, granulocytes and natural killer (NK) cells. The humoral arm consists of acute-phase proteins and the complement system. This 'unspecific' immune system represents the first line of defence.

The specific part of the immune system of higher organisms including humans is the 'adaptive' immune system, consisting of the cellular arm of the T and B cells and the humoral arm of specific antibodies. This system includes higher functions like memory and can be conditioned. In case of a re-exposition to a specific antigen, this system can recognize the enemy and initiate a specific immune response (Table XVII-5.1).

The 'innate' and 'adaptive' immune systems are functionally balanced. Within the 'adaptive' immune system is another balance regarding the activation of the cellular and the humoral immune system. The cellular arm of the adaptive immune system is mainly activated by the T-helper-1 (TH-1) system: helper cells, which produce the activating 'immunotransmitters' IL-2, IL-12, IFN-γ and TNF-α. The humoral arm of the adaptive immune system is mainly activated via the TH-2 system: helper cells, which produce mainly IL-4, IL-10 and IL-6.

RELATIONSHIP BETWEEN CLINICAL CHARACTERISTICS AND IMMUNE ALTERATIONS

Signs of an inflammatory disease process in schizophrenia have been observed in a subgroup of schizophrenic patients (Körschenhausen *et al.*, 1996). Clinical features of this subgroup showing signs of an immunological or inflammatory disease have been studied by several groups of researchers. It was observed, however, that the symptomatology, e.g. paranoid symptoms or negative symptoms (Cazullo *et al.*, 1998; Müller and Ackenheil, 1995), acuity (Korte *et al.*, 1998; Sperner-Unterweger *et al.*, 1992; Wilke *et al.*, 1996) and drug treatment (Özek *et al.*, 1971; Saunders and Muchmore, 1964; Maes *et al.*, 1995; Masserini *et al.*, 1990; Müller *et al.*, 1991, 1997b, 1997c, 1999; Pollmächer *et al.*, 1996) influence the immunological parameters. Also the clinical response to treatment with neuroleptics seems to be related to immune parameters (Müller *et al.*, 1993a). The present author's own data show that the IgG content of CSF is especially high in patients with pronounced schizophrenic negative symptoms, which are associated with an unfavourable course of the disorder and often with treatment resistance (Müller and Ackenheil, 1995). Those findings fit with the suggestion that the inflammatory response system is activated in patients which are treatment resistant to antipsychotic medication (Maes *et al.*, 2000).

However, a clear-cut differentiation between immunological subgroups in schizophrenia is not yet established. The discussion of this relationship between clinical characteristics of schizophrenia and parameters of the immune system may help to define subgroups which are characterized by disturbances of the immune system.

GENETICS AND SCHIZOPHRENIA

There is no doubt that environmental and genetic factors play a role in schizophrenia. Studies in monozygotic twins showed that the risk of a healthy monozygotic twin of a schizophrenic twin is about 50% to develop a schizophrenic disorder as well, i.e. about 50% of the risk is attributed to genetic factors and 50% to environmental factors. One of the strongest findings of genetic linkage studies in schizophrenia points to the chromosomal region 6p22–23. Several genes are located very near to this region which are associated with immune function. The HLA region (6p23.1) is crucially involved in immune function, e.g. during (viral) infection (Lindholm *et al.*, 1999; Schwab *et al.*, 1995).

A common genetic vulnerability locus has been suggested on the HLA class II allele DQB1*0602 for schizophrenia, multiple sclerosis and narcolepsy, although confirmation studies are still lacking (Großkopf *et al.*, 1998). For African-American schizophrenics, a negative association could be found in two studies (Nimgaronkar *et al.*, 1992; Nimgaronkar *et al.*, 1997) and in one study for Chinese schizophrenics (Nimgaronkar *et al.*, 1995). Those interesting but divergent results underline that further investigations of HLA class II in schizophrenia should be carried out.

This finding is of special interest because there is one more link to the immune function and schizophrenia on chromosome 6: Retroviral control elements have been identified on chromosome 6 near the HLA region in patients with insulin-dependent diabetes mellitus (IDDM: Badenhoop *et al.*, 1996), whereas a marked vulnerability for IDDM has been found in first-degree relatives of schizophrenic patients (Wright *et al.*, 1996). IDDM, however, is inversely associated with schizophrenia (Finney, 1989). Another candidate for HLA investigations is the DRB1*04 gene because it is positively associated with rheumatoid arthritis, an autoimmune disease that is inversely associated with schizophrenia (Eaton *et al.*, 1992). A negative genetic association of DRB1*04 with schizophrenia has been found (Wright *et al.*, 1996) and there is evidence that this might be a robust result (Wright *et al.*, 2001), e.g. the preferential non-transmission of DRB1*04 alleles from heterozygous parents to schizophrenic offspring (Wright *et al.*, 1997).

Another methodological approach is the investigation of characteristics in the immune function of the schizophrenic disorder and HLA genes. An example for such an approach is the association between HLA genes and the maternal immune response of mothers from schizophrenic offspring (Wright *et al.*, 1993).

Our own study showed a strong relationship between cytotoxic gamma/delta T cells in unmedicated schizophrenic patients, the blood–brain barrier and the HLA allele DPA 02011 (Müller *et al.*, 1998).

The introduction of HLA genotyping has effectively eliminated the inaccuracies associated with HLA serotyping and has revealed the genetic polymorphism within the HLA system as a complexity previously unimagined.

Many HLA investigations were performed before the era of HLA genotyping, and different associations especially between the HLA class I system and schizophrenia have been reported without conclusive results (overview: Wright *et al.*, 2001).

However, studies of the association between specific characteristics of the schizophrenic disorder and the HLA class I system showed some interesting results. Several studies focused on the association of HLA class I and response to antipsychotic treatment or increased risk for adverse effects of neuroleptics.

These studies found an association between HLA-Bw44 and the titres of auto-antibodies to chlorpromazine in chronic schizophrenics (Canoso *et al.*, 1982). A significant difference in the treatment

response to γ-endorphin in schizophrenia with respect to HLA-B15 has also been described (DeJongh *et al.*, 1982). Moreover, there have been reports showing HLA-B44 to be associated with an increased risk of developing tardive dyskinesia (Canoso *et al.*, 1986) and drug-induced Parkinsonism (Metzer *et al.*, 1989). In a preliminary study a positive association between HLA-A1 and an increased risk for neuroleptic-induced akathisia was observed (Brown and White, 1991). A cluster-analytic study (Müller *et al.*, 1993) showed that the altered immune function in schizophrenia, especially the enhanced $CD3^+$ and $CD4^+$ cells and the reduced inhibition of the activated immune system ('suppressor cell function'; see above), was significantly related to the HLA class I system and to the family history of psychiatric diseases. This statistical method, which analyses in a combined manner immune-functional, immune-genetic and clinical data, showed two independent factors: the HLA system and the family history of psychiatric diseases contributing to the altered immune function in schizophrenia.

Genetic determinants of infection include genes which modulate the immune response, the expression of viral receptors and the susceptibility of cells to soluble factors generated during the course of viral infection (Yolken *et al.*, 1999).

VIRUS HYPOTHESIS OF SCHIZOPHRENIA

Different case reports of patients presenting with psychiatric symptoms during viral infections with herpes simplex encephalitis, varicella zoster encephalitis, or subacute sclerosing panencephalitis led to the suggestion that viral infections might play a role in the pathogenesis of schizophrenia. Epidemiological data of an increased number of manifestations of schizophrenia in winter-born people (Torrey *et al.*, 1977), of geographic variations (Torrey, 1987), and of an increased incidence of schizophrenia in children of mothers infected by an epidemic influenza during pregnancy in England in 1957 (O'Callaghan *et al.*, 1991) point to a virus pathogenesis as well (Yolken and Torrey, 1995). Numerous studies have detected raised titres of antibodies against certain viruses (herpes simplex, measles, cytomegalovirus, varicella, Borna virus) in schizophrenic patients as compared to controls and/or increased cerebrospinal fluid (CSF)/serum ratios of viral antibodies, indicating a local synthesis of antibodies in the brain or CSF (Hoechtlen and Müller, 1992; Torrey *et al.*, 1977; Albrecht *et al.*, 1980; Bechter *et al.*, 1999; Gottlieb-Stematsky *et al.*, 1981), but the significance of these findings is debated. Viruses or a virus genome were not detectable in the CSF or brain tissue of schizophrenic patients by most authors (Stevens *et al.*, 1984; Taylor and Crow, 1986; Alexander *et al.*, 1992).

It may be assumed that viruses may be detectable only during certain states of virus-induced diseases ('hit and quit'). Thus, the fact that viruses or a virus genome could not be isolated does not exclude the involvement of viruses in the pathogenesis of the disease. On the other hand, comparable findings were described in multiple sclerosis (MS), a disease with a probable autoimmune pathogenesis. In MS, raised virus antibody titres are discussed as representing a polyspecific local synthesis of antibodies against neurotropic viruses, particularly measles, rubella and varicella zoster virus (MRZ reaction) (Stevens, 1988). The polyspecific antiviral response seems to indicate an immune dysregulation in accordance with an unspecific activation of antibody-producing B cells, although an involvement of viruses in the aetiology of MS has not been excluded until now. MS and schizophrenia show some further parallels, like waxing and waning course, genetic factor and early onset in a number of cases (Felgenhauer 1990). Moreover, MS cases sometimes appear as schizophrenia-like psychosis (Pisiur-Strehlow *et al.*, 1988), too.

The virus and the autoimmune hypotheses of schizophrenia may be connected with regard to the role of viruses in molecular mimicry: viruses induce cells to change their identity, probably via expressing surface antigens; thus they are not recognized as 'self', but as 'non-self' and therefore are attacked by their own immune cells. From that point of view, our findings of an immune activation in schizophrenia are compatible with both the virus and the autoimmune hypotheses of schizophrenia.

Recent studies indicate that schizophrenia is associated with the upregulation of a number of different RNA species (Yolken *et al.*, 1999). These RNAs are supposed to represent genomic or messenger RNA derived from novel viral agents. In this regard it is of interest that several of the (predicted) proteins are homologous to viruses of non-human origin. The authors discuss the possibility that certain unidentified human retroviruses may contribute to schizophrenia together with genetic determinants — viral infection of neuronal cells can, in combination with genetic determinants of susceptibility, result in the profound alteration of brain function typical of schizophrenia (Yolken *et al.*, 1999). Interestingly, a case of subclinical Borna disease viral encephalitis presenting as schizophrenia has been described recently. While schizophrenic positive symptoms improved during antipsychotic pharmacological treatment, negative symptoms did not. Therefore, the authors decided to use the CSF filtration method, an experimental therapeutic method for treatment of therapy-resistant patients suffering from Guillain–Barré syndrome. The schizophrenic negative symptoms rapidly improved, as did neurological soft signs and cognitive function. The authors suggest that toxic factors are removed by the filtration (Bechter *et al.*, 1999). From a scientific point of view, those therapeutic approaches are interesting but of course need further evaluation.

NEURODEVELOPMENTAL HYPOTHESIS

The excess of late winter/early spring births in patients with schizophrenia has been attributed to infectious, nutritional or other environmental factors (Torrey *et al.*, 1997; Mortensen *et al.*, 1999). The hypothesis that an infection of pregnant mothers is related to an increased risk for schizophrenia in the offspring implicates sequelae for the development of the unborn offspring. The pathological mechanisms underlying prenatal and perinatal risk factors for schizophrenia, including infection, remain largely unstudied. Infection during pregnancy is one of various factors defined as obstretical complication. Cytokines are known to regulate normal brain development and have been implicated in abnormal brain development (Merill, 1992; Mehler *et al.*, 1995; Mehler and Kessler, 1997). It has been hypothesized that pro-inflammatory cytokines generated by the immune system are important mediators of the association between maternal infection, abnormal brain development and increased risk for schizophrenia and other neurodevelopmental disorders (Leviton, 1993; Jarskog *et al.*, 1997). There is no doubt about the significant association between obstretical complications and the ultimate development of schizophrenia (Geddes and Lawrie, 1995; Jones *et al.*, 1998; Dalman *et al.*, 1999; Hultman *et al.*, 1999).

Not only are influenza and other viral infections during pregnancy known to be associated with an increased incidence of schizophrenia in an offspring, but it is likely that the association between *in utero* or early post-natal exposure to obstretical complications and schizophrenia is a more general phenomenon not limited to a single aetiological viral agent such as influenza. Infectious processes in general but also other pathological factors such as hypoxia are known to lead to an altered cytokine production in the brain.

Pro-inflammatory cytokines are neurotoxic to a variety of developing neurones *in vitro*. For example, IL-1β, IL-6 and TNF-α decrease survival of foetal dopaminergic and serotonergic neurones *in vitro* (Jarskog *et al.*, 1997). Moreover, IL-1β decreases neurone

survival in primary cultures of embryonic rat hippocampus (Arajujo and Cotman, 1995) and TNF-α potentiates glutamate excitotoxicity in cultures of foetal cortical neurones (Chao and Hu, 1994). In an animal model of prenatal exposure to infection with pregnant rats it could be shown that *Escherichia coli* lipopolysaccharides (LPS) in different doses lead to alterations of cytokine levels. Low-dose LPS was associated with significant increase of IL-1β, IL-6 and TNF-α in the placenta and IL-6 in the amniotic fluid. High-dose LPS was associated with a significant elevation of IL-6 and TNF-α in the placenta, and of TNF-α in the amniotic fluid, but with a significant decrease of TNF-α in the foetal brain. These observations show that maternal exposure to infection alters pro-inflammatory cytokine levels in the foetal environment, which may have a significant impact on the developing brain (Urakubo *et al.*, 2001).

BLOOD–BRAIN BARRIER IMPAIRMENT

Abnormalities of the CSF are regularly reported in about 20–30% of psychiatric patients (Kirch *et al.*, 1985; Naber *et al.*, 1986). Investigations of schizophrenic patients have shown increased total protein content (>45 mg%) or a blood–brain barrier (BBB) impairment in about one-third, and an intrathecal IgG production in 15% of patients (Müller and Ackenheil, 1995). The increased immunoglobulins and the BBB disturbance are likely to be part of an immune process whose pathophysiological relevance is not yet clear. Nevertheless, a correlation of schizophrenic psychopathology with IgG content suggests a close relationship between the immune process and the schizophrenia in those patients who show negative symptoms (Müller and Ackenheil, 1995).

The physiological function of a CNS immune activation is, however, the defence against invading antigens. For example, it could be shown that an infection with herpes virus which cannot activate glia cells takes a more disadvantageous course than an infection with herpes virus involving activated glial cells (astrocytes) in the immune response (Lewandowski *et al.*, 1994). Infection with measles virus *in vitro* shows that the levels of cytokine release in glia cells depend on the duration of infection (Schneider-Schaulies *et al.*, 1993).

On the other hand, a local CNS immune activation may be controlled insufficiently due to a lack of communication between the peripheral immune system and the CNS. T cells invading from the peripheral blood into the CNS are able to control an immune activating process in the CNS. There are hints that a T cell penetration through the BBB is necessary for control of several inflammatory processes (Reich *et al.*, 1992; Shankar *et al.*, 1992).

A BBB disturbance is accompanied by an involvement of astrocytes, because the capillary endothelial cells, which are connected by tight junctions, are surrounded almost completely by astrocytes that modulate the BBB in the small vessels (Benveniste, 1992; Cancilla and DeBault, 1983). The present author's investigations have shown that antipsychotic treatment and psychopathological improvement are associated with a significant increase in the adhesion molecule expressing T-helper cells VLA-4$^+$/CD4$^+$ and the VLA-4$^+$/CD8$^+$ lymphocytes in the blood; moreover, a strong relationship between VLA-4$^+$/CD4$^+$ and LFA-1$^+$/CD4$^+$ lymphocytes and both BBB disturbance and CSF parameters (total protein, albumin, IgG) was observed (Müller *et al.*, 1997c). VLA-4 and LFA-1 are surface markers which are expressed on lymphocytes in order to mediate adhesion of lymphocytes to endothelial cells and a penetration into the tissue (Fabry *et al.*, 1992, 1994; Oppenheimer-Marks *et al.*, 1991). One speculative explanation of these findings is that VLA-4/CD4$^+$ and LFA-1/CD4$^+$ lymphocytes are involved in an immunoregulatory mechanism taking place between astrocytes and/or microglial cells and lymphocytes from the blood that is supported by the action of neuroleptics (Müller *et al.*, 1997c, 1999).

IL-2 and IL-6 play a pivotal role in the disturbance of the BBB. The peripheral application of IL-2 can cause an alteration of the BBB (Rosenstein *et al.*, 1986; Ellison *et al.*, 1987, 1990; Saris *et al.*, 1988) and a disturbance of the BBB seems to be involved in the CNS effects of peripherally applied IL-2. The alteration of the BBB by intrathecal application of IL-2 (Watts *et al.*, 1989) suggests that an increased intracerebral IL-2 production may contribute to the opening of the BBB.

As mentioned above, in many cases of schizophrenic patients obstretical complications such as pre-, peri- or post-natal CNS damage are suggested to contribute to the pathogenesis (Murray and O'Callaghan, 1991). Moreover, in those cases, a BBB disturbance may contribute to the pathological process, because birth complications are often connected with a disturbed BBB (Ebbesen and Knudsen, 1992), which promotes the invasion of immune activating components, such as infectious agents, into the CNS.

IL-6 also alleviates the invasion of lymphocytes into the CNS and promotes impairment of the BBB (Muraguchi *et al.*, 1988).

Due to an increased permeability of the BBB, a primarily local CNS process may spread into the periphery and activate the peripheral immune system. An increased communication between CNS immune system and peripheral immune system may include both a regulatory process leading to elimination of activating antigens (Reich *et al.*, 1992; Shankar *et al.*, 1992) and damage to CNS structures by invading auto-aggressive cells, as was suggested in MS (Ransohoff and Bö, 1996).

ACTIVATION OF THE INNATE 'UNSPECIFIC' IMMUNE SYSTEM IN SCHIZOPHRENIA

Although systematic investigations of the innate immune system are lacking in schizophrenia there are several hints that this part of the immune system may be more activated in schizophrenic patients than in controls. There is a report that monocytes are increased in schizophrenic patients compared to controls (Wilke *et al.*, 1996) and our own investigations of unmedicated schizophrenic patients also showed increased amounts of monocytes in schizophrenia compared to controls (unpublished results). An increase of cells of the 'first immune barrier' was also found in $\gamma\delta^+$CD8$^+$ cells in unmedicated schizophrenics (Müller *et al.*, 1998).

One of the key cytokines which initiates the immune response and in particular activates the B cell system is IL-6. Activated monocytes and macrophages are the major sources of the production and release of IL-6. Vice versa, increased levels of IL-6, as mentioned in the next paragraph, might be the result of the activation of the monocyte/macrophage system, leading to an overproduction of IL-6 by the innate immune system.

Since a significantly higher proportion of mononuclear phagocytes/macrophages in the CSF of schizophrenics has been reported compared to neurological controls, which was followed by a normalization of the cytological picture at a follow-up investigation in a small subgroup of patients during antipsychotic treatment, it seems that similar immunological processes take place in the peripheral and the CNS immune systems (Nikkilä *et al.*, 1999).

IL-6 IN THE CNS

IL-6 is a pleiotropic cytokine which is released from different cell types in the blood (macrophages, monocytes, T and B cells). One function of IL-6 is to activate B cells to synthesize antibodies (Plata-Salaman, 1991). However, like several other cytokines, IL-6 is not only synthesized and released in immune cells of the peripheral blood, but is also produced by activated astrocytes and microglia cells in the CNS. Several findings suggest that IL-6 may

mediate the exacerbation of autoimmune disorders in the CNS (Dunn, 1992); e.g. IL-6 supports the differentiation of B cells, the local IgG synthesis in the CNS and a BBB disturbance (Frei *et al.*, 1989; Muraguchi *et al.*, 1988). In the hypothalamus, IL-6 can induce the release of growth hormone-releasing hormone and thyroid-stimulating hormone, and it stimulates *in vitro* the secretion of prolactin and growth hormone from pituitary cells (Spangelo *et al.*, 1989).

A strong relationship between IL-6 and neurotransmitter production has been reported by different studies. IL-6 can stimulate neurones *in vitro* to secrete dopamine and probably other catecholamines as well (Hama *et al.*, 1991). The peripheral application of IL-6 in animal experiments enhanced the dopaminergic and serotonergic turnover in the hippocampus and frontal cortex, without affecting noradrenaline (Zalcman *et al.*, 1994). Conversely, noradrenaline can stimulate astrocytes to release IL-6 (Dunn, 1992). Both observations point to a direct influence of activating cytokines, especially IL-6, to the catecholaminergic neurotransmitter system.

IL-6 AND SCHIZOPHRENIA

Several reports showed increased serum IL-6 levels in schizophrenia (Ganguli *et al.*, 1994; Maes *et al.*, 1995; Frommberger *et al.*, 1997; Lin *et al.*, 1998). Two of the report's authors described a relationship between increased IL-6 levels and clinical features of schizophrenia: high serum- IL-6 levels were related to the duration of the disorder (Ganguli *et al.*, 1994) and to treatment resistance (Lin *et al.*, 1998). These findings suggest that IL-6 serum levels might be especially high in patients with an unfavourable course of the disease. On the other hand, methodological concerns have to be considered: IL-6 is a cytokine which is released mainly paracrinely and the function of serum levels is not yet fully established. Moreover, the cytokine IL-6, like IL-2, IL-1 and other cytokines, is not stable and many methodological pitfalls have to be considered.

The soluble IL-6 receptor (sIL-6R) is a more stable marker of the IL-6 system, both in the blood and in the CSF. Investigations of the sIL-6R levels in the CSF, however, showed that high levels of sIL-6R can be found especially in schizophrenic patients with a more marked paranoid–hallucinatory syndrome (Müller *et al.*, 1997b). These investigations also point to a more altered IL-6 system in patients with an unfavourable course of the disease: longer duration of illness, treatment resistance or more marked paranoid–hallucinatory symptomatology.

Another study found reduced levels of sgp130 in the CSF of schizophrenic patients compared to depressed patients and psychiatric healthy controls (Schwarz *et al.*, 2001). This result supports the view of a disturbance in the IL-6 system in schizophrenia, because gp130 is part of the IL-6 system. The soluble protein spg130 is acting as an antagonist to the gp130 receptor and mediates the inhibition of the IL-6 system (Narazaki *et al.*, 1993). Functionally, decreased sgp130 levels in the CSF point to a decrease in the inhibition of the IL-6 system and a functional increase in the activation.

ANTIPSYCHOTIC THERAPY AND THE IL-6 SYSTEM

There are several observations indicating that antipsychotic therapy with neuroleptics is accompanied by a functional decrease of the IL-6 system. A significant decrease of IL-6 during therapy with neuroleptics was described by Maes and co-workers (1995). Two studies found a significant decrease of sIL-6R levels during antipsychotic therapy with neuroleptics (Maes *et al.*, 1995; Müller *et al.*, 1997a). Studies from human CNS cell cultures also showed an inhibitory effect of different neuroleptics to the production of IL-6 after stimulation with lipopolysaccharides, which was more marked in phenothiazines compared to butyrophenones (unpublished results). Similar observations have been described by other authors (Lin *et al.*, 1998).

There are indications for a time-dependent effect of antipsychotic treatment to IL-6: one study reported that short-term treatment with clozapine (median 12 days) induced an increase in IL-6 levels (Maes *et al.*, 1997) and another group found an increase of IL-6 after 2 weeks of clozapine treatment but a decrease after a further 4 weeks of treatment (Pollmächer *et al.*, 1996).

T-HELPER-2 CELL ACTIVATION IN SCHIZOPHRENIA

IL-6 is not only a product of macrophage/monocyte activation, but also of the activation of the TH-2 system. Therefore it cannot be differentiated whether a functional increase of the IL-6 system is a product of TH-2 activation or of the monocyte/macrophage line. However, other results point to an activation of the TH-2 system in schizophrenia.

IL-10 is a cytokine that is produced by TH-2 cells. An increase of IL-10 in schizophrenic patients compared to healthy controls was reported (Cazzullo *et al.*, 1998). Another study observed a strong relationship between IL-10 levels and schizophrenic negative symptoms in the CSF of 62 unmedicated schizophrenics (van Kammen *et al.*, 1997). In medicated schizophrenics treated with haloperidol, a significant relationship between CSF IL-10 levels and the severity of schizophrenic psychosis, as measured by the Bunney–Hamburg psychosis rating scale, was found (van Kammen *et al.*, 1997). These findings indicate that IL-10 levels in the CSF are related to the severity of the psychosis, especially to the negative symptoms.

Another characteristic cytokine that is produced by TH-2 cells is IL-4. An increase of IL-4 levels in the CSF of juvenile schizophrenic patients has recently been reported (Mittleman *et al.*, 1997). The production of IgE is also an indicator for the activation of the TH-2 immune response. Increased levels of IgE in schizophrenic patients compared to controls have been observed (Ramchand *et al.*, 1994). The latter findings indicate that the probable increase of the TH-2 system in schizophrenia is not only a phenomenon of the peripheral immune system, but that it also seems to play a role in the CNS immune system.

Earlier descriptions of elevated $CD3^+$ and $CD4^+$ cells in unmedicated schizophrenics are consistent with the hypothesis of a shift to the TH-2 system, with a diminished TH-1 immune response in schizophrenia (Müller *et al.*, 1991; Sperner-Unterweger *et al.*, 1999).

As shown in Figure XVII-5.3 there is a functional balance between the TH-1 and TH-2 system. It would be expected that an

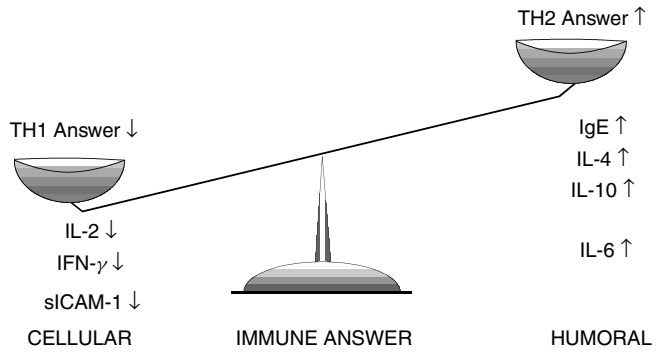

Figure XVII-5.3 Dysbalance of the immune system in schizophrenia. Insufficient activation of the TH-1 answer and relative overactivation of the TH-2 system

overactivation of the TH-2 system is associated with an underactivation of the TH-1 system. Many different findings over decades point to a decreased activation of the TH-1 system in schizophrenia.

T-HELPER-1 SYSTEM AND SCHIZOPHRENIA

The key characteristics of the TH-1 system are the production of IFN-γ and IL-2. One of the often replicated findings in schizophrenia is the decreased *in vitro* production of IL-2 (Villemain *et al.*, 1989; Ganguli *et al.*, 1995; Hornberg *et al.*, 1995; Bessler *et al.*, 1995; Cazzullo *et al.*, 1998). This phenomenon has often been interpreted as the consequence of an exhaustion of the lymphocytes after overproduction of IL-2; however, it might also reflect the reduced capacity of lymphocytes to produce IL-2. The observation of a decreased production of IL-2 fits well with another finding: the decreased production of IFN-γ (Wilke *et al.*, 1996; Rothermund *et al.*, 1996). Both findings point to a blunted production of TH-1-related cytokines and to an underactivation of the TH-1 system in schizophrenia. A lack of activation of the TH-1-related cellular immune system has also be postulated by other researchers (Sperner-Unterweger *et al.*, 1999). Our own findings of the decreased production of lymphocytes after stimulation with different specific antigens also might reflect the reduced capacity for a TH-1-mediated immune answer in schizophrenia. Especially after stimulation with tuberculin, which provokes a TH-1-mediated immune answer, the reaction was blunted (Müller *et al.*, 1991).

DECREASED LEVELS OF sICAM-1 IN UNMEDICATED SCHIZOPHRENICS

Recently, decreased levels of the soluble intercellular adhesion molecule 1 (sICAM-1) in the serum of schizophrenic patients have been described (Wieselmann, personal communication; Schwarz *et al.*, 2000). ICAM-1 is a molecule that mediates the adhesion of lymphocytes to other lymphocytes, to endothelial cells and to parenchymatic cells, but it also mediates the signal for the activation of the cellular immune system. ICAM-1 is part of the TH-1 immune response (van Seventer *et al.*, 1990; Kuhlmann *et al.*, 1991). Therefore, decreased levels of the soluble form of ICAM-1, which is shedded from lymphocytes, seem to represent the state of activation of the TH-1 system. However, reduced sICAM-1 levels have been found not only in the serum but also recently in the CSF of schizophrenic patients (Schwarz *et al.*, in preparation). The latter finding indicates that the blunted activation of the TH-1 system may not be restricted to the peripheral immune system, because CSF parameters reflect more directly the immune pathology of the CNS.

One of the 'classical' epidemiological findings in schizophrenia research is the negative association between schizophrenia and rheumatoid arthritis (Vinogradov *et al.*, 1991). This negative association can be interpreted as two sides of the TH-1/TH-2 balance coin, represented by increased sICAM-1 levels in rheumatoid arthritis and decreased sICAM-1 levels in schizophrenia. SICAM-1 is also a key molecule that mediates the inflammatory reaction in rheumatoid arthritis: increased sICAM-1 levels are regularly found in rheumatoid arthritis (Neidhart *et al.*, 1995). Rheumatoid arthritis is a disorder that is primarily mediated by the cellular TH-1-related immune system.

BLUNTED REACTION OF THE CUTANEOUS CELLULAR IMMUNE RESPONSE IN SCHIZOPHRENIA

The blunted cutaneous reaction to antigens of the Multitest Mérieux which can be observed in schizophrenic patients (Müller *et al.*, in preparation) also points to a blunted response of cell-mediated immunity in schizophrenia. A very early description of this phenomenon, long before the era of neuroleptics, concerned the hyposensitivity to guinea-pig serum, injected intracutaneously in schizophrenic patients, compared to a control group (Molholm, 1942). Such a blunted reaction of a cell-mediated (TH-1) immune response was not only observed in the cutaneous reaction to antigens, but also a decreased antibody production after vaccination with salmonella was found in unmedicated schizophrenic patients (Özek *et al.*, 1971). The descriptions of immune reactions before the era of neuroleptics exclude the possibility that the observed phenomena may be artefacts of the effects of neuroleptics.

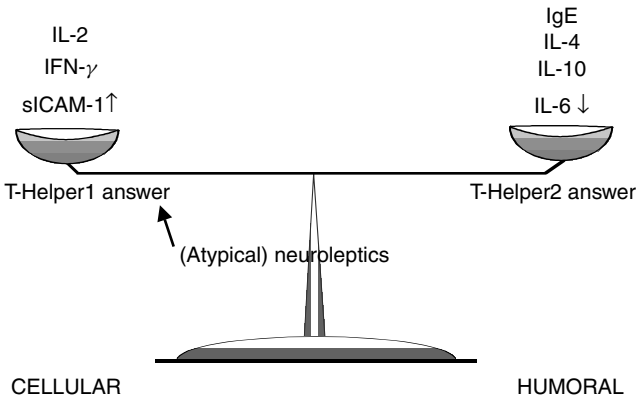

Figure XVII-5.4 Different markers of the immune system point to an immune activation of the TH-1 response during therapy with (atypical) antipsychotics

Antipsychotic therapy, however, is associated with different effects on the immune system. Nevertheless, these effects may counter-regulate the immune effects of the disease. Neuroleptics mainly activate the TH-1 system.

TH-1 SYSTEM AND ANTIPSYCHOTIC THERAPY

Recent findings indicate that neuroleptics may have TH-1-stimulating effects. *In vitro* studies show that the blunted IFN-γ production becomes normalized after therapy with neuroleptics (Wilke *et al.*, 1996). The increase of soluble IL-2 receptors has been described by several groups (Maes *et al.*, 1994; Pollmächer *et al.*, 1995; Müller *et al.*, 1997a). Since sIL-2R are shedded from activated T cells, the increase might reflect an increase of activated, IL-2-bearing T cells.

An increase in $CD4^+CD45RO^+$ cells during therapy with neuroleptics was observed by different groups (Cazzullo *et al.*, 1998; Müller *et al.*, 1997c). $CD4^+CD45RO^+$ cells ('memory cells') are one of the main sources of interferon-γ production. The increase of this sub-population during therapy may contribute to an increase in IFN-γ production. Reduced sICAM-1 levels in the serum of schizophrenics do not normalize during short-term antipsychotic therapy, but a statistically non-significant tendency to increased sICAM-1 could be observed (Schwarz *et al.*, 2000). On the other hand, the leucocyte function antigen 1 (LFA-1) molecule on $CD4^+$ cells shows increased expression during antipsychotic therapy (Müller *et al.*, 1999). LFA-1 is the counter-molecule to ICAM-1. Moreover, the blunted reaction to vaccination with salmonella was not observed in patients who were treated with neuroleptics (Özek *et al.*, 1971). These studies indicate that the TH-1-mediated immune response increases during antipsychotic therapy. Recently,

an elevation of IL-18 serum levels has been described in medicated schizophrenics (Tanaka *et al.*, 2000). Since IL-18 plays a pivotal role in the TH-1 response, this finding fits with other descriptions of a TH-1 activation during antipsychotic treatment.

Interestingly, there is a recent report describing a correlation between the dose of short-term antipsychotic treatment and the mononuclear cell count in the CSF, the CSF macrophage count, as well as the total lymphocyte count (Wahlbeck *et al.*, 2000). The correlation was found especially in patients treated with chlorpromazine-like antipsychotics. This is the first report that describes a relationship between antipsychotic treatment and cellular immunity in the CSF; further investigations have to elucidate whether lymphocytes and macrophages are activated — an activation of the cellular immune system also within the CNS could reflect an activation of the peripheral immune system. Moreover, it would be interesting to know whether a special subgroup of schizophrenics shows an increase of immune cells in the CSF — the total cell count was within the normal range.

B CELLS AND ANTIPSYCHOTIC TREATMENT

Activated B cells are antibody-producing cells. Several observations in the literature indicate that B cells are activated during antipsychotic treatment and antibody production might increase. As early as the 1970s, *in vitro* studies showed an increase of antibody production after stimulation with phenothiazines (Gallien *et al.*, 1977; Zarrabi *et al.*, 1979).

Many studies have described increased antibody production in schizophrenic patients, those observations leading to the discussion of an autoimmune origin of schizophrenia (Ganguli *et al.*, 1987). Nevertheless, the role of antipsychotic treatment has not been considered in several of these studies. Although findings have repeatedly reported that about 20–35% of schizophrenic patients show features of an autoimmune process (Müller and Ackenheil, 1998), the role of actual or former therapy with neuroleptics may not have been taken sufficiently into consideration.

An increase of IgG — antibodies are mainly IgG antibodies — in the CSF has been described, especially in patients with predominant negative symptoms (Müller and Ackenheil, 1995). Increased antibodies against heat shock protein 60 is one of the recent interesting findings in schizophrenia, because it may reflect a mechanism of loss of neuronal protection (Schwarz *et al.*, 1998b, 1999; Kilidireas *et al.*, 1992). However, antibodies against heat shock protein 60 are found especially in patients during antipsychotic therapy.

An increased number of patients with activated B cells ($CD5^+CD19^+$ cells) compared to healthy controls have been described in schizophrenia treated with antipsychotics. Our own study shows an increase of activated B cells during antipsychotic therapy (Müller *et al.*, 1997c). It seems that not only the TH-1 cell system is activated during antipsychotic therapy, but also antibody production by activated B cells.

During antipsychotic therapy, both arms of the specific 'adaptive' immune system seem to become activated: the specific cellular immunity of the TH-1 system, and also the B cell system with its humoral arm, antibody production. An activation of the unspecific 'innate' immune system can be found mainly in unmedicated schizophrenic patients, the latter indicating that this may reflect the disease process and not the result of antipsychotic treatment.

OUTLOOK

Different immunological methods and different clinical features of schizophrenic patients regarding the acuity or chronicity, subtype, course or state of medication have to be taken into account in the discussion of heterogeneous results of immunological studies. Moreover, it has to be realized that schizophrenia is not a disease entity but a syndrome. Therefore, results have to be interpreted cautiously.

However, alterations of the immune system in schizophrenia have been observed for a long time. In the context of both modern sophisticated immunological methods and the current knowledge of the different and multiple functions of immune cells, cytokines, soluble factors etc., more precise suggestions and explanations regarding the interrelationship of the immune functions are possible. The functional differentiation between the innate and adaptive immune system and the T-helper-1 and T-helper-2 conceptualization, especially, allow functional deficits in schizophrenia to be defined. This leads to a more consistent theory of the immune disturbances in schizophrenia, the role of antipsychotic therapy and implications for an immune therapy in schizophrenia.

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XVII-6

Psychophysiology Studies of Schizophrenia

J.G. Csernansky

INTRODUCTION

The last twenty years of schizophrenia research have brought us significant advances in our understanding of the neurobiology of schizophrenia. Psychophysiological studies of subjects with schizophrenia have played an important part in this effort, especially by offering the opportunity to explore relationships between clinical symptoms, cognitive deficits, structural and functional brain abnormalities in subjects with schizophrenia. This chapter has been organized into sections. First, consideration will be given to the various types of psychophysiological abnormalities that have been found in subjects with schizophrenia. Then, studies employing psychophysiological measures to better understand the neuroanatomy and aetiology of schizophrenia will be reviewed. Finally, the use of psychophysiological measures as markers of psychotropic drug action will be considered. The emphasis of this chapter will be on recent studies, which should serve as an efficient entry into earlier literature.

WHAT IS A PSYCHOPHYSIOLOGICAL VARIABLE?

Before beginning a review of the literature on psychophysiology and schizophrenia, it might be helpful to define what is and what is not a psychophysiological variable. A psychophysiological variable may be defined as any quantitative measurement of the activity of one of the body's physiological systems. Such variables may be peripheral, such as measurements of skin conductance or heart rate, or more closely related to the central nervous system (CNS), such as the electroencephalogram (EEG). Prior to the advent of modern techniques for structural and functional neuroimaging, psychophysiological variables were widely used to investigate the neurobiology of schizophrenia. Over years of such investigation, investigators gradually came to use psychophysiological variables that reflected as much as possible the CNS (e.g., quantitative EEG) in favour of variables that were peripheral in nature. For this reason, less attention will be given in this chapter to psychophysiological markers of peripheral types. Further, the assessment of CNS psychophysiological variables in the context of specific, induced cognitive events (e.g., the event-related potential (ERP)) became a preferred strategy to link the study of hypothesized neurobiological substrates with cognitive or clinical phenomena known to be characteristic of subjects with schizophrenia.

The development of psychophysiological variables related to the CNS has been guided largely by the desire to identify abnormalities of neural circuits that can inform us about the pathophysiology of schizophrenia, including the explanation of its behavioural abnormalities. However, schizophrenia has long been thought to be an aetiologically heterogeneous disorder, and these same variables have been used in attempts to discover aetiological factors to differentiate one form of schizophrenia from another. Finally, psychophysiological variables have been employed to monitor the effects of drug treatments.

PSYCHOPHYSIOLOGICAL ABNORMALITIES IN SUBJECTS WITH SCHIZOPHRENIA

A variety of psychophysiological abnormalities have been demonstrated in subjects with schizophrenia using four predominant approaches: (1) measurement of electrodermal responses to experimental stimuli, (2) quantitative eye movements, (3) the resting EEG, and (4) time-averaged EEG responses to experimentally induced cognitive events (i.e., the ERP). Because the first two approaches employ variables that are peripheral in nature, they will be reviewed in less detail here. However, excellent recent reviews of electrodermal measures (Nuechterlein and Dawson, 1995) and eye movements (Levy *et al.*, 1993; Broerse *et al.*, 2001) are available elsewhere.

Electrodermal Measures

Abnormal electrodermal measures of two types have been found in subjects with schizophrenia: abnormalities of resting skin conductance levels and abnormalities of changes in skin conductance elicited by an experimental stimulus. Studies in this area suggest that schizophrenia subjects can be divided into two groups: responders and non-responders (Ohman, 1981). Responders are those subjects who respond normally to orienting stimuli (e.g., an unexpected noise) and have ordinary levels of baseline skin conductance. Non-responders fail to respond normally to orienting stimuli and have exaggerated or unusually variable levels of baseline skin conductance. More recent studies of schizophrenia subjects in different clinical states (i.e., psychotic and non-psychotic) suggest that while the baseline level of skin conductance may be state-dependent (i.e., higher levels are associated with psychotic states), failure to respond normally to orienting stimuli may be a trait phenomenon (Dawson *et al.*, 1992).

Quantitative Eye Movements

Abnormal eye movements during the tracking of a smoothly moving object have been observed for many years. Approximately half of individuals with schizophrenia demonstrate the intrusion of unusual saccadic movements while attempting to follow such a target across their visual field (Holzman, 1987). This abnormality does not appear to improve with treatment and is not found as commonly in individuals with other psychiatric disorders, such as bipolar disorder. Moreover, the same abnormality has been found in the

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relatives of individuals with schizophrenia (Iacono and Clementz, 1993). Thus, abnormal visual tracking appears to be a trait of schizophrenia, and possibly related to the genetic risk of acquiring the disease.

EEG

The EEG has been widely used to provide a physiological assessment of the activity of the CNS in subjects with schizophrenia. Compared to other approaches, such as positron emission tomography (PET) or single photon emission computed tomography (SPECT), it is non-invasive and it can be repeated as often as needed. Moreover, the EEG has been shown to be sensitive to changes in states of consciousness and cognitive events. While the recent development of functional magnetic resonance imaging has begun to overshadow the EEG as a tool for investigating the neuroanatomical basis of cognition, it has continued to offer unique psychophysiological information about CNS function in subjects with schizophrenia.

Abnormalities of the resting EEG have been reported in subjects with schizophrenia for many years. A common finding has been the presence of excessive slow wave activity, especially across EEG leads that reflect the frontal cortex (Morihisa *et al.*, 1983). When schizophrenia subjects who are psychotic and in remission from psychosis are compared, excessive slow wave activity appears to be related only to the psychotic state (Koukkou and Manske, 1986). Other studies have shown that the resting EEG is overly reactive to simple changes in physiological state, such as opening the eyes (Shagass, 1991). This latter observation has led experts to hypothesize that EEG abnormalities in schizophrenia may be more related to the dysregulation of neural circuits rather than the steady presence of either hyper- or hypo-arousal (Shagass, 1991).

As mentioned above, EEG responses to an experimentally induced cognitive event (i.e., the ERP) have been widely used to search for further evidence of neural circuit dysregulation in schizophrenia. The ERP has two major advantages over the resting EEG. First, time-averaging of the EEG signal reduces unwanted, random noise, and second, the electrophysiological responses of a preselected brain region to specific perceptual or cognitive events can be studied. By selecting a perceptual or cognitive stimulus known to discriminate schizophrenia subjects from healthy controls or from subjects with another neuropsychiatric disorder, psychophysiological information specific to schizophrenia can be collected.

After an experimental stimulus, several waveforms are usually observed in the ERP. These waveforms are named according to their electrical direction (i.e., positive (P) or negative (N)) and their latency (in milliseconds) with respect to the stimulus. In some cases, progress has been made in identifying the neuroanatomical sources of these waveforms. Two of these waveforms have attracted the special interest of schizophrenia researchers over the years: the P50 and the P300.

One of the earliest ERPs following an experimental stimulus is the P50, which appears to reflect an orienting response of the CNS to a new perceptual event. Some investigators have suggested that the neuroanatomical sources of the P50 can be traced to the temporal lobe (Reite *et al.*, 1988; Goff *et al.*, 1980). Because of clinical observations that schizophrenia subjects cannot properly filter out non-salient perceptions, several groups have been interested in using the P50 to assess sensory gating mechanisms in schizophrenia. The typical experimental paradigm involves assessing the magnitude of reductions in the amplitude of the P50 during paired presentations of an auditory stimulus. Because the amplitude of the P50 is less than the amplitude of the background EEG signal, several pairings are averaged to extract the signal. This phenomenon is akin to other forms of behavioural desensitization or habituation, and has also been termed pre-pulse inhibition. In healthy subjects, intact sensory gating mechanisms are reflected by substantial reductions in P50 amplitude (Roth and Kopell, 1969). In subjects with schizophrenia, such reductions have been observed to be substantially blunted (Judd *et al.*, 1992; Freedman *et al.*, 1996).

The presence of an abnormal P50 ERP in schizophrenia subjects appears to have the characteristics of both a trait and a state. P50 abnormalities have been observed in outpatients with schizophrenia who were in relative remission from their psychosis (Dawson *et al.*, 1993). Also, P50 ERP abnormalities have been found among the relatives of subjects with schizophrenia, which suggests that such abnormalities may be a trait linked to the genetic risks of acquiring the disease (see below and Coon *et al.*, 1993). However, in contrast to these findings, P50 ERP abnormalities have been observed to be reversible in subjects with schizophrenia in some studies. For example, cigarette smoking and the administration of nicotine appear to mitigate P50 gating deficits in some subjects with schizophrenia (Adler *et al.*, 1993). This observation has been especially intriguing because of other evidence linking dysfunction of α-7 acetylcholine receptors stimulated by nicotine located on interneurones within the hippocampus to the pathophysiology of schizophrenia (Freedman *et al.*, 1995).

P300 is a later ERP, which appears to be a cognitive response to an unexpected stimulus. Because of the experimental characteristics of this ERP, it has been suggested to be related to working memory (Donchin and Coles, 1988). Both auditory and visual stimuli are capable of producing P300 ERPs, and the amplitude of the P300 can be modulated by a variety of experimental protocol parameters, such as adjusting the rarity of the unexpected stimulus (Pfefferbaum *et al.*, 1989). Unfortunately, it has been difficult to localize the neuroanatomical source or sources of the P300; many experts believe that it may be generated by a multitude of sources across the cerebral cortex. Nonetheless, the widespread nature of the P300 ERP allows one to investigate and compare its topography in neuropsychiatric disorders (Salisbury *et al.*, 1999).

P300 in subjects with schizophrenia compared to healthy controls has been observed to have both a reduced amplitude and a longer latency (Ford *et al.*, 1992). Again, this psychophysiological abnormality has the features of both a trait and state marker. In favour of the hypothesis that P300 abnormalities reflect a long-standing trait in subjects with schizophrenia, P300 amplitude reductions have been noted in schizophrenia subjects who are in remission from their psychosis (Mathalon *et al.*, 2000a). Moreover, as with the P50, P300 abnormalities have been found among the relatives of subjects with schizophrenia (Frangou *et al.*, 1997). The effects of antipsychotic drug treatment on P300 is less clear, however. In some studies, no changes in P300 have been noted during treatment with antipsychotic drugs (Ford *et al.*, 1994; Turetsky *et al.*, 1998a). However, in other studies, correlations between schizophrenia symptoms and the degree of P300 amplitude reduction have been reported (Pfefferbaum *et al.*, 1989; Turetsky *et al.*, 1998a; Mathalon *et al.*, 2000a).

Recent studies have focused on the neurobiological implications of a prolonged P300 latency in subjects with schizophrenia. In two cross-sectional studies, correlations have been noted between longer P300 latencies and longer durations of illness (O'Donnell *et al.*, 1995; Mathalon *et al.*, 2000b). Combined with evidence from *in vivo* neuroimaging studies that suggest the presence of progressive cortical grey matter losses in some subjects with schizophrenia (Gur *et al.*, 1998), these findings support recent proposals that schizophrenia is a progressive disease process in at least some individuals. Moreover, these studies provide support for the notion that P300 latency may be a useful method of detecting the phenomenon.

Other ERPs and EEG-based psychophysiological measures continue to be developed to investigate the neurobiology of schizophrenia. A negative ERP with a long latency, the N400, has been

shown to have a diminished amplitude and prolonged latency in schizophrenia subjects compared to healthy controls (Koyama *et al.*, 1991; Olichney *et al.*, 1993). Because this ERP is sensitive to components of language processing, in particular semantic congruity (Kutas and Hillyard, 1984), N400 abnormalities in schizophrenia have been interpreted as evidence of a fundamental defect in language processing that may underlie disorganized thinking. In addition to ERPs, new techniques for recording specific EEG resonances in response to repeated auditory and visual stimulation are being used to study schizophrenia. Abnormal synchronization in the alpha (Jin *et al.*, 2000) and gamma (Kwon *et al.*, 1999) ranges of EEG frequencies have been recently reported in subjects with schizophrenia. These EEG variables offer a complementary approach to ERPs for testing hypotheses related to the presence and characteristics of abnormal cortical circuitry in subjects with schizophrenia.

PSYCHOPHYSIOLOGICAL VARIABLES AS MARKERS OF NEURAL CIRCUIT DYSFUNCTION

Abnormalities of brain structure have been shown to be a key biological characteristic of schizophrenia. Moreover, these abnormalities appear to involve a group of functionally related structures in the temporal and frontal lobes. *In vivo* magnetic resonance (MR) imaging has uncovered evidence of volume reductions within the temporal lobe (Suddath *et al.*, 1989) and specifically involving the hippocampus (see Nelson *et al.*, 1998 for a meta-analysis of results) of schizophrenia subjects. Moreover, the computerized analysis of hippocampal shape has revealed that these volume losses are localized to the head of the hippocampus (Csernansky *et al.*, 1998) where projections from the hippocampus to the frontal cortex are plentiful. Volume reductions have also been reported in the parahippocampal gyrus, which forms the major input to the hippocampus (Shenton *et al.*, 1992) and in the superior temporal gyrus (McCarley *et al.*, 1993).

In vivo MR studies have also provided evidence for reductions in the total volume of the frontal lobe (Andreasen, 1986), the grey matter volume of the frontal lobe (Zipursky *et al.*, 1992) and the white matter volume of the frontal lobe (Breier *et al.*, 1992). While there have not yet been *in vivo* MR studies of the cingulate gyrus, post-mortem studies of that brain structure also suggest the presence of anatomical irregularities. Specifically, Benes and co-workers demonstrated decreased numbers and somal size of GABA interneurones, as well as compensatory increases in GABA receptors on pyramidal cells (Benes *et al.*, 1991, 1992) of the cingulate gyrus.

Moreover, abnormalities of brain structure listed have been observed early in the course of illness in subjects with schizophrenia and appear to show little progression with age in most subjects (Weinberger, 1986; Csernansky and Bardgett, 1998). This lack of progression has led many to suggest that brain structure abnormalities in schizophrenia may occur, at least in part, through defects in a neurodevelopmental process (Csernansky and Bardgett, 1998; Pearlson and Marsh, 1999). The development of cortical–cortical and subcortical–cortical connections is a critical step in successful brain organization (Goldman-Rakic, 1987). When neurones do not achieve their proper location within a brain structure due to neurodevelopmental aberrations they may not be supplied with their required growth factors and die. Thus, disturbances of neuronal division and migration are plausible explanations for the observed changes in neuronal density and grey matter volume (Bloom, 1993).

Evidence from psychophysiological studies has provided complementary evidence for functional abnormalities within this network of frontal and temporal lobe structures in schizophrenia. As reviewed above, the sources for the P50 appear to include structures within the temporal lobe, while sources for the P300 may exist within the frontal cortex. With the development of multi-electrode arrays, the topography of ERPs has begun to be investigated in subjects with schizophrenia. Using a 64-channel array, Potts *et al.* (1998) showed that P300 amplitude reductions were asymmetric; i.e., P300 amplitudes in left temporal lobe leads were more reduced than in right temporal lobe leads. Moreover, this same group has shown that this topography of P300 amplitude reductions may be relatively specific to schizophrenia, as a different topography of P300 reductions was found in subjects with manic psychosis (Salisbury *et al.*, 1999).

Other research groups have also provided evidence of a specific topography for P300 abnormalities in schizophrenia subjects. Bruder *et al.* (1999) found asymmetric reductions (left > right) of this late positive ERP in subjects with schizophrenia. Turetsky *et al.* (1998b) applied principal components analysis to data collected from a 16-electrode array and extracted three subcomponent to the P300 ERP. Specific abnormalities were then found for individual P300 subcomponents related to gender and clinical presentation (i.e., deficit versus non-deficit symptoms).

Combining evidence from high-resolution structural brain imaging and multi-array ERP studies, a picture of structural and functional abnormalities within a circuit of connected structures in the temporal and frontal lobes is beginning to emerge to explain the pathophysiology of schizophrenia. As additional information is gained from studies of functional MR imaging, this picture should come into greater focus. However, it should be kept in mind that structures within the temporal and frontal lobes are connected indirectly by pathways passing through the basal ganglia, and that the basal ganglia, especially the ventral striatum, have been hypothesized to be an important site of action for antipsychotic drugs (Csernansky and Bardgett, 1998). Thus, additional experimental approaches may be needed that include examinations of these deeper brain structures to add to our understanding of the neurobiology of schizophrenia.

PSYCHOPHYSIOLOGICAL STUDIES AND AETIOLOGICAL FACTORS

Two types of factors have been brought forward to explain the aetiology of schizophrenia: (1) genetic factors, and (2) environmental trauma to the CNS early in life. One experimental approach that has provided evidence of the genetic basis of schizophrenia has been the search for neurobiological abnormalities among the relatives of schizophrenia subjects. However, evidence to support environmental trauma as the aetiological basis of schizophrenia has been more difficult to collect, partly because detailed information about pregnancy and labour difficulties is often not available for analysis.

Several research studies have suggested that the relatives of subjects with schizophrenia may have brain structure abnormalities that are similar, but of lesser magnitude, to those observed in subjects with the disease. In a study of twins discordant for schizophrenia, Suddath *et al.* (1990) found a reduction in the volume of the left temporal lobe and hippocampus in affected twins compared to the unaffected co-twins. Interestingly, however, the 'unaffected' co-twins demonstrated brain structure volumes that were intermediate between those demonstrated by the ill co-twins and healthy controls without schizophrenia or a family history of schizophrenia. Also, Seidman and colleagues found that the non-psychotic siblings of schizophrenia patients had small decreases in the volume of the hippocampus–amygdala complex (Seidman *et al.*, 1997, 1999) and the thalamus (Seidman *et al.*, 1999) as compared to healthy controls without a family history of psychiatric disorders. Such studies provide preliminary but powerful evidence that genetic factors may be involved in the development of brain structure abnormalities in schizophrenia.

Psychophysiological studies of the relatives of subjects with schizophrenia suggest that individuals at genetic risk for developing schizophrenia have functional abnormalities related to the same group of brain structures. For example, abnormalities of P50 suppression have been reported among the relatives of schizophrenia subjects and in individuals with disorders thought to be genetically related to schizophrenia. Freedman *et al.* (1997) reported that the failure to suppress P50 after an auditory stimulus was present in relatives of schizophrenia probands. Moreover, this group discovered evidence of a linkage between this psychophysiological abnormality and a genetic locus on chromosome 15. In support of this exciting finding, Clementz *et al.* (1998) reported that similar P50 suppression abnormalities could be demonstrated in both schizophrenia subjects and their first-degree relatives. Finally, failure to suppress the P50 ERP after an auditory stimulus has been found recently in individuals with schizotypal personality disorder, a disorder found more commonly among the relatives of schizophrenia subjects than in the general population (Cadenhead *et al.*, 2000).

In keeping with the hypothesis that ERP abnormalities are present in individuals at risk for schizophrenia, Frangou *et al.* (1997) reported that both schizophrenia subjects and their first-degree relatives have prolonged P300 latencies compared to healthy controls without a family history of schizophrenia. Also, P300 amplitudes among these relatives were intermediate between schizophrenia subjects and healthy controls. Similar results have been found recently by Karoumi *et al.* (2000), who reported prolonged P300 latencies among the well siblings of subjects with schizophrenia. However, in contrast, Weisbrod *et al.* (1999) have recently reported reduced P300 amplitudes, but not prolonged P300 latencies, among the discordant monozygotic twins of subjects with schizophrenia. Discrepancies between these various results may be due to small sample sizes and the likelihood that there is some neurobiological heterogeneity among subjects with schizophrenia. In keeping with this hypothesis, Frangou *et al.* (1997) also reported that there was a bimodal distribution of P300 latencies among the first-degree relatives of schizophrenia subjects, with only 35% of such subjects demonstrating prolonged P300 latencies.

Somewhat different results were found in a study of the children of subjects with schizophrenia studied as part of the New York High-Risk Project (Friedman *et al.*, 1995). In this ground-breaking study, the children of subjects with schizophrenia and affective disorders were studied and compared to the children of subjects with mood disorders and controls longitudinally for several years. ERP testing was carried out when these children were adolescents (ages 11–19) and correlated with clinical testing as young adults (ages 20–28). Unfortunately, no differences were observed in P300 amplitudes among the three groups of adolescents, and no relationship was found between reduced P300 amplitudes in the adolescent children of the schizophrenia subjects and the occurrence of a later diagnosis of psychosis.

Despite some contradictory results, most studies suggest that individuals at risk for developing schizophrenia have ERP abnormalities that at least resemble the abnormalities found in individuals with the disease. Especially exciting have been suggestions that ERP abnormalities, such as sensory gating deficits, may be used as quantitative traits in future genetic studies of schizophrenia. However, because of the likelihood of genetic heterogeneity among individuals with schizophrenia, ERP abnormalities may not be found in all individuals with schizophrenia nor in all individuals at risk for developing schizophrenia.

PSYCHOPHYSIOLOGICAL VARIABLES TO MONITOR THE EFFECTS OF TREATMENT

Psychophysiological variables have also been employed to monitor the effects of treatments in subjects with schizophrenia. Of course, experiments where changes in psychophysiological measures are sought as an index of successful treatment must be based on observations that such measures are state-dependent. In addition, EEG markers have sometimes been used to document the activity of an experimental drug in subjects with schizophrenia, independent of any hypothesis that such subjects are characterized by abnormalities of such EEG measures.

Early studies comparing subjects with schizophrenia that were 'drug-free' or treated with conventional antipsychotic drugs suggested that conventional antipsychotic drugs were not capable of ameliorating P50 abnormalities (Freedman *et al.*, 1983). However, somewhat at odds with this apparent irreversibility of P50 abnormalities, administration of the indirect dopamine agonist, amphetamine, to normal subjects was found to disrupt the suppression of P50 after an auditory stimulus (Light *et al.*, 1999). This latter observation suggests that a reversible schizophrenia-like psychophysiological state can be produced. Perhaps helpful in resolving the uncertainty as to whether failure to suppress P50 after an auditory stimulus is reversible or irreversible, Light *et al.* (2000) have recently reported that while conventional antipsychotic drugs were not able to normalize P50 suppression in schizophrenia subjects, treatment with three second-generation antipsychotic drugs (i.e., risperidone, olanzapine and clozapine) did so. These findings suggest that while dopaminergic excesses may disrupt normal P50 suppression mechanisms, blockade of dopamine receptors (by conventional antipsychotic drugs) is not necessarily adequate to restore such disruptions when they are found in individuals with schizophrenia. The actions of second-generation antipsychotic drugs at neuroreceptors other than dopamine, such as serotonin or noradrenaline (norepinephrine), appear to be necessary to accomplish this task.

The question of whether P300 abnormalities in schizophrenia subjects can be normalized by antipsychotic medications also remains controversial. In a placebo-controlled study of schizophrenia subjects treated with the selective dopamine receptor antagonist, raclopride, treatment was not found to normalize P300 amplitudes (Ford *et al.*, 1994). However, in a more recent study of schizophrenia subjects treated with two other dopamine receptor antagonists, remoxipride or haloperidol, abnormalities in both P300 amplitude and latency were reversed (Coburn *et al.*, 1998). Unfortunately, the effects of other second-generation antipsychotic drugs on P300 abnormalities in schizophrenia subjects have not yet been well investigated. However, given the results obtained to date in reversing P50 abnormalities using second-generation antipsychotic drugs (see above), such treatments may have more promising effects with respect to normalizing P300 and other psychophysiological abnormalities.

CONCLUSIONS

A variety of psychophysiological abnormalities have been found in subjects with schizophrenia (see Table XVII-6.1 for a summary). These abnormalities often have the characteristics of both state- and trait-dependent factors. In keeping with psychophysiological abnormalities as neurobiological traits, similar abnormalities have been found in individuals at genetic risk for developing schizophrenia. In addition, studies combining these psychophysiological measures with cognitive assessments or *in vivo* structural neuroimaging suggest that schizophrenia may be related to dysfunction of a circuit of frontal and temporal lobe brain structures. Research has yet to determine whether psychophysiological abnormalities can be reversed by treatment in subjects with schizophrenia. While treatment with conventional antipsychotic drugs has been disappointing in this regard, treatment with second-generation antipsychotic drugs has offered more promising results.

Table XVII-6.1 Summary of the characteristics of psychophysiological variables in subjects with schizophrenia

Variables	Source	Reversible	Present in at-risk subjects
Skin conductance responses	Peripheral	No	Unknown
Eye tracking movements	Frontal lobe	No	Yes
Resting EEG	Cerebral cortex	Unknown	Unknown
P50 ERP	Temporal lobe	Variable[a]	Yes
P300 ERP	Frontal lobe	No	Yes

[a] Reversible by second-generation antipsychotic drugs, but not by conventional antipsychotic drugs.

Psychophysiological variables will continue to be important variables in studies of the neurobiology of schizophrenia. Because of our ability to relate these variables to both cognitive elements and neuroanatomical structures, they provide essential information for forming a comprehensive understanding of the neurobiology of schizophrenia. Because of the rapid rise of methods for functional MR imaging, future research is likely to involve the combination of such methods with psychophysiological measures. Further, techniques for psychophysiology research will continue to develop through improvements in EEG technology and more specific cognitive stimuli.

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The Neuropsychology of Schizophrenia

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INTRODUCTION

Schizophrenia is a major psychiatric disorder characterized by a disruption in affective, cognitive and social domains, which results in compromised ability to adapt to a changing environment and to function adequately in the community. The point prevalence, expressed as the number of cases per 1000 persons at risk, is estimated at between 1.4 and 1.6, and the lifetime prevalence ranges from 0.5% to 1.0% of the population (Jablensky, 1995). The disorder usually manifests itself between the ages of 18 and 25, but according to current neurodevelopmental models of schizophrenia may already begin during prenatal development. More specifically, it is hypothesized that a failure occurs during the period of cell migration, leading to non-optimal connections between brain areas (e.g., Weinberger, 1987). The onset of psychotic symptoms is so much later, when the affected part of the brain matures and is called 'on line' (Weinberger, 1987).

With respect to the neuropsychology of schizophrenia, the notion of deterioration in cognitive and behavioural functions was implicit in the initial conceptualization of the disorder. Kraepelin (1919) labelled the disorder 'dementia praecox', defining it as an irreversible deteriorative condition. In past decades, numerous studies have yielded evidence for cognitive deficits in patients with schizophrenia. In the 1960s, investigations into disrupted attentional functions started with systematic experimental investigations of reaction time parameters in the differentiation of schizophrenics from non-psychiatric individuals. Clinical research in the same period had shown that schizophrenic patients performed abnormally on measures of general intellectual functioning (e.g., Heaton and Crowley, 1981). Indeed, it turned out that when a group of patients with schizophrenia are compared to healthy controls on a neuropsychological test battery, performance of the patient group was worse on almost all of the tests and comparable to the performance of patients with known neurological damage. However, a description of the symptoms, cognition and behaviour of schizophrenics in terms of the dichotomy 'organic' versus 'functional' has not proved very fruitful. Nowadays, schizophrenia is considered as a psychiatric disorder with neuropsychological correlates. This more accurately characterizes the illness and enables the investigation of specific brain–behaviour relationships which underlie the variability of symptoms and neurobehavioural systems which may be affected.

In this chapter we present the neuropsychological aspects of schizophrenia, with an emphasis upon the major cognitive and behavioural functions that are affected. We do not attempt to give an elaborate overview of clinical neuropsychological research which has shown, generally, deficits in performance of schizophrenic subjects. Rather, we focus on neurocognitive processes involved in the expression of the disease and elaborate on possibly relevant modulatory factors, and on current models which attempt to understand the behavioural expression of the disease. Attentional functions, executive functioning and memory — notably working memory — are described in relation to the various experimental techniques used to measure neurocognitive processes. Important notions are evaluated such as those regarding the processing of context, theory of mind and monitoring. We will also consider vulnerability factors such as genetic and environmental factors and the relation with factors such as medication, cognitive therapy and the course of deficits. Closing paragraphs are devoted to a description of the functional significance of the neurocognitive and behavioural changes to allow better understanding of the symptoms and neurobehavioural profile of schizophrenics. The chapter does not contain material concerning neurological or 'brain localizational' aspects of schizophrenia, because Chapter XVII-8 in this book is dedicated to that subject.

THE NATURE OF THE NEUROCOGNITIVE DEFICITS IN SCHIZOPHRENIA

The range of deficits in schizophrenia is extremely broad. Roughly, when a battery of neuropsychological tests is administered to a group of patients with chronic schizophrenia, mean performance of the group will be significantly below normal performance on at least half of the tests. Reviewing all findings would be nearly impossible, and would probably obscure the central issues in this review. Therefore, this section summarizes research on three cognitive domains: attention, memory and executive function. These were chosen because impairments on these domains have been consistently implicated in schizophrenia research, and sophisticated paradigms have been developed to elucidate the nature of dysfunction in these domains.

Attentional Functions

Both clinical descriptions and subjective accounts of schizophrenia emphasize attentional deficits. Attention is a multifactorial construct, including the ability to maintain an alert state, to orient to novel stimuli, to filter relevant information and to rapidly discriminate stimuli for some duration of time. Two aspects of attention that have received particular attention in schizophrenia research are sustained and selective attention. Sustained attention refers to the capacity to maintain a state of readiness to respond to small changes in the environment. Selective attention is the capacity to focus while ignoring irrelevant information.

Sustained Attention

A widely used test to assess sustained attention is the Continuous Performance Test (CPT). In this test, stimuli, usually letters or

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digits, are presented briefly one at a time in a random order. The subject has to respond when a certain target stimulus appears — in some variants of the task only when preceded by a certain cue. Patients with schizophrenia usually display reduced sensitivity on the CPT (Addington and Addington, 1997; Nuechterlein *et al.*, 1994; Van den Bosch *et al.*, 1996). This deficit is particularly pronounced on versions with high processing loads; for example, tasks with rapid stimulus presentation, stimulus degradation or when the previous stimulus must be remembered to make the current decision (Nelson *et al.*, 1998; Nuechterlein *et al.*, 1994). Although impaired CPT performance is a robust finding, there is as yet no consensus concerning the precise mechanism of the impaired performance. Cohen and Servan-Schreiber (1993) have constructed a computer simulation model of the CPT and other tasks to test their hypothesis that the core cognitive deficit in schizophrenia is 'an impairment in maintaining contextual information over time and in using that information to inhibit inappropriate responses'. They showed that a single disturbance to the module responsible for representing and maintaining context yielded performance impairments similar to those seen in schizophrenia. In a subsequent study, a variant of the CPT designed to elicit deficits in the processing of contextual information was administered to patients with first-episode schizophrenia and patients later in the course of their illness (Servan-Schreiber *et al.*, 1996). Specifically, the frequency of cue–target sequences (e.g., A–X) was increased, to introduce a strong tendency to respond to the letter X. Thus, in the non-cue followed by target condition (e.g., B–X), subjects had to rely on the previous letter as a context to inhibit the response to X. Two delay intervals between cue and target were used, namely 750 ms and 5 s. The prediction was that patients with schizophrenia would perform worse in the long delay condition, particularly with regard to the number of non-cue–target errors, because of a reduced capacity to maintain context. Indeed, the performance of unmedicated patients followed the pattern predicted by the context hypothesis. The deficit was worse in unmedicated patients with a longer course of the illness. The authors interpreted the results as support for the view that a single deficit in the processing of context may underlie the cognitive impairments in schizophrenia.

However, a recent study has called this explanation into question and instead suggested that a problem in encoding is at the core of deficits on this type of tasks. This study used variations of the CPT to investigate possible mechanisms of impairment, including stimulus–response mapping, use of context and working memory (Elvevåg *et al.*, 2000). The amount of stimulus–response mapping that was required was varied by using two kinds of cue–target pairs, namely with and without inherent instructions (e.g., 'ready' followed by 'press' versus 'one' followed by "nine"). As in the study by Servan-Schreiber and colleagues (1996), the sensitivity to context problems was manipulated by varying the frequency of the target stimuli; to investigate the involvement of working memory, two delay intervals between the cue and the target were used (namely, 1000 ms and 3000 ms). These tasks were administered to 20 inpatients with schizophrenia and 30 healthy volunteers. As expected, the patients performed significantly worse on all tasks than did the comparison subjects. Contrary to expectations, however, there was no differential impact of any of the task manipulations on the speed of performance of the patients. The only effect of the task manipulations was that patients made more errors at short delay intervals. This pattern of results suggests difficulties in the rapid encoding of information, leading to a differentially impaired performance in short delay intervals between stimuli, where the second stimulus interferes with constructing a representation of the first. In sum, impairment on the CPT is a robust finding in schizophrenia, but there might be more than one pathway to poor performance. One explanation suggests a deficit in the processing of context, but it has also been proposed that a dysfunction of rapid encoding is at the core of impaired CPT performance.

Selective Attention

Selective attention is often assessed using the Stroop Colour–Word Test (SCWT; Stroop, 1935). The SCWT has an interference component that requires the subject to inhibit an automated response (word reading) in favour of a less salient aspect (colour naming). A typical Stroop task consists of three conditions: (1) word reading; (2) colour naming; and (3) interference (colour names printed in conflicting colours). Several studies have demonstrated impaired performance on the SCWT, particularly in the interference condition (Barch and Carter, 1998; Krabbendam *et al.*, 2000; Liddle and Morris, 1991). Recently, investigators have begun to use single-trial versions of the Stroop task, instead of lists of stimuli printed on cards, and included a congruent condition in which the word and the print colour are the same. Typically, reaction times are shorter in congruent compared to neutral conditions, when there is no relation between colour and semantic meaning (e.g., the word chair printed in blue). This phenomenon is known as Stroop facilitation. Studies using a single-trial Stroop task have yielded evidence for an increased facilitation effect in schizophrenia (Carter *et al.*, 1992; Cohen *et al.*, 1999). This effect was further examined by Barch and colleagues (1999). Forty patients with schizophrenia and 20 healthy controls were administered blocks of congruent, incongruent and neutral trials. In each trial, a stimulus was presented in one of four colours: red, blue, green or purple. The task was to name the colour in which the stimulus was printed as quickly as possible. The congruent trials consisted of colour names presented in its own colour. The incongruent condition consisted of colour names presented in one of the three remaining colours. The neutral trials consisted of either words or squares, printed in one of the four colours. Patients with schizophrenia showed increased facilitation compared to normal controls in both word and non-word neutral conditions. In the non-word condition, the stimulus does not interfere with the (verbal) colour-naming response. Thus, an increased facilitation effect in this condition means that part of this effect can be attributed to a greater influence of the word in the congruent condition. Apparently, patients are less able than controls to ignore the word dimension in this condition, possibly due to selective attention deficits.

Memory

Verbal Memory and Intentional Learning

Memory impairment in patients with schizophrenia has been reported in several studies (Landrø, 1994; McKenna *et al.*, 1990; Saykin *et al.*, 1991). Yet, poor memory may be the consequence of a number of factors, including medication or psychopathology. To the extent that this is true, these factors cannot completely account for the memory deficit in schizophrenia. A recent meta-analysis of 70 studies documented a significant and wide-ranging deficit, which was not due to potential confounding factors such as medication status, age or severity of psychopathology (Aleman *et al.*, 1999). Verbal memory, including story recall and word list learning, seems to be more severely affected than non-verbal memory (Saykin *et al.*, 1991), although this difference was not significant in the meta-analysis by Aleman and colleagues (1999). Free recall is particularly affected (Beatty *et al.*, 1993; Paulsen *et al.*, 1995), indicating a deficit of retrieval of information. However, impaired recognition is sometimes observed, at least in the more severely disturbed patients (Clare *et al.*, 1993; Landrø, 1994), which suggests that consolidation of information may also be impaired. It has been suggested that the memory deficit in schizophrenia is in some respects analogous to the classical amnesic syndrome with intact implicit memory, relative sparing of short-term recall, an accelerated forgetting and poor long-term recall (McKenna *et al.*,

1990). This view is not supported by the meta-analysis by Aleman and colleagues, which yielded evidence for an impaired short-term memory and a normal rate of forgetting (Aleman *et al.*, 1999). Nevertheless, a relative sparing of implicit learning and memory has been reported in several studies (Clare *et al.*, 1993; Kern *et al.*, 1997).

Working Memory

An aspect of memory that has received particular attention in schizophrenia research is working memory. Working memory is the type of memory that is active and relevant only for a short period of time and is defined in content and duration by the action it subserves (Baddeley and Hitch, 1974; Fuster, 1989). Working memory accounts for the ability to guide behaviour by cognitive representations in the face of varying degrees of distraction and thus to base behaviour on thoughts and intentions rather than on direct stimulation. The term is related to the notion of context introduced in the previous section. According to Baddeley and Hitch, working memory consists of a superordinate central executive system and two slave systems for the transient storage of visuospatial and verbal information (the visuospatial sketchpad and phonological loop). Unfortunately, the term working memory is used in different ways (Baddeley and Hitch, 1994; Perry *et al.*, 2001). A major source of confusion is that sometimes only involvement of the slave systems is required to perform the working memory tasks, and sometimes the central executive system as well. Perry *et al.* (2001) propose to distinguish between transient 'online' storage, when only storage and retrieval of information are required, and executive-functioning working memory, when in addition to storage and retrieval manipulation of the information is required. The distinction between forward and backward digit spans may correspond roughly to the distinction between transient storage and executive function working memory. Another example of transient online storage is the delayed response paradigm employed by Goldman-Rakic and colleagues to investigate working memory in non-human primates (Funahashi *et al.*, 1989; Goldman and Rosvold, 1970). There are numerous tasks that tap into executive-functioning working memory, such as the Tower of London (Shallice, 1982) and the Wisconsin Card Sorting Test (WCST; Berg, 1948; Heaton, 1981). Although these tasks may not be designed specifically to assess working memory, they do require to keep in mind instructions, goals and concepts (Goldman-Rakic, 1994). Does the working memory impairment in schizophrenia involve both transient 'online' storage and executive functioning, or is the first aspect relatively intact? In general, the findings point to an impairment of both functions, although the picture is not entirely consistent. Several studies have demonstrated impaired performance on transient storage tasks in schizophrenia (e.g., Fleming *et al.*, 1997; Park and Holzman, 1992; Spitzer, 1993; Pantelis *et al.*, 1997). For example, Pantelis *et al.* (1997), using tests from the Cambridge Neuropsychological Test Automated battery (CANTAB), found evidence for impaired performance on a task that required the subject to remember a sequence of squares presented on a screen. Park and Holzman (1992) also found evidence for impaired transient storage on a spatial working memory task. However, in the same sample auditory working memory assessed with digit span forward was intact. Also, in a comparison of monozygotic twins discordant for schizophrenia the affected twin performed worse than the healthy twin in the backward but not in the forward condition (Goldberg *et al.*, 1990). However, most studies point to significant impairments in both digit span tasks. This is also apparent from the meta-analysis by Aleman *et al.* (1999). Data from 18 studies on forward span and seven studies on backward span yielded no significant difference in effect size between the two spans.

Semantic Memory

The term semantic memory refers to stored information that is impersonal, and includes knowledge of words and their meanings, knowledge about objects and their categorizations, and general information about the world. The term was introduced by Tulving to distinguish this form of memory from episodic memory, the memory for personally experienced events (Tulving, 1983). Both forms of memory are considered to be closely related and in continuous interaction with each other. The notion of impaired semantic knowledge in schizophrenia dates from several decades ago. Cameron has suggested that the core feature of thought disorders in schizophrenia was overinclusive thinking, a vagueness of boundaries between concepts making them overextensive and able to accommodate logical contradictions (Cameron, 1939). Subsequent studies have provided support for this notion (Chen *et al.*, 1994; Cutting *et al.*, 1987; Payne, 1973). The study by Chen and colleagues used a task that required subjects to verify words as members or non-members of a conceptual category. The words differed in their degree of semantic relationship to the category. Control subjects had the longest reaction time for items at the borderline of the category (e.g., penguin in the category of birds). In contrast, patients with schizophrenia took longest to respond to items that were outside the category, but semantically related to it (e.g., airplane in the category of birds). According to the authors, this pattern of results suggests an outward shift of semantic category boundaries in schizophrenia. Overinclusive thinking may be explained in terms of an activated semantic network, in which activation spreads faster than normal to other related concepts and thereby crosses over the usual boundaries of concepts (Spitzer, 1993). Such heightened activation has been experimentally demonstrated using semantic priming paradigms. Patients with schizophrenia show more semantic priming (Kwapil *et al.*, 1990; Maher *et al.*, 1996), most notably when the prime and the target are indirectly semantically related (e.g., lemon — sweet) (Spitzer *et al.*, 1993). Thus, semantic memory in schizophrenia may be qualitatively different from semantic memory in healthy controls.

Executive Functions

The Wisconsin Card Sorting Test

The executive functions comprise capacities for volitional activity, forward planning and self-regulation (Lezak, 1995). The most widely used test of executive functions is the Wisconsin Card Sorting Test (WCST). The WCST is a complex test involving concept formation, working memory and cognitive flexibility. A large amount of literature suggests impaired performance in schizophrenia in terms of increased numbers of perseverative errors and fewer categories achieved, when compared to healthy controls (Goldberg *et al.*, 1994; Haut *et al.*, 1996; Morice, 1990; Sullivan *et al.*, 1993). However, performance on the WCST seems to be heterogeneous (Braff *et al.*, 1991) with poor performance restricted to a subgroup of patients characterized by the presence of other cognitive deficits (Bellini *et al.*, 1991; Goldstein *et al.*, 1996), as well as negative symptoms and more hospitalizations (Butler *et al.*, 1992). Other authors have directed attention to the unusual and strong relationship between performance on the WCST and general intellectual ability, ageing and education, which suggests that non-specific factors account for a substantial proportion of WCST performance in schizophrenia (Heinrichs, 1990; Stratta *et al.*, 1993; Heinrichs and Zakzanis, 1998). Given the complexity of the WCST, several studies have attempted to identify specific processes that underlie test performance. Gold *et al.* (1997) have suggested that working memory, assessed by a novel measure called 'letter–number span', is a critical determinant

of WCST performance in schizophrenia. Yet, even this simpler task involves functions other than working memory, for example, aspects of executive functions. This measure involved the auditory presentation of a mixed series of alternating numbers and letters, the task being to respond by first saying the numbers in order from the smallest to the largest, followed by saying the letters in alphabetical order. In another study, a significant correlation between WCST perseverative errors and time spent on the interference test of the SCWT was observed and the authors concluded that these tests have the involvement of mental control and cognitive flexibility in common (Rossi *et al.*, 1997a). In sum, the WCST is a widely used measure of executive functions, but due to its complexity it is unclear which cognitive processes underlie poor performance, while several studies have indicated that part of the variance in WCST performance is shared with general factors, such as intellectual ability, ageing and number of hospitalizations.

Tower of London

Shallice (1982) introduced this test, derived from the Tower of Hanoi puzzle. The task is to move a number of coloured beads placed on three upright poles so as to reproduce a certain pattern. The subject must look ahead and divide the task into a series of subtasks, and carry these out in the right order to obtain the solution in a minimum number of moves. Pantelis *et al.* (1997) used an automated version of this task, which is part of the CANTAB. This allowed for the registration of planning and execution latencies. Performance of hospitalized patients with schizophrenia ($N = 36$) was compared to that of normal subjects and patients with neurological disorders. The patients with schizophrenia were able to complete the task, but made fewer solutions within the minimum number of moves, and required more moves for completion compared to the healthy volunteers. Also, time needed for completion was longer for the patients with schizophrenia than for the healthy volunteers. The patients with schizophrenia were not impaired in their 'initial thinking' (planning) latencies, but had significantly prolonged 'subsequent thinking' (execution) latencies. This pattern resembled that of the group with frontal lobe lesions and contrasted with the prolonged 'initial thinking' time seen in Parkinson's disease. However, in a sample of 24 outpatients with schizophrenia, performance on the Tower of London was normal, whereas on the WCST and the Behavioural Assessment of the Dysexecutive Syndrome (BADS; see next section) performance was impaired (Krabbendam *et al.*, 1999). Although this study did not record time needed for completion, the findings do suggest that the Tower of London is less sensitive to deficits of planning than the other two tests.

The Behavioural Assessment of the Dysexecutive Syndrome

It has been difficult to develop satisfactory methods for demonstrating and quantifying executive functions, because the structured nature of most neuropsychological examinations gives the patient insufficient opportunity to make use of these functions (Lezak, 1982). Moreover, the standard tests tend to focus on individual components of executive functioning. Yet, what is impaired in patients with impaired executive functions is the ability to initiate, integrate and monitor the use of these components (Shallice and Burgess, 1991). Patients with gross difficulties in daily life may therefore perform within normal limits on the standard executive tests. To overcome these shortcomings, a test battery has been developed, with the aim of being able to predict which everyday problems would arise as a result of impaired executive functioning. The Behavioural Assessment of the Dysexecutive Syndrome (BADS; Wilson *et al.*, 1996) presents the subject with a series of unstructured tasks that are designed to reflect daily life situations. For example, in the subtest Modified Six Elements the subject is told to do six tasks (dictation, simple arithmetic and picture naming, each of which has two sections) in 10 minutes. It is impossible to complete all tasks in 10 minutes, but the subject should do at least something of all six sections, without breaking certain rules. Recent findings suggested that the BADS can indeed identify executive deficits in schizophrenia (Evans *et al.*, 1997; Krabbendam *et al.*, 1999). Significant impairments on the BADS were even found in a subgroup who displayed intact general intellectual functioning (Evans *et al.*, 1997).

General Intelligence

Global intellectual decline is a common finding in schizophrenia (Braff *et al.*, 1991; Blanchard and Neale, 1994; see Aylward *et al.*, 1984, for a review of earlier studies), even in first-episode patients (e.g., Bilder *et al.*, 2000; Mohamed *et al.*, 1999). However, a decline in intelligence quotient (IQ) may not be universal. A cluster analysis of neurocognitive data of 104 patients with chronic schizophrenia yielded a five-cluster solution, two being characterized by average IQ and a normal cognitive profile, except for motor impairments and slightly higher than normal recall intrusion rates, respectively (Heinrichs and Awad, 1993; Heinrichs *et al.*, 1997). Only one cluster (24% of the patients) suggested generalized impairment, the other two clusters comprising patients with low-average IQ and a selective executive or executive-motor dysfunction. In a sample of 117 consecutively admitted patients with chronic schizophrenia, Weickert and colleagues (2000) found a general intellectual decline of 10 points or more from premorbid levels, estimated by means of reading scores, in 60 patients (51%). The remaining patients showed no significant decline relative to estimated premorbid IQ. Half of these patients (i.e., 25% of the total sample) had impaired premorbid IQs and half displayed average estimated premorbid intellectual levels. This latter group showed cognitive profiles similar to normal, except for the presence of executive function deficits and, possibly, attention and encoding. This pattern of compromised executive functions in the presence of normal intelligence has been described previously (Elliot *et al.*, 1995; Evans *et al.*, 1997; Heinrichs and Awad, 1993; Shallice *et al.*, 1991).

The concept of intelligence encompasses a range of cognitive functions and abilities. Therefore, intelligence tests are not well suited to examine specific patterns of deficits, unless the findings are considered in the context of detailed cognitive assessments. Nonetheless, one advantage is associated with the use of intelligence tests. That is, because intelligence tests are widely used in schools, conscript cohorts and hospitals, large data sets are available. This allows for the possibility to investigate the course of the intellectual impairment from premorbid to postmorbid state. Findings based on these data will be discussed below in the sections on course of the deficits and on cognitive vulnerability indicators.

Integrative Approaches

Generalized Deficit

An issue that continues to raise considerable debate is whether the cognitive deficit in schizophrenia can best be characterized in terms of a generalized or a specific impairment. Given the broad range of cognitive deficits, the most parsimonious explanation would be that patients with schizophrenia have a generalized deficit and perform poorly on all tests (Blanchard and Neale, 1994; Mohamed *et al.*, 1999). However, not all deficits fit such an explanation. Some abnormalities of attention and information processing can be demonstrated on tasks that require minimal effort, for example defective sensory gating. Other deficits result in superior rather than

deficient performance, for example on latent inhibition paradigms (Braff, 1993; see also below). Moreover, within the context of this generalized deficit, the question becomes whether patients perform disproportionately worse on some tests (Green, 1998).

Several candidates for such a differential deficit have been proposed, notably attention (Weickert *et al.*, 2000), verbal learning and memory (Bilder *et al.*, 2000; McKenna *et al.*, 1990; Saykin *et al.*, 1991) and executive functions (Evans *et al.*, 1997; Riley *et al.*, 2000; Weickert *et al.*, 2000). However, the findings of a differential deficit should be interpreted in the context of the psychometric limitations of the neuropsychological tests. As Chapman and Chapman (1978) have pointed out, a differential deficit in performance does not necessarily indicate a differential deficit in ability. To measure differential deficit in ability, tests must be matched on psychometric characteristics of test reliability and test difficulty. In addition, differential discriminating power of the various tasks may obscure any differential deficits in ability. The study by Saykin and co-workers (1991) used standardized residual scores (Chapman and Chapman, 1989) as an alternative strategy for the elaborate procedures for matching tasks on their psychometric properties. However, Blanchard and Neale (1994) have argued that this method does not allow for inferences regarding differential abilities, when used in the context of a generalized performance deficit. Thus, given the psychometric limitations of currently available cognitive tests, findings of generalized and differential deficits must be interpreted with caution.

Processing of Context

In normal information processing, perception is dependent on an interaction between the external stimuli and memories of regularities in previous input. When appropriate material from memory is activated, this results in expectancies and response biases, so that temporal and spatial redundancy is used to reduce information-processing demands. Hemsley (1994, 1987) has proposed that the rapid and automatic assessment of the significance of the sensory input on the basis of past regularities is impaired in schizophrenia. According to this model, it should be possible to develop tasks at which patients with schizophrenia perform better than normal controls, namely when the latter group forms expectancies which are inappropriate to the stimulus presented. The latent inhibition paradigm, derived from animal studies, is an example of a task at which patients display superior performance (Hemsley, 1993; Lubow and Gewirtz, 1995). In the first stage of the paradigm, a stimulus is repeatedly presented to the subjects; in the second stage, this pre-exposed stimulus is paired with reinforcement in a classical or instrumental learning procedure. Pre-exposed subjects learn this association much more slowly than subjects who did not receive the first stage of pre-exposure. This effect is interpreted as a reduction in the deployment of attention to a stimulus that is redundant, since it has no consequences according to the learned regularity. Patients with acute schizophrenia perform better than normals in the pre-exposure condition, apparently due to their continuing to attend to the redundant stimulus (Baruch *et al.*, 1988; Gray *et al.*, 1992). Patients with chronic schizophrenia and patients who have been treated with antipsychotic medication for several weeks show no differences in performance on latent inhibition tasks compared to healthy individuals. The link between abnormal latent inhibition and psychotic symptoms may be that, following a loosening of the perceptual context, attention may be captured by incidental details of the environment. These details would normally not reach awareness, but the conscious registration initiates a search for the reasons for its occurrence (Hemsley, 1994). According to the model, symptoms such as poverty of speech, social withdrawal and retardation are learned over time to cope with the high levels of information overloads. However, this model cannot easily account for the finding that negative symptoms may be present at a very early stage of the illness (Arndt *et al.*, 1995).

Computational Models

Based on computer simulation models that specify mechanisms of normal information processing underlying task performance, Cohen and Servan-Schreiber (1992; see also above) have also emphasized the role of context information. According to their model, a single mechanism that is responsible for the representation and maintenance of context information may account for the pervasive cognitive impairment in schizophrenia. Given the similarities between definitions of context and working memory, this view is in line with the proposal by other authors that a reduction of working memory capacity is the core dysfunction in schizophrenia (Fleming *et al.*, 1994; Goldman-Rakic, 1994). Processing of context information may be relevant to almost all cognitive tasks, although it is particularly important in tasks that require to maintain information over time and to inhibit habitual responses. Cohen and Servan-Schreiber have constructed computer simulation models of three tasks: the SCWT, the Continuous Performance Test and a lexical disambiguation task (Cohen *et al.*, 1999; Cohen and Servan-Schreiber, 1992). Each model consisted of an input module for representing externally presented stimuli, an output module for producing a response, an associative module for relating stimuli to appropriate responses, and a context module that can regulate the flow of information through the associative module. Reciprocal connections between the associative module and the context module allowed stimuli presented to the network to help establish the context, which in turn can influence the processing of subsequent stimuli. The context module carried out both the maintenance of information and the inhibition of irrelevant information, indicating that both memory and inhibition reflect the operation of a single mechanism. When this context module was disturbed, the model predicted changes in performance similar to the actual performance deficits of patients with schizophrenia. This was the case for each of the three computer models.

Course of the Deficits

Two contrasting hypotheses have been postulated concerning the natural course of the cognitive deficits in schizophrenia. According to the first, the cognitive deficits follow a course analogous to progressive dementia, consistent with the view that schizophrenia involves a neurodegenerative process. In the second view, the cognitive deficits are considered relatively stable, in accordance with a neurodevelopmental model of schizophrenia. During recent decades, both longitudinal and cross-sectional studies of cognitive deficits have mainly supported the neurodevelopmental hypothesis (Goldberg *et al.*, 1993b; Rund, 1998). Mild cognitive deficits may be present premorbidly, as indicated by studies following large samples of children of parents with schizophrenia from childhood into the age of risk, like the Israeli High-Risk Study (Mirsky *et al.*, 1995b) and the New York High-Risk Project (Cornblatt *et al.*, 1999). Prospective studies of large birth cohorts have supported the association between cognitive deficits in childhood and adult psychosis (Cannon *et al.*, 1999; Jones *et al.*, 1994a, 1994b; Kremen *et al.*, 1998). Also, there is evidence that subgroups of patients with schizophrenia show lower estimates of premorbid IQ than controls on the basis of reading scores (Crawford *et al.*, 1992; Gilvarry *et al.*, 2000).

Following the onset of the first psychotic episode, most patients experience a substantial decline in cognitive functions compared to their premorbid level (Goldberg *et al.*, 1993b; Weickert *et al.*, 2000). That onset of the psychotic symptoms and the cognitive

decline do not occur in childhood but so much later may be because only then does the affected part of the brain mature and is called 'on line' (Weinberger, 1987). Most cognitive deficits persist during clinical remission (Cantor Graae *et al.*, 1995; Goldberg *et al.*, 1993b; Nopoulos *et al.*, 1994; Nuechterlein *et al.*, 1994). Once established early in the course of the disorder, the deficits seem to be relatively stable over the years (Goldberg *et al.*, 1993b; Rund, 1998). Thus, cross-sectional studies of first-episode patients have shown patterns of deficits similar to patients later in the course of their illness (Bilder *et al.*, 2000; Heaton *et al.*, 1994; Mohamed *et al.*, 1999; Saykin *et al.*, 1994). Furthermore, cross-sectional studies across a wide age range have suggested stable cognitive impairments (Eyler Zorrilla *et al.*, 2000). Most recent longitudinal studies also point to a stability of cognitive functioning (Censits *et al.*, 1997; Gold *et al.*, 1999), early in the course of the disorder (Hoff *et al.*, 1999; Nopoulos *et al.*, 1994) but also in older patients (Harvey *et al.*, 1995). Yet, the time between baseline and follow-up was 1 or 2 years in most studies, except for the study by Gold *et al.* (5 years) and Hoff *et al.* (5 years), indicating the need for studies of longer duration.

However, the neurodevelopmental model cannot account for all findings. A subgroup of geriatric patients with chronic schizophrenia displays severe cognitive and functional impairments that implicate decline at some time point after the onset of illness (Friedman *et al.*, 1999a; Harvey *et al.*, 1999a, 1999b). Also, it has been suggested that progressive age-related deterioration in schizophrenia occurs in specific cognitive functions, notably abstraction ability (Fucetola *et al.*, 2000) or processing resources (Granholm *et al.*, 2000). This is in line with the view recently put forward by Lieberman (1999), that it is premature to reject a role for neurodegenerative processes in the pathophysiology of schizophrenia.

SYMPTOMS OF SCHIZOPHRENIA AND COGNITIVE DEFICITS

Positive and Negative Syndromes

Attempts to link the cognitive deficits to symptoms of schizophrenia have mainly focused on the relation with positive and negative syndromes (see Strauss, 1993, for a review of earlier studies). The main approach has been to correlate the degree of cognitive deficits to the severity of symptoms of both dimensions. Negative symptoms have been found to be associated with deficits in verbal and visual memory, visual/motor processing and impaired executive functions (Berman *et al.*, 1997; Capleton, 1996; O'Leary *et al.*, 2000; Rossi *et al.*, 1997b). Positive symptoms have been found to co-occur with impaired auditory attention and verbal memory (Berman *et al.*, 1997; Green and Walker, 1986), but the evidence is more in favour of independence between positive symptoms and neuropsychological deficits (O'Leary *et al.*, 2000; Voruganti *et al.*, 1997). In accordance with this, several studies have shown that the paranoid subtype, characterized by delusions and hallucinations, is associated with better performance in memory, attention and executive functions, compared to the other subtypes (Seltzer *et al.*, 1997; see Zalewski *et al.*, 1998, for a review).

Liddle (1987) has proposed that the symptoms of disorganization can best be seen as a separate cluster within the positive dimension, which has been confirmed by subsequent factor analyses (Johnstone and Frith, 1996). The dimension of disorganization might be associated with impaired cognitive functions, notably impaired selective attention and inhibition (Liddle and Morris, 1991; Van der Does *et al.*, 1996); low verbal IQ and impaired concept attainment (O'Leary *et al.*, 2000); and impaired visual and auditory attention (Brekke *et al.*, 1995).

However, correlations between symptoms and deficits are generally quite modest. It seems that most of the variance of symptoms must be attributed to factors other than neurocognition. In fact, several studies have found that symptoms and neurocognition are independent. This is particularly the case for the positive symptom cluster (Brekke *et al.*, 1995; O'Leary *et al.*, 2000; Voruganti *et al.*, 1997), but similar results have been found for negative symptoms as well (Seaton *et al.*, 1999; Suslow *et al.*, 1998). Further, a major drawback of this correlational approach is that it is descriptive instead of explanatory, which means that it offers no insight into the reason for the association (Green, 1998). An alternative approach has been to take individual symptoms of psychosis as the starting point and examine possible cognitive mechanisms that may underlie them. In the next section, two cognitive accounts of psychotic symptoms will be discussed, namely theory of mind and self-monitoring.

Theory of Mind

Theory of mind refers to the ability to explain the behaviour of others in terms of their mental states (Premack and Woodruff, 1978). In normal children, this ability emerges in the pre-school years, manifested for example in the use of mental state expressions in their language and the understanding of the consequences of false belief, (e.g., understanding the actions of characters within stories that are a result of their false beliefs about the state of the world) (Leslie, 1987). In recent decades, most research on theory of mind, in humans has focused on children with autism. There is evidence that autistic children have a specific deficit in theory of mind that is apparent, for example, from deficits in pretend play and difficulties in the understanding of false belief (Baron-Cohen *et al.*, 1985). In schizophrenia, interest in theory of mind abilities has been less explicit, although several studies of social competence in schizophrenia did refer to the ability to infer mental states in others (e.g., Cutting and Murphy, 1990; Diamond, 1956). For example, Diamond (1956) presented evidence to support the notion that patients with schizophrenia are unable adequately to internalize the point of view of others. Frith (1992) has applied the concept of theory of mind to specific symptoms of schizophrenia, namely delusions of reference, misidentification and persecution, as well as third-person hallucinations. According to Frith, these experiences are by their very nature characterized by a misinterpretation of another person's behaviour or intentions. Whereas autistic children may never develop mentalizing ability, patients with schizophrenia may lose an ability they once had (Frith and Corcoran, 1996).

In a series of experiments, Frith and his co-workers were able to demonstrate impaired theory of mind in subgroups of patients with schizophrenia on a number of newly devised tasks (Corcoran *et al.*, 1995, 1997; Doody *et al.*, 1998; Frith and Corcoran, 1996). Patients with behavioural signs (i.e., negative symptoms and disorganization) and those with paranoid delusions had problems on a hinting task that requires the ability to infer intentions behind indirect speech, in contrast to patients with passivity experiences and patients in remission, who performed normally (Corcoran *et al.*, 1995). A similar pattern of results was found in a subsequent study using stories that involved false beliefs, so that to understand the stories subjects had to infer the mental states of the characters (Frith and Corcoran, 1996). Again, patients with behavioural signs and those with paranoid delusions were impaired on this task, while patients with passivity experiences and those in remission did not differ from normal controls. There were no differences in IQ score between the patients with paranoid delusions and those with passivity experiences or remitted patients, making it unlikely that the observed differences in theory of mind ability could be ascribed to general cognitive impairment. In contrast, the poor performance of patients with behavioural signs was mediated by

their poor general cognitive abilities. Another study indicated that impaired performance on theory of mind tasks is found in patients with schizophrenia, but not in patients with affective disorders with psychotic symptoms (Doody *et al.*, 1998). Although these studies confirmed the initial hypothesis that impaired theory of mind is associated with specific symptoms, evidence from other studies is mixed. Using a series of comic strips, Sarfati and colleagues found that only patients with thought disorganization had difficulties interpreting the behaviour of the characters (Sarfati and Hardy Bayle, 1999; Sarfati *et al.*, 1997), independent of the mode of presentation, verbal or pictorial (Sarfati *et al.*, 1999). A study by another group included three groups: patients with schizophrenia, patients with depression and deluded patients with psychotic disorders other than schizophrenia (Drury *et al.*, 1998). Patients were investigated during the acute phase and following recovery. In line with the hypothesis, patients with schizophrenia displayed impaired theory of mind abilities compared to non-schizophrenia patients, during psychosis but not following recovery. However, more subtle theory of mind tasks, like the interpretation of irony, failed to distinguish between the groups, which is difficult to reconcile with Frith's account. Furthermore, there were no differences in performance between patients with delusions of reference and persecution, and non-deluded patients, a finding not in line with the notion that impaired theory of mind is the critical mechanism underlying these delusions.

Taken together, the findings indicate that patients with schizophrenia certainly have difficulties performing theory of mind tasks. These difficulties are state rather than trait variables. The impairments seem to be particularly pronounced in patients with negative symptoms or positive behavioural signs, while the predicted association with delusions has been found in some studies. General cognitive abilities seem to mediate only part of the associations between symptoms of schizophrenia and theory of mind abilities.

Source Monitoring

Source monitoring refers to the processes involved in making attributions about the origin of memories, knowledge and beliefs (Johnson *et al.*, 1993). This decision-making process can apply to various types of distinctions, namely between memories of internally generated information and externally derived information, for example memories for imagination versus memories for perceived events ('reality monitoring'), or between different external sources ('external source monitoring') or between internally generated sources, for example memories of what one said versus memories of what one thought ('internal source monitoring') (Johnson *et al.*, 1993). The notion of reality monitoring may be particularly relevant to psychosis. The nature of several symptoms of psychosis suggests that patients with this disorder are unable to monitor the generation of their own thoughts and actions, which then results in their conclusion that these thoughts or actions came from an external source (Bentall, 1990; Frith, 1992). This may apply to hallucinations, thought insertion, made feelings and other experiences of alien control (Frith, 1992).

Action Monitoring

A number of studies have applied source-monitoring paradigms to schizophrenia. These paradigms have mainly focused on verbal monitoring (for example, (Bentall *et al.*, 1991; Brébion *et al.*, 1997; Johns *et al.*, 2001), whereas some have investigated the monitoring of actions (Franck *et al.*, 2001; Frith and Done, 1989; Mlakar *et al.*, 1994; Stirling *et al.*, 1998). Studies of action monitoring performance have mainly used tasks that require subjects to keep track of the actions they had just performed in the absence of visual feedback (Frith and Done, 1989; Mlakar *et al.*, 1994; Stirling *et al.*, 1998). The results of these three studies have supported the hypothesis that patients with experiences of alien control have difficulties monitoring their actions internally, particularly when the actions are self-generated rather than being directed by external stimuli. In the study by Franck *et al.* (2001) visual feedback was not absent but distorted. The image of a virtual hand was superimposed on a subject's real hand holding a joystick, so that the subject only saw the virtual hand. Subjects executed discrete movements, while angular biases and temporal delays were randomly introduced in some trials. The task was to decide immediately after each trial whether the movement they saw corresponded exactly to the one they made with there own hand. In the trials with angular biases patients with delusions of influence made more errors than controls and non-influenced patients with schizophrenia. This result suggests that a difficulty in keeping track of one's own actions is a critical mechanism underlying delusions of influence.

Verbal Monitoring

In the verbal monitoring paradigm developed by Johnson *et al.* (1981), subjects are required to generate answers to clues, consisting of a category and a letter, and to listen to paired associates consisting of a category and a particular. To manipulate the amount of cognitive effort involved, half of the items were easy clues or high-probability associations (e.g., 'Think of a type of dwelling beginning with H. . .', 'A type of footwear — shoe') and half were difficult clues or low-probability associations (e.g., 'Think of a fruit beginning with T. . .', 'A country — Norway'). In normals, confusion between memories of perceived and imagined events increases with decreases in the amount of cognitive effort that is associated with the self-generated event (Johnson *et al.*, 1981). After 1 week, subjects are presented a list in which their answers to the clues are mixed with the associates and with words not previously presented. Their task was to identify the source of each item on the list. Bentall *et al.* (1991) used this task to compare performance between three groups: patients with schizophrenia who were currently hallucinating, patients with delusions but no history of hallucinations, diagnosed with schizophrenia or delusional disorder, and normal controls. Both patient groups were less accurate on the source-monitoring task compared to controls. A specific bias was present in the patients with hallucinations: they more often misattributed self-generated answers to the experimenter than either the psychiatric or the healthy controls, a difference that was significant for the items that required high cognitive effort. Using a similar paradigm, another study also reported a bias toward attributing self-generated items to an external source (Brébion *et al.*, 1997). Keefe *et al.* applied multinomial modelling analysis to source-monitoring data, a method that yields separate and independent measures of recognizing information, remembering its source and response biases (Keefe *et al.*, 1999a). This analysis indicated that patients were impaired in reality monitoring, as well as internal and external monitoring. In case of ambiguity, when someone did not remember the source of the information, patients with schizophrenia showed a stronger bias than controls to report that it came from an external source.

Online Monitoring

The tasks used in the studies described so far require subjects to identify the source of events some time after they had been presented or generated. Johns *et al.* (2001) developed a paradigm that involves immediate monitoring, which might be more relevant to the mechanisms underlying the psychotic symptoms. Participants read single words aloud while they received feedback of their own voice or alien feedback (someone else's voice). In some trials, the acoustic characteristics of the speech were distorted. Immediately

after reading aloud the words, subjects had to identify the source of the speech they heard. Both patients with hallucinations and delusions and patients with delusions but no hallucinations made more errors than the control group when reading aloud with distorted feedback of their own voice. The patients with hallucinations had the strongest tendency to misattribute their distorted voice to someone else, particularly when the words presented had a negative emotional content.

Bentall (1990) has argued that the misattribution may be influenced by top-down processes, such as beliefs and expectations about the self and the world. This cognitive bias may operate in addition to deficits in discriminating between external and internal events. The bias may be reinforced by the anxiety reduction that is associated with attributing negative thoughts to an external source. The results reported by Morrison and Haddock (1997) supported the role of a cognitive bias. Subjects rated their response on a word association task in terms of how much a self-generated thought was their own. Patients with hallucinations had lower internality rating than both the psychiatric and the normal control group, particularly with emotional material. A subsequent study indicated that these internality ratings were associated with metacognitive beliefs about uncontrollability and danger (Baker and Morrison, 1998).

In sum, a number of studies have documented robust deficits of reality monitoring in schizophrenia, with a tendency to attribute events to an external source in case of ambiguity. Other forms of source monitoring, namely internal and external monitoring, may be impaired as well. The results of different studies are not entirely consistent regarding the associations with specific symptoms of psychosis. In general, if a relation between impaired source monitoring and symptoms is found, it is mainly with symptoms of alien control rather than hallucinations *per se*. However, the presence of an external attribution bias may be specifically involved in hallucinations.

COGNITIVE DEFICITS AS VULNERABILITY FACTORS

Deficits in specific functions, like source monitoring and theory of mind, are most likely state-dependent variables. However, this is certainly not the case for all cognitive deficits in schizophrenia. Strong evidence for trait-like deficits comes from studies involving non-psychotic individuals with a genetically mediated vulnerability to schizophrenia. According to the vulnerability/stress model, some individuals have a predisposition to schizophrenia that is largely determined by genetic and neurodevelopmental factors (Nuechterlein and Dawson, 1984; Zubin and Spring, 1977). Many studies have attempted to find markers of this vulnerability in first-degree adult relatives of patients with schizophrenia and in individuals with a higher risk of developing schizophrenia than the general population.

Cognitive Deficits in Non-Psychotic Relatives

Adult Relatives

Several studies have suggested that adult relatives of patients with schizophrenia have mild cognitive impairments (Faraone *et al.*, 1995, 1999; Krabbendam *et al.*, 2001; see Kremen *et al.*, 1994, for a review). The study by Krabbendam *et al.* (2001) suggested that the pattern of deficits in relatives is similar, albeit milder, to that found in patients. They found that for both patients and relatives attention, verbal memory and working memory independently discriminated from control performance, with a similar pattern in the order of the effects. A 4-year follow-up study by Faraone and colleagues indicated that the neuropsychological deficits in the non-psychotic relatives are stable over time (Faraone *et al.*, 1995, 1999). In a subsequent study, these authors demonstrated that non-psychotic individuals with two first-degree relatives with schizophrenia have lower estimated intelligence and worse performance on visual and verbal memory, compared to persons with only one first-degree relative with schizophrenia. Thus, the cognitive deficits in the relatives reflect their degree of genetic predisposition to schizophrenia.

A likely explanation for the cognitive deficits in non-psychotic adult relatives is that the deficits are transmitted within families of patients with schizophrenia, as markers of genetic risk. If this is the case, specific cognitive deficits may possibly be used as intermediate phenotypes to facilitate genetic studies of schizophrenia (Egan *et al.*, 2000; Tsuang, 1993). The advantage of this approach compared to using the schizophrenia diagnosis as the phenotype is that it may be possible to identify subgroups that are genetically more homogeneous. Further, the intermediate phenotypes may have a simpler genetic architecture. To assess whether cognitive deficits are useful as phenotypes for genetic studies, one needs to examine heritability estimates. Only few studies have done this. Chen *et al.* (1998) found very high relative risk for sustained attention in a Taiwanese cohort, which suggests that this phenotype is highly heritable. In the study by Egan and colleagues, relative risk of impaired sustained attention was in the moderate range (Egan *et al.*, 2000). Although it is too early to conclude that cognitive deficits can be used as phenotypic markers, these findings at least suggest that it would be useful to explore this possibility further.

This, however, does not mean that cognitive deficits in schizophrenia cannot be due to environmental mechanisms. Specifically, obstetric complications may be associated with schizophrenia (Dalman *et al.*, 1999) as well as with compromised intellectual development (Aylward *et al.*, 1989). In the study reported by Gilvarry and colleagues (2000, 2001), relatives of patients who have been exposed to obstetric complications performed significantly better on measures of IQ and verbal fluency than did relatives of patients not exposed to obstetric complications. This suggests that impaired IQ and verbal fluency were not transmitted in the families of the patients with a history of obstetric complications, but were a consequence of the patient's exposure to obstetric complications.

Children of Patients with Schizophrenia

The high-risk research typically involves children of a parent with schizophrenia, assessed during childhood and adolescence. A number of studies have suggested that offspring of patients with schizophrenia may display impairments in attention skills (Hans *et al.*, 1999; Mirsky *et al.*, 1995a), executive functions, memory and intellectual function (Byrne *et al.*, 1999). Unlike the adult relatives, children of patients with schizophrenia form a heterogeneous group, because some of the children will eventually develop schizophrenia themselves and others will not. Long periods of follow-up have been performed to identify the factors that are associated with later development of schizophrenia (Erlenmeyer-Kimling *et al.*, 1993; Mirsky *et al.*, 1995b). The NIMH–Israeli High-Risk Study conducted a long-term follow-up of children having a parent with schizophrenia (Mirsky *et al.*, 1995b). One hundred children were originally included, 50 of them having a parent with schizophrenia, and 50 control children. Cognitive assessments were conducted at ages 11, 17, 26 and 32. Sixty-three subjects could be included in the last assessment. The children who eventually developed schizophrenia spectrum disorders, including schizophrenia, were identifiable by attentional deficits at age 11. Results of the New York High-Risk Project have indicated that childhood deficits in attention, verbal memory and gross motor skills distinguish children who develop schizophrenia-related psychoses in adulthood from children at risk for schizophrenia who did not develop schizophrenia-related psychoses in adulthood

(Cornblatt *et al.*, 1999; Erlenmeyer-Kimling *et al.*, 2000). In this project, the offspring of patients with schizophrenia were tested neuropsychologically and behaviourally at 7–12 years of age and assessed again in mid-adulthood for psychiatric diagnoses. In sum, several cognitive abnormalities, notably attentional deficits, are present in offspring of patients with schizophrenia, particularly in those children who eventually develop schizophrenia spectrum disorders themselves.

Several population-based studies of IQ and later development of schizophrenia have been conducted (e.g., David *et al.*, 1997; Jones *et al.*, 1994a, 1994b). These studies have indicated that low IQ in childhood or young adolescence is a risk factor for subsequent development of schizophrenia. Based on a 19-year longitudinal study of a community sample, Kremen and colleagues have reported that an unexpected drop in IQ between the ages of 4 and 7 was strongly and independently associated with later psychotic symptoms (Kremen *et al.*, 1998). Two possible mechanisms may account for the relation between childhood IQ and schizophrenia. IQ could be indirectly associated with schizophrenia, with any factors causing lower IQ, such as genetic predisposition or abnormal brain development, increasing the risk for schizophrenia. Alternatively, the association could be causal, with low IQ reducing the capacity to cope with stressors or leading to false beliefs and perceptions.

Cognitive Deficits in Schizotypy

An alternative approach to identify factors associated with schizophrenia liability has been to study schizotypic subjects, either individuals diagnosed with DSM-IV schizotypal personality disorder, or individuals who display deviance on psychometric tests for schizotypy ('psychosis proneness'). This approach starts from the assumption that schizophrenia is the extreme end of a continuum that ranges from mild to severe, with schizotypy being part of this continuum (Claridge, 1994). Family studies have reported elevated rates of schizotypy in relatives of patients with schizophrenia, indicating that schizotypy shares a common genetic vulnerability to schizophrenia (Kendler *et al.*, 1993, 1994). To the extent that this is the case, schizotypal subjects may have the same pattern of cognitive deficits as individuals with schizophrenia, albeit to a lesser degree.

Based on studies involving either subjects with schizotypal personality disorder (Cadenhead *et al.*, 1999; Diforio *et al.*, 2000; Trestman *et al.*, 1995; Voglmaier *et al.*, 1997, 2000) or psychometrically identified 'psychosis-prone' subjects (Lenzenweger and Gold, 2000; Lenzenweger and Korfine, 1994; Park *et al.*, 1995), findings have indeed supported the notion of a shared cognitive liability. Cadenhead and co-workers (1999) reported widespread deficits in 20 subjects with schizotypal personality disorder, including deficits of attention, verbal working memory, abstract reasoning and general intellectual functioning. The deficits were of lesser magnitude than those observed in the patients with schizophrenia. Deficits of executive functions were found in adolescents with schizotypal personality disorder, particularly in individuals with negative symptoms (Diforio *et al.*, 2000). However, the deficits were only apparent on one test of executive function, the Modified WCST, and not on the Tower of London or verbal fluency tests, which may reflect that these tests may not be sensitive enough to detect the mild cognitive impairments in schizotypal personality disorder. In a comparison of undergraduate students who scored high and low, respectively, on the Perceptual Aberration Scale (Chapman *et al.*, 1978), high scores were associated with deficits of visuospatial working memory (Park *et al.*, 1995), but not auditory working memory (Lenzenweger and Gold, 2000). This latter finding was somewhat unexpected, given that auditory working memory is impaired in patients with schizophrenia (Aleman *et al.*, 1999; Gold *et al.*, 1997) and that the modal finding in schizotypy research is that the cognitive profile of schizotypic individuals resembles that of patients, although in attenuated form. According to the authors, one possible explanation for this result could be that the task used to assess auditory working memory (namely, letter–number span) involved discrete trials and so did not require subjects to hold online the information over a delay period, in contrast to the task used to assess visuospatial working memory.

Some studies have explored the notion of reduced cognitive inhibition in schizotypy (Beech *et al.*, 1991; Moritz and Mass, 1997; Peters *et al.*, 1994; Williams, 1995). Cognitive inhibition refers to the active suppression of irrelevant information, one of the functions of the context module in the computational model by Cohen and Servan-Schreiber described above (Cohen *et al.*, 1999; Cohen and Servan-Schreiber, 1992). These studies have used a negative priming paradigm (Tipper, 1985), which typically consists of a target word presented subsequently to a semantically related, or identical, ignored distractor word. Negative priming refers to the longer reaction times following the ignored distractor word, a phenomenon that is thought to reflect reduced inhibition. The findings converge on the point that every study reported increased negative priming, particularly in those subjects who scored high on the scales for positive schizotypal symptoms.

FUNCTIONAL CONSEQUENCES AND TREATMENT ISSUES

Impact of Cognitive Deficits on Daily Functioning

Given the scope of the cognitive impairment in schizophrenia, an important question is in what way this impairment affects daily functioning. Despite its clinical relevance, this question has been largely ignored until roughly a decade ago. Nevertheless, some trends emerge from the available empirical data.

In a review of 17 studies, Green (1996) divided the literature into three areas of functional outcome, namely community outcome, social problem solving and skill acquisition. Measures of community outcome included assessments of occupational functioning, quality of social networks and degree of independent living. Although there were large differences between studies with regard to cognitive as well as outcome measures, a replicated finding was that baseline verbal episodic memory and executive functions, assessed with the WCST, predicted community functioning at follow-up (Green, 1996; Velligan *et al.*, 2000). Social problem solving is considered to be a component of community functioning. Most studies have assessed social problem solving using laboratory measures that presents the subjects with videotaped vignettes depicting an interpersonal problem (e.g., a waitress writing down an order incorrectly; Donahoe *et al.*, 1990). The task of the subject is to describe the problem, to derive a solution and to enact this solution in a role-played simulation. Despite some variety between studies in the selection of the outcome measures, a consistent finding was that both verbal memory and vigilance, or sustained attention, were related to social problem solving, whereas executive functions were not (Green, 1996). The majority of the studies in the review were cross-sectional. Subsequent 2-year longitudinal studies of cognitive functioning and social problem solving have reported that both predictive ability of baseline cognitive functions (Addington and Addington, 2000) and the level of social functioning (Dickerson *et al.*, 1999) are stable over time.

Acquisition of psychosocial skills is another determinant of community functioning. Rehabilitation programmes involve skill acquisition, as these programmes instruct patients with regard to interpersonal skills, leisure activities and vocational skills. Studies that have investigated the relation between cognitive functions and skill acquisition have generally assessed cognition at baseline and progress of psychosocial skills during the course of a rehabilitation

programme (Green, 1996). Verbal memory was associated with skill acquisition, as it was with community functioning and social problem solving. Also, verbal working memory and vigilance were associated with skill acquisition. Executive functions did not consistently predict success of rehabilitation programmes.

This summary of findings suggests some specificity in the associations between cognitive functions and outcome. Some of the observed relations have clear face validity, for example, the finding that verbal memory predicts skill acquisition. Yet, it should be noted that the absence of relations between other cognitive functions and outcome might as well have resulted from lack of power due to small sample sizes. In fact, Velligan and colleagues (2000) found evidence for many other associations between cognitive functions and community functions. According to these authors, the multitude of associations may be due to the fact that both cognitive test performance and functional skills do not involve isolated abilities, but instead are determined by several cognitive abilities.

Eight studies that were included in the review by Green (1996) evaluated the relationship between psychotic symptoms and functional outcome. None of them reported a significant relation. However, these studies used composite measures of psychotic symptoms, and so the possibility remains that negative effects of single symptoms, for example thought disorder, were obscured. Negative symptoms showed replicated links with social problem solving, but not with skill acquisition, whereas relations with community functioning were inconsistent. According to the path model by Velligan and colleagues (1997; see also Green, 1998), the pattern of associations between symptoms, cognitive functions and functional outcome suggests that cognitive functions mediate the relation between negative symptoms and outcome. That is, when cognitive impairment is used to predict functional outcome, the contribution of negative symptoms to the prediction is very much reduced. This emphasizes the necessity of addressing cognitive impairments in pharmaceutical or psychosocial interventions.

Effect of Medication on Cognitive Functions

Conventional Antipsychotics

The therapeutic effects of conventional antipsychotic drugs are largely confined to the positive symptoms. These drugs were not designed to improve cognitive functions. Given their impact on several neurotransmitter systems that are implicated in arousal, attention as well as other cognitive functions, it is likely that they do influence cognition, either positively or negatively. Studies involving healthy volunteers have yielded evidence for negative effects, particularly for medication with sedating effects, such as thioridazine (Hindmarch and Tiplady, 1994; King, 1994). In patients, investigating the effects of antipsychotic medication on cognition is hindered by methodological and ethical concerns. For example, discontinuation or delay of drug treatment to study the course of cognitive functioning is often not advisable. Nevertheless, several reviews have concluded that the deleterious effects of antipsychotic medication on cognitive functioning in patients are only minor (Medalia *et al.*, 1988; Spohn and Strauss, 1989). For example, neuroleptically naive patients with schizophrenia had a similar pattern of cognitive deficits as did previously treated patients (Saykin *et al.*, 1994). In another study, a substantial dose reduction of conventional antipsychotic medication did not have marked effects on cognitive functioning (Seidman *et al.*, 1993). There may even be a slight beneficial effect of antipsychotic medication on cognition (Spohn and Strauss, 1989; Verdoux *et al.*, 1995).

There is one possible exception to this, namely the effect of anticholinergic medication or of the anticholinergic properties of antipsychotic drugs. Anticholinergics are known to impair memory and it seems plausible that this effect could contribute to the memory impairment seen in patients with schizophrenia (Goldberg *et al.*, 1993a; King, 1990; Spohn and Strauss, 1989) or to the failure of conventional antipsychotics to improve memory performance in schizophrenia (Mortimer, 1997).

Atypical Antipsychotics

With the development of atypical antipsychotic medication, such as clozapine, risperidone and olanzapine, the treatment of cognitive dysfunctions has become an important focus. Do these drugs really improve cognition? Keefe *et al.* (1999b) have recently performed a quantitative analysis of 15 efficacy studies, studying the effects of clozapine (10), risperidone (4), zotepine (1), ziprasidone (1) and aripiprazole (1). The authors first developed methodological standards against which studies in these area may be judged. None of the included study met all methodological requirements; for example, only three studies were double-blind whereas 12 studies were open-label studies. Despite these methodological concerns, the authors felt sufficiently confident to conclude that atypical antipsychotic drugs improve cognitive functioning in schizophrenia, when compared to conventional antipsychotics. Verbal fluency, digit–symbol substitution, fine motor function and executive functions were the strongest responders to the novel antipsychotics. Yet, even on these tests, performance of the patients with schizophrenia did not reach normal levels in any of the studies reviewed. This emphasizes the necessity to investigate the usefulness of cognition-enhancing drugs in schizophrenia, such as those that activate certain dopaminergic receptors or cholinomimetic drugs that increase cortical cholinergic activity (Friedman *et al.*, 1999b).

Cognitive Remediation

Until a few years ago, there had been little research on cognitive remediation in schizophrenia, despite the fact that it has been established for decades that cognitive deficits persist even after the psychosis subsides. However, the cognitive deficits are now viewed as having direct implications for treatment and findings in the area of neuropsychological interventions are emerging rapidly (Green and Nuechterlein, 1999).

The interventions can take many forms, but generally involve laboratory-based exercises that are related to the cognitive process being trained. Some training programmes use cognitive rehabilitation software. For example, Medalia *et al.* used a software program entitled 'Where in the USA is Carmen Sandiego?' to remediate problem-solving skills in patients with schizophrenia (Medalia *et al.*, 2001). The purpose of this task was to solve a simulated detective case by interpreting information given to identify the suspect in order to make an arrest. Some studies focus on single tasks, for example, the WCST, to investigate whether performance can be enhanced by inducing specific task modifications, such as reinforcement or feedback (Goldberg and Weinberger, 1994; Green *et al.*, 1992; Hellman *et al.*, 1998). There are large differences between training programmes regarding the intensity and duration of the training. For example, Bell and colleagues (2001) applied neurocognitive enhancement therapy, in which every participant receives feedback about his or her cognitive functioning on a biweekly schedule, as well as cognitive exercises for up to 5 hours each week for 26 weeks and a weekly social processing group. In contrast, the computer-aided training by Medalia *et al.* (2000, 2001) consisted of 10 sessions of 25 minutes, twice weekly for 5 weeks.

Despite the growing literature, there is a paucity of controlled studies. In a recent systematic review by the Cochrane group, only three studies could be included that used a randomized design in which the impact of cognitive rehabilitation on people with schizophrenia was compared to a placebo intervention, another intervention or standard treatment (Hayes and McGrath, 2000).

This review did not include comparisons of different types of cognitive rehabilitation. Suslow and colleagues (2001) identified nine peer-reviewed studies of attention training in schizophrenia, which incorporated a control group and used external measures of outcome. Both reviews concluded that data are inconclusive and provide no evidence for or against cognitive remediation or attention training in schizophrenia. An important issue is the generalizability of the improvement. Performance on the training task can often be improved (Green *et al.*, 1992; Hellman *et al.*, 1998) but it is doubtful whether these effects generalize meaningfully (Bellack *et al.*, 1996).

Among the few methodologically adequate studies are the study by Wykes and colleagues (1999) and the one by Medalia and colleagues (2000, 2001). The study by Wykes *et al.* (1999) investigated the effects of an intensive cognitive rehabilitation programme. The intervention was targetted at deficits of executive functions, based on procedural learning and the principles of errorless learning, targetted reinforcement and massed practice. It consisted of individual daily sessions of 1 hour for up to 3 months. The control therapy was occupational therapy, matched for length of treatment and therapist contact. Seventeen patients received the cognitive treatment and 16 received occupational therapy. Some improvement in cognition followed both therapies, but the cognitive intervention was superior to the occupational therapy with regard to tests of cognitive flexibility and memory. There were no systematic changes in symptoms or social functioning in either group, with one exception: patients in the cognitive treatment showed a significant increase in self-esteem. The study by Medalia *et al.* (2000, 2001) applied computer-aided training programmes to remediate memory and problem-solving deficits. A sample of 54 patients with schizophrenia were randomly assigned to a memory-training group, a problem-solving group or a control group. Both training groups received 10 sessions of computer training. The control group participated in routine unit activities. The memory-training group improved on the training tasks during the course of the training, but they did not make greater gains on external outcome measures of memory. The subjects failed to apply the mnemonic strategies that they learned successfully within training tasks to other general measures of memory and learning. In contrast, the problem-solving group showed a significant improvement on the outcome measure that assessed problem-solving skills required for independent living, an effect that was not present in the other two groups. The authors concluded that patients with schizophrenia are responsive to problem-solving training techniques.

THE CONTRIBUTION OF CLINICAL NEUROPSYCHOLOGY

The cognitive profile in schizophrenia is characterized by a broad range of deficits. However, there is no single deficit with sufficient specificity to be useful as a diagnostic instrument. Deficits of attention, memory and executive functions may also be present in other psychiatric conditions, such as unipolar or bipolar depression, although in schizophrenia deficits are generally more severe compared to other psychiatric disorders (Martinez Aran *et al.*, 2000; Zakzanis *et al.*, 1999). Furthermore, test results of a patient with schizophrenia may be indistinguishable from those obtained in neurological disorders, such as mild traumatic brain injury (Zakzanis *et al.*, 1999). Therefore, the clinical neuropsychologist cannot rely on quantitative neuropsychological profiles to differentiate schizophrenia and a neurological condition. At the same time, no single test completely discriminates patients with schizophrenia from healthy controls. According to the quantitative review by Zakzanis *et al.* (1999), the effects of the cognitive variables are associated with a 30–40% overlap between schizophrenia and control distributions. Indeed, substantial numbers of patients with schizophrenia have normal neuropsychological profiles (Palmer *et al.*, 1997). Thus, cognitive deficit is not an inclusive feature of the illness. In sum, neuropsychological test profiles are of limited use in differential diagnosis and can only be interpreted in the context of qualitative aspects of the assessment as well as information about psychopathology, course of the deficits and premorbid functioning.

Same performance on a test does not necessarily point to the same underlying dysfunction (Keefe, 1995). Patients with schizophrenia may perform poorly for reasons that involve other cognitive and brain processes than patients with neurological disorder. Moreover, clinical neuropsychological tests were designed to be sensitive to the type of dysfunction seen with brain lesions. These tests are sensitive to a range of cognitive deficits in schizophrenia as well, but they do not tell us much about the origins of the characteristic symptoms of this disorder. Perhaps the current neuropsychological instrumentarium should be supplemented with tests that fit the psychopathology in schizophrenia, for example tests involving source monitoring or theory of mind. This may give clues to the cognitive mechanisms that may be involved in vulnerability for psychosis.

Clinical neuropsychological assessment does contribute to individual health care, but this contribution most likely concerns treatment and prognosis rather than psychiatric diagnosis. Deficits of attention, memory and executive functions predict functional outcome, possibly even more so than the psychotic symptoms do (Green, 1996; Velligan *et al.*, 2000; see 'Impact of cognitive deficits on daily functioning', above). Thus, the cognitive assessment can be used to adjust the rehabilitation programme to the strengths and weaknesses of the individual patient.

CONCLUDING REMARKS

The cognitive profile in schizophrenia is characterized by deficits across several domains, notably attention, memory and executive functions. The attentional deficits include sustained attention, the capacity to maintain a state of readiness to respond to small changes in the environment, as well as selective attention: the capacity to focus while ignoring irrelevant information. Memory functions that are particularly affected include verbal memory and learning, working memory and semantic memory. The aspects of executive functions that are compromised in schizophrenia are the capacities for volitional activity, forward planning and self-regulation. Regarding the course of the deficits, the evidence is mostly in favour of a relatively stable course, with no further deterioration once the deficits have been established during the first few years following the onset of the first psychotic episode.

An issue that continues to raise considerable debate is whether the cognitive deficit in schizophrenia can best be characterized in terms of a generalized or a specific impairment. Studies that attempt to resolve this issue have to deal with the methodological problems associated with measuring a differential deficit against a background of general impairment, that is, a deficit that is more pronounced than any other cognitive deficit. Given the psychometric limitations of the currently availably cognitive tests, findings of both generalized and specific deficits must be interpreted with caution.

A related issue is whether a single mechanism can be found that can account for the range of deficits in schizophrenia. An influential hypothesis is that a single deficit in the processing of context is sufficient to produce all cognitive deficits as well as certain symptoms of schizophrenia. Strong support for this hypothesis has come from computer models. It appeared from these models that the pervasive deficit seen in schizophrenia could be simulated by disturbance of a single context module that carried out the maintenance of information and the inhibition of irrelevant information.

The psychopathology in schizophrenia is notoriously diverse. Several classifications have been proposed, either categorical or dimensional, and numerous studies have tried to identify distinct cognitive profiles associated with these subtypings. This correlational approach has yielded some interesting results, although typically associations between symptoms and neurocognitive deficits are only modest. An alternative approach to the relation between symptoms and cognition has been to test cognitive models of individual symptoms. Two paradigms that have received substantial empirical support are theory of mind and reality monitoring, in relation to delusions and hallucinations respectively. Impairments of theory of mind seem to be particularly pronounced in patients with negative symptoms or positive behavioural signs, while the predicted association with delusions has been found in some studies. A number of studies have documented robust deficits of reality monitoring in schizophrenia, with a tendency to attribute events to an external source in case of ambiguity. Other forms of monitoring may also be impaired. In general, if a relationship between impaired reality monitoring and symptoms is found, it is mainly with symptoms of alien control rather than hallucinations *per se*. However, the presence of an external attribution bias may be specifically involved in hallucinations.

While the symptom approach tries to identify those cognitive abnormalities that may underlie specific symptoms of psychosis, the focus of the vulnerability approach is on cognitive deficits that have a trait character, independent of the symptoms. According to the vulnerability/stress model, some individuals have a predisposition to schizophrenia that is largely determined by genetic and neurodevelopmental factors. Studies have attempted to find markers of this vulnerability in first-degree adult relatives of patients with schizophrenia and in individuals with a higher risk of developing schizophrenia than the general population. It appeared from this research that cognitive deficits are robust markers of schizophrenia vulnerability, although further research should investigate whether they are useful as alternative phenotypes in genetic studies.

Although the functional relevance of the cognitive deficits in schizophrenia has not received much attention, the available data suggest that deficits of verbal episodic memory, vigilance and executive functions influence functional outcome. Deficits in these, and presumably other domains of cognitive functioning restrict the possibilities for functional recovery, possibly even more so than do the symptoms of psychosis. This indicates that successful treatment goes beyond the reduction of the symptoms of psychosis. Cognitive deficits should be a target for intervention, whether pharmaceutically or psychologically. The therapeutic effects of conventional antipsychotic medication on cognition are generally considered to be minor. There is some evidence that atypical antipsychotic drugs improve cognitive functioning in schizophrenia, when compared to conventional antipsychotics. Yet, in none of the empirical studies does performance of patients with schizophrenia reach normal levels. This emphasizes the necessity to investigate the usefulness of cognition-enhancing drugs in schizophrenia. Few studies have been conducted that examine the effects of cognitive remediation on cognitive and daily functioning in schizophrenia in a controlled design. Therefore, there is as yet no evidence for or against cognitive remediation as a treatment in schizophrenia. However, given the impact of cognitive deficits on functioning in schizophrenia, the field invites exploration.

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XVII-8

Schizophrenia and Brain Imaging

S.-J. Blakemore

INTRODUCTION: SCHIZOPHRENIA HAS A BIOLOGICAL AETIOLOGY

It is widely accepted that schizophrenia has a biological aetiology. However, the shift towards this agreement is recent, and the aetiology of schizophrenia has been the subject of lengthy and intense debate. The debate has been split between those who propose a biological aetiology and those who postulate a psychodynamic origin to schizophrenia. In the latter camp, non-biological factors such as family interaction and stressful life events (Kuipers and Bebbington, 1988) have been proposed to play a causal role in the acquisition of schizophrenia. However, these theories have received little empirical support. In addition, in the past 50 years two main lines of evidence supporting a biological role in its aetiology have become apparent. First, there was the discovery of antipsychotic drugs in the 1950s (Delay *et al.*, 1952) and, second, the demonstration of a significant hereditary contribution to the disorder (Gottesman and Shields, 1982). These observations strongly suggest that schizophrenia has a biological basis.

However, current methodologies present challenges for research on the genetic and dopamine theories of schizophrenia. As a consequence, research has become increasingly focused on attempts to elucidate some structural or functional brain abnormality since it is widely held that schizophrenia is a disease of the brain (e.g. Ron and Harvey, 1990). The theory that some gross brain lesion characterizes schizophrenia seems unlikely. Instead, it is generally accepted that schizophrenia is characterized not by structural damage, but by functional abnormalities. This is supported by the relapsing and remitting course of the illness, fluctuations in symptoms and response to pharmacological interventions. Therefore the advent of functional neuroimaging has been important in the study of mental illness because it enables brain function and its abnormalities to be investigated. As stated by Weinberger *et al.* (1996): 'Functional neuroimaging in psychiatry has had its broadest application and greatest impact in the study of schizophrenia.'

A major problem is that images of brain function reflect the current mental state of the patient (i.e. symptoms) and these are very variable. Symptoms include disorders of inferential thinking (delusions), perception (hallucinations), goal-directed behaviour (avolition) and emotional expression. Current thinking generally distinguishes symptoms that comprise the presence of something that should be absent (positive symptoms) and the absence of something that should be present (negative symptoms; Crow, 1980). Factor-analytic studies of symptoms suggest that positive symptoms should be subdivided further into a psychotic group (comprising delusions and hallucinations) and a disorganized group (comprising disorganized speech, formal thought disorder, disorganized behaviour and inappropriate affect; Liddle, 1987). The diversity of symptoms in schizophrenia makes it unlikely that the pathophysiology can be accounted for by a single localized brain dysfunction. Instead, the strategy of attempting to localize specific symptoms to specific brain regions or connections between regions is encouraging, and this chapter will evaluate the results of such studies.

STRUCTURAL STUDIES

Computed Tomography (CT) Studies

The main finding from CT scan studies is that the lateral ventricles are enlarged in schizophrenic patients compared with normal controls. In the first study using this technique, Johnstone *et al.* (1976) found that 17 chronically hospitalized schizophrenic patients had significantly enlarged ventricles compared to normal controls. In a review of the CT literature Andreasen *et al.* (1990) noted that 36 out of 49 subsequent studies have replicated this finding to some extent. There have also been two meta-analyses of the CT scan data (Raz and Raz, 1990; Van Horn and McManus, 1992), which supported the finding of enlarged ventricles in schizophrenic patients. However, the extent of ventricular enlargement in schizophrenia is often small (e.g. Weinberger *et al.*, 1979) or even non-existent in some studies (Smith and Iacano, 1986). Positive findings might be due to the control group having smaller ventricles rather than the schizophrenic patients having larger ventricles. In support of this suggestion, Van Horn and McManus (1992) found in their meta-analysis that choice of control group was a contributing factor to the variability of control ventricle:brain ratio (VBR).

Correlation between CT findings and symptoms has been investigated. Lewis (1990) reviewed 41 CT scan studies that addressed the issue of heterogeneity of schizophrenic symptoms. Only one of 18 studies found any association between increased ventricular area and chronicity of illness. Five out of 18 found evidence of a relationship with negative symptoms. Poor treatment response was found to be associated with increased ventricular area in about half the studies. Chua and McKenna (1995) reviewed the CT literature and concluded that although the finding of increased ventricular area in schizophrenic patients is widespread and replicated, the difference between schizophrenic patients and normal controls is clearly small and depends significantly on the control group chosen.

Magnetic Resonance Imaging (MRI) Studies

MRI improves on CT owing to its better spatial resolution and ability to differentiate white and grey matter. There have been several replicated findings using structural MRI scans to investigate the structure of schizophrenics' brains. A number of studies have shown evidence for reductions in overall brain size, but most of these have used poor control groups and/or small numbers of schizophrenic patients (e.g. Andreasen *et al.*, 1986; Harvey *et al.*, 1993). In addition this finding has not been replicated by other

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studies (DeMyer *et al.*, 1988; Andreasen *et al.*, 1990). Many MRI studies provide evidence that schizophrenic patients have larger lateral ventricles than normal controls (e.g. Coffman and Nasrallah, 1986; Kelsoe *et al.*, 1988; Suddath *et al.*, 1990; Andreasen *et al.*, 1990; Woodruff *et al.*, 1997a; Sharma *et al.*, 1998; Lieberman *et al.*, 2001). However, the results are conflicting, and there are several negative findings (e.g. Smith *et al.*, 1987; Johnstone *et al.*, 1989; Rossi *et al.*, 1994).

Temporal lobe reductions in schizophrenia have been reported, and are especially prominent in the hippocampus (Fukuzako *et al.*, 1997; Sigmundsson *et al.*, 2001), parahippocampal gyrus and the amygdala (Yurgelun-Todd *et al.*, 1996a). Suddath *et al.* (1990), using the identical twins of schizophrenic patients as controls, found evidence for reductions of the left temporal lobe, including the hippocampus. Kwon *et al.* (1999) recently replicated the finding of a reduction in volume of the left temporal lobe. Cannon *et al.* (1998) suggested that frontal and temporal structural changes might reflect genetic (or shared environmental) effects. In a study using a large group of patients and well-matched controls (the patients' non-psychotic siblings and a group of normal controls), they found that volume reductions of the frontal and temporal lobes were present in patients with schizophrenia and in some of their siblings without schizophrenia. However, temporal lobe reductions are equivocal, with some negative findings in the literature (e.g. Young *et al.*, 1991). There have been many conflicting and negative results in studies attempting to locate clinical correlates with temporal abnormalities. Many studies have found no association between chronicity and temporal lobe size (e.g. Kelsoe *et al.*, 1988; Young *et al.*, 1991), although one study found an inverse relationship between these two factors (DeLisi *et al.*, 1991). Size of the superior temporal gyrus has been associated with hallucinations (Barta *et al.*, 1990) and formal thought disorder (Shenton *et al.*, 1992). Bilateral reduction in volume of the hippocampal formation has been associated with the severity of disorganization syndrome (Fukuzako *et al.*, 1997).

Some studies have found frontal lobe reductions in schizophrenic patients (Harvey *et al.*, 1993; Cannon *et al.*, 1998; Sigmundsson *et al.*, 2001). However, the results are inconsistent, especially in relation to the precise localization of the frontal abnormalities (Raine *et al.*, 1992). Buchanan *et al.* (1998) improved on this by subdividing the prefrontal cortex (PFC) into superior, middle, inferior and orbital regions and found that patients with schizophrenia exhibited selective grey matter volume reductions in the inferior PFC bilaterally. There was no difference between the schizophrenic and control groups in any other region of the frontal lobes.

There are some MRI data that provide support for the hypothesis of disconnection between brain areas in schizophrenia. Breier *et al.* (1992) found that schizophrenic patients, compared with matched healthy controls, had reductions in the right and left amygdala, the left hippocampus and prefrontal white matter. Moreover, the right prefrontal white matter volume in schizophrenic patients was significantly related to right amygdala/hippocampal volume, suggesting there might be abnormal connections between these areas. Buchsbaum *et al.* (1997) found evidence for a decreased left hemispheric volume in frontal and temporal regions in schizophrenic patients. This result was supported by Woodruff *et al.* (1997a), who found that interregional correlations were significantly reduced in schizophrenics between prefrontal and superior temporal gyrus volumes. The authors propose that these results support the existence of a relative 'fronto-temporal dissociation' in schizophrenia.

Reversal or reduction of normal structural cerebral asymmetries may be related to the pathogenesis of schizophrenia (Crow, 1995). Lack of normal asymmetry has been especially associated with early onset of schizophrenia (Fukuzako *et al.*, 1997; DeLisi *et al.*, 1997). Maher *et al.* (1998) found that low levels of hemispheric asymmetry in the frontal and temporal areas were associated with early onset of schizophrenia, the association with frontal volume being more marked than with temporal volume. These findings are consistent with the hypothesis that failure to develop asymmetry is an important component of the pathology underlying some forms of schizophrenia.

Voxel-Based Morphometry

Voxel-based morphometry is a new approach for looking at structural brain abnormalities using MRI. It is a data-led technique in which the brain images are normalized, then differences between groups anywhere in brain are identified (Wright *et al.*, 1995). Andreasen *et al.* (1994) created normal and schizophrenic average brains, compared the latter with the former, and found decreased thalamus size in schizophrenic patients. Wolkin *et al.* (1998) used linear intersubject averaging of structural MR images to create a single averaged brain for the schizophrenic group ($n = 25$) and for the control group ($n = 25$). The signal intensity differences between these average images were consistent with cortical thinning/sulcal widening and ventricular enlargement. Recently, this technique was used in a well-controlled study to compare regional grey matter in 42 schizophrenic patients and 52 controls (Wright *et al.*, 1999). The authors found a significant reduction in paralimbic (bilateral temporal pole and insula), limbic (right amygdala) and cortical (left dorsolateral prefrontal cortex) regions in patients compared with controls.

FUNCTIONAL IMAGING

Positron-Emission Tomography (PET) Resting Studies

There are many PET metabolism studies in the literature, and this chapter is not exhaustive. The main finding from data from PET metabolism studies is that there is less metabolism in the frontal lobes of schizophrenic patients compared to normal controls. This has become known as 'hypofrontality'. The first study using isotope imaging was by Ingvar and Franzen (1974), who compared 15 normal controls with 11 patients with dementia, and two groups of schizophrenics (one group comprised nine chronic, elderly patients; the other comprised 11 younger patients). Whereas the demented patients showed a lower level of overall metabolism, both schizophrenic groups showed some evidence for reduced blood flow in anterior, relative to posterior, regions (hypofrontality; see Figure XVII-8.1).

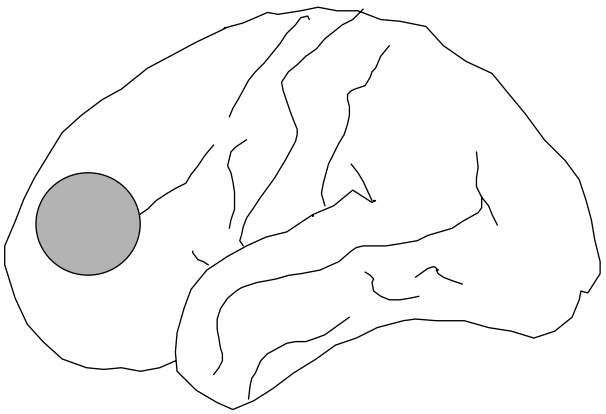

Figure XVII-8.1 Approximate average localization of hypofrontality found by Liddle *et al.* (1992), Weinberger *et al.* (1986), Daniel *et al.* (1991), Volz *et al.* (1997), Ragland *et al.* (1998), Spence *et al.* (1998), Yurgelun-Todd *et al.* (1996b) and Fletcher *et al.* (1998)

However, disagreement about the definition of hypofrontality has caused inconsistencies in the results. Studies vary on the frontal areas in which activity was measured. Some have included all anterior regions; others have looked at frontal or prefrontal subdivisions only. The earlier studies tended to use the frontal:occipital ratio as a measure of hypofrontality, whereas recently most studies have used absolute frontal flow values with or without correction for mean total brain blood flow rates. The majority of studies, using any of these definitions, have found little evidence for statistically significant hypofrontality, and in several studies hypofrontality was due to an elevated flow in posterior regions (Mathew *et al.*, 1988; Siegel *et al.*, 1993). In other studies, the differences between control and schizophrenic frontal cortex metabolism are small. It is claimed that hypofrontality is not due to the drug status of the patients at scanning (Waddington, 1990). However, recently antipsychotic medication has been found to affect brain metabolism (Miller *et al.*, 1997). Several studies have looked at clinical correlates associated with hypofrontality, but the results are inconsistent. Among those showing a positive relationship with hypofrontality are chronicity (Mathew *et al.*, 1988), negative symptoms (e.g. Ebmeier *et al.*, 1993) and neuropsychological task impairment (e.g. Paulman *et al.*, 1990).

Researchers have attempted to correlate regional cerebral blood flow (rCBF) levels with symptom severity scores for Liddle's three clinical subdivisions in 30 schizophrenic patients (Liddle *et al.*, 1992). They demonstrated that the psychomotor poverty syndrome, which has been shown to involve a diminished ability to generate words, was associated with decreased perfusion of the dorsolateral prefrontal cortex (DLPFC) at a locus that is activated in normal subjects during the internal generation of words. The disorganization syndrome, which has been shown to involve impaired suppression of inappropriate responses (e.g. in the Stroop test), was associated with increased perfusion of the right anterior cingulate gyrus at a location activated in normal subjects performing the Stroop test. The reality distortion syndrome, which might arise from disordered internal monitoring of activity, was associated with increased perfusion in the medial temporal lobe at a locus activated in normal subjects during the internal monitoring of eye movements. Therefore the abnormalities of brain metabolism underlying each of the three syndromes might be widely distributed over the brain.

Using data from the same patients, Friston *et al.* (1992) examined correlations between rCBF and a measure of psychopathology receiving equal contributions from each of Liddle's three subsystems. The degree of psychopathology correlated highly with rCBF in the left medial temporal region, and mesencephalic, thalamic and left striatal structures. The highest correlation was in the left parahippocampal region, and the authors proposed that this might be a central deficit in schizophrenia. A canonical analysis of the same data highlighted the left parahippocampal region and left striatum (globus pallidus), in which rCBF increased with increasing severity of psychopathology. Friston and colleagues suggested that disinhibition of left medial temporal lobe activity mediated by fronto-limbic connections might explain these findings.

Neuroreceptor Imaging of Antipsychotics using PET

Dopamine Receptors

Many studies have shown evidence that the density of dopamine (DA; in particular D2) receptors is increased in schizophrenic brains (Wong *et al.*, 1986; Breier *et al.*, 1997). Early PET and single photon emission computed tomography (SPECT) receptor imaging studies focused on striatal D2 receptors. However, Okubo *et al.* (1997) used PET to examine the distribution of D1 and D2 receptors in the brains of drug-naive or drug-free schizophrenic patients. Although no differences were observed in the striatum relative to control subjects, binding of the radioligand to D1 receptors was reduced in the prefrontal cortex of schizophrenics. Other studies using PET have produced contradictory results or only very weak evidence of an abnormality in DA receptor number in schizophrenia (e.g. Crawley *et al.*, 1986; Farde *et al.*, 1987; Pilowsky *et al.*, 1994).

In a meta-analysis of 15 brain-imaging studies comparing indices of dopamine function in drug-naive or drug-free patients with schizophrenia, Laruelle (1998) found that, compared to healthy controls, patients with schizophrenia present a significant but mild elevation of D2 receptor density parameters. However, other meta-analyses have shown that a significant proportion (up to a third) of schizophrenic patients cannot be discriminated from normal healthy controls in terms of D2 dopamine density (Zakzanis and Hansen, 1998; Soares and Innis, 1999). The discrepant results might in part be due to the diversity of PET methodology used in these studies.

Receptor Imaging and Antipsychotic Drug Action

PET and SPECT receptor imaging can be used to explore receptor targets for antipsychotic drug action in living patients. It is widely accepted that the 'typical' antipsychotics (such as haloperidol and perphenazine) bind mainly to the D2 receptor (Kapur, 1998). There is also broad agreement that unwanted extrapyramidal (parkinsonian) side effects of antipsychotic drugs result from high striatal D2/D3 receptor blockade by these drugs. PET receptor-binding studies have found that 60–80% D2 occupancy provides optimal antipsychotic response with little extrapyramidal side effects (Kapur, 1998).

Recent attention has been focused on the involvement of serotonin (5-HT) in the pathophysiology of schizophrenia and its role in mediating antipsychotic drug effects, especially for 'atypical' antipsychotics, such as clozapine, olanzapine, risperidone and Quetiapine. At clinically relevant doses, atypical antipsychotics tend to have a higher affinity for 5-HT receptor subtypes than for D2 receptors and are associated with few extrapyramidal side effects (Lieberman *et al.*, 1998; Silvestri *et al.*, 2001). Atypical antipsychotics differ widely in their D2 occupancy. The D2 occupancy of risperidone had been found to be within the typical range (over 60%), while that of clozapine is significantly lower (under 60%). Some atypical antipsychotics such as Quetiapine have very low (Gefvert *et al.*, 2001) and only transient D2 occupancy, which nevertheless seems to be sufficient for mediating an antipsychotic effect (Raedler *et al.*, 1999; Kapur *et al.*, 2000a).

The D2 occupancy seems to be the important mediator of response and side effects in antipsychotic treatment (Kapur *et al.*, 2000b). Freedom from motor side effects results from low D2 occupancy, not from high 5-HT2 occupancy (Kapur and Seeman, 2001). If the D2 occupancy is too high (it exceeds 80%), antipsychotics lose some of their 'atypical' properties and produce a higher incidence of extrapyramidal side effects (Kapur *et al.*, 1999; Kapur and Seeman, 2001) and negative subjective experiences such as depression (de Haan *et al.*, 2000).

Cognitive Activation Studies

Functional neuroimaging is most frequently used to evaluate the regional cerebral responses to a particular cognitive or sensorimotor process. Typically, subjects are scanned while performing an activation task, which engages the cognitive/sensorimotor process of interest, and a baseline task, which engages all components of the activation task except the cognitive/sensorimotor process of interest. Regions that show significantly more activity in the experimental state than in the baseline state are considered to be involved in the cognitive/sensorimotor process of interest. These task-specific

activity patterns can then be compared between patients and control subjects to determine how the condition affects brain function. Several types of task that have been used to evaluate brain function in schizophrenic patients are discussed in this chapter.

Task-Based Studies of Executive Function

In behavioural studies the most striking impairments are seen when schizophrenic patients perform the various complex executive tasks associated with the frontal lobes. Brain imaging makes it possible to identify the abnormal pattern of brain activity associated with this abnormal performance.

Wisconsin Card Sorting Task (WCST)

Schizophrenia is largely characterized by impairments in planning and execution and therefore tasks that involve this kind of planning and modification of behaviour have been exploited in the scanner. Several researchers have investigated brain activity in schizophrenic patients while they perform a version of the WCST. This task is known to activate the DLPFC in normal controls, and is particularly sensitive to damage to DLPFC (Berman *et al.*, 1995; Nagahama *et al.*, 1996). In the typical computerized version of the WCST subjects view a computer screen that displays a number of stimuli. These stimuli differ along three dimensions: colour, shape and number. On each of a series of trials the subjects have to match a target stimulus with one of the four standard stimuli. However, the match is not exact, but has to made in terms of either colour, shape or form. Subjects are not informed of how to make the match, but are informed after each choice whether they are right or wrong. They have to determine from trial and error which dimension is correct. After subjects have made a series of correct responses, the rule is changed and subjects must determine the new rule for matching.

Weinberger *et al.* (1986) measured rCBF using Xe^{133} inhalation SPECT in 20 medication-free patients with chronic schizophrenia and 25 normal controls during the WCST and a number-matching control task. During the WCST but not during the control task normal subjects showed increased DLPFC rCBF, whereas patients did not (patient performance was worse than that of the controls). Furthermore, in patients, DLPFC rCBF correlated positively with WCST performance. The authors suggest that this result shows that the better DLPFC was able to function, the better patients could perform the task. However, this conclusion is based on an ill-founded assumption. It is impossible to determine whether task-related underactivation *causes* or *reflects* poor task performance. This is a crucial issue in cognitive activation studies, and will be discussed in more detail throughout the chapter.

Daniel *et al.* (1991) used SPECT to study the effect of amphetamine (a DA agonist) and a placebo on rCBF in 10 chronic schizophrenic patients while they performed a version of the WCST and a matched control task. On placebo no significant activation was seen during the WCST compared with the control task. In contrast, significant activation of the left DLPFC occurred on the amphetamine trials. Daniel and colleagues point out that patients' performance improved with amphetamine relative to placebo and that with amphetamine, but not with placebo, a significant correlation was found between activation of DLPFC and performance on the WCST task. Again this is a finding that is difficult to interpret: did amphetamine facilitate task performance, which then caused an increased rCBF in the PFC? Or did amphetamine cause PFC activity to increase, which facilitated performance?

Volz *et al.* (1997) used functional magnetic resonance imaging (fMRI) to investigate activity during the WCST in 13 chronic schizophrenics on stable neuroleptic medication. They also showed evidence for lack of activation in the right PFC and a trend towards increased left temporal activity during the WCST compared to normal controls. However, again the task performance was different between the two groups and therefore the results remain ambiguous. In addition, this study was limited because a one-slice imaging technique was used, so no information about the activation pattern in adjacent brain regions was obtained.

Better WCST performance correlated with rCBF increase in prefrontal regions for controls and in the parahippocampal gyrus for patients in two recent studies (Ragland *et al.*, 1998; Riehemann *et al.*, 2001). The results suggest that schizophrenia may involve a breakdown in the integration of a fronto-temporal network that is responsive to executive and declarative memory demands in healthy individuals.

Tower of London Task

The Tower of London task involves high-level strategic planning among a number of other processes (Shallice, 1982). In this task subjects have to rearrange a set of three balls presented on a computer screen, so that their positions match a goal arrangement also presented on the screen. The complexity of the task, in terms of the number of moves necessary to complete the task, can be varied.

Andreasen *et al.* (1992) used SPECT during the Tower of London task in three different groups: 13 neuroleptic-naive schizophrenic patients; 23 non-naive schizophrenic patients who had been chronically ill but were medication free for at least 3 weeks; and 15 healthy normal volunteers. The Tower of London task activated the left mesial frontal cortex (probably including parts of the cingulate gyrus) in normal controls, but not in either patient group. Both patient groups also lacked activation of the right parietal cortex, representing the circuitry specifically activated by the Tower of London in normal controls. Importantly, decreased activation occurred only in the patients with high scores for negative symptoms. The authors therefore suggested that hypofrontality is related to negative symptoms and is not a long-term effect of neuroleptic treatment or of chronicity of illness. Again, schizophrenic patients performed poorly on the tasks involved, so whether less activation of the PFC was due to poorer performance or vice versa cannot be resolved.

The Component Processes Underlying Executive Function

One problem with studies that employ complex executive tasks is that these tasks involve many processes. For example, the WCST involves choosing a strategy, remembering the previous responses in order to learn by trial and error, attending to one dimension rather than another, and so on. In the absence of a series of carefully constructed comparison tasks it is not possible to relate the various brain regions activated with each of the component processes. In the following section studies that employed simpler tasks with far fewer component processes are reviewed.

Motor Tasks

Even tasks that require no more than the production of a simple sequence of movements can be associated with abnormal patterns of brain activity in schizophrenia. Mattay *et al.* (1997) studied seven patients with schizophrenia and seven normal subjects while they performed a finger movement task of increasing complexity. Patients showed greater ipsilateral activation in the primary sensorimotor and lateral premotor regions and had a significantly lower laterality quotient than normal subjects. These functional abnormalities increased with the complexity of the task. The authors proposed that these results demonstrate a functional disturbance in the cortical motor circuitry of schizophrenic patients. Schröder

et al. (1999) asked 12 patients and 12 healthy controls to produce sequences of movements at three different speeds during fMRI. Both groups showed increasing activity with increasing speed in sensorimotor cortex and supplementary motor area (SMA). However, the patients showed less overall activation than the controls. The differences were most marked in a subgroup of patients who were drug free at the time of testing. Both these studies raise the possibility of a fundamental but subtle problem of motor control associated with schizophrenia.

Willed Action

Willed action involves a 'higher' stage in the control of action. There is a fundamental distinction between actions elicited by external stimuli and actions elicited by internal goals (acts of will). Routine actions are specified by an external stimulus. In contrast, in willed (or self-generated) acts, the response is open-ended and involves making a deliberate choice. Willed actions are a fundamental component of executive tasks. In normal subjects, willed acts in two response modalities (speaking a word, or lifting a finger), relative to routine actions, were associated with increased blood flow in the DLPFC (Brodmann area 46; Frith *et al.*, 1991). Schizophrenic patients typically show abnormalities of willed behaviour. In chronic patients, intentions of will are no longer properly formed and so actions are rarely elicited via this route. This gives rise to behavioural negative signs (e.g. poverty of speech and action) (Frith, 1992).

In a recent PET study, subjects had to make voluntary joystick movements in the experimental condition, stereotyped (routine) movements in the baseline condition, and do nothing in a control rest condition (Spence *et al.*, 1998). The authors analysed data from 13 schizophrenic patients, comparing two occasions when symptoms were severe and when they had subsided, and included data from a normal control group to clarify the role of the left DLPFC in volition. The DLPFC was activated by normal controls for the free choice task only. However, it was not activated by schizophrenics with symptoms, but became activated when their symptoms decreased. The authors noted that the DLPFC was also activated during the stereotyped joystick movement task in schizophrenic patients in remission, in contrast to a control group. Spence and colleagues concluded that, since hypofrontality was evident in schizophrenics who can perform the experimental task, the DLPFC is not necessary for that task. In addition, hypofrontality seems to depend on current symptoms. They suggested the reason for previous equivocal hypofrontality results is that schizophrenics with a varying amount and combination of symptoms are being compared with normal controls. However, these results provoke another question. What is the functional significance of DLPFC activation in normal controls and patients without symptoms if schizophrenic patients with symptoms can perform the task without recruiting the DLPFC?

Verbal Fluency

Verbal fluency tasks involve subjects having to generate words to a given cue. For example, subjects might have to produce a word beginning with a certain letter, a different letter being presented every 5 seconds. This can be seen as a task that involves willed action since subjects have to choose for themselves precisely which word to say. Verbal fluency tasks engage a distributed brain system similar to that engaged by motor response selection tasks associated with willed action (Frith *et al.*, 1991).

Schizophrenic patients showed reduced left PFC activation and increased left temporal activation relative to control subjects during a verbal fluency task in an fMRI study (Yurgelun-Todd *et al.*, 1996b). However, the lack of frontal activation by cognitive tasks in schizophrenic patients has not consistently been located in the PFC. Dolan *et al.* (1995) and Fletcher *et al.* (1996) used a factorial design in PET to test the effect of apomorphine, a non-selective dopamine agonist, which when given in very low doses as in this experiment acts primarily on auto-receptors, thus decreasing the release of endogenous dopamine. Brain systems engaged by a paced verbal fluency task in unmedicated schizophrenic patients and normal controls were studied. Activation of the DLPFC was normal, but they found a failure of task-related activation in anterior cingulate cortex and deactivation of the left superior temporal gyrus in the schizophrenic subjects (see Figure XVII-8.2). Fletcher and colleagues therefore suggested that schizophrenia is associated with both segregated (anterior cingulate) and integrative (fronto-temporal) functional abnormalities. Cingulate activation was restored by low-dose apomorphine in schizophrenics. Additionally, the abnormal fronto-temporal pattern of activation in schizophrenic subjects was normalized by this neuropharmacological intervention. Overall, in schizophrenic subjects the effect of apomorphine was to modify the pattern of brain activity, making it more similar to that seen in control subjects. The interpretation of the apomorphine-induced reversal of the deactivation in the left temporal lobe in schizophrenic subjects is unclear. It might reflect a direct influence of apomorphine on the temporal lobe; alternatively the reversal could be due to a 'downstream' effect of the change in anterior cingulate function. The authors interpret the absence of the normal reciprocal interaction between the frontal and the superior temporal cortex in schizophrenia (the failure of task-related deactivation of the superior temporal gyrus in the schizophrenic group) as suggesting the presence of impaired functional integration. This is an important concept, especially given the relatively large amount of evidence showing a lack of temporal deactivation in the presence of a lack of frontal activation in schizophrenia.

The finding of normal prefrontal activation found in this study is in agreement with a study in which task performance was optimized by pacing the task (Frith *et al.*, 1995). Using PET, these researchers investigated rCBF of 18 chronic schizophrenic patients and six normal controls matched for age, sex and premorbid IQ, while they performed (a) paced verbal fluency, (b) paced word categorization and (c) paced word repetition. The schizophrenic patients were split into three groups according to their verbal fluency task performance level. All patient groups showed the *same* pattern of left PFC activation as control subjects, independent of their level of performance. However, in the left superior temporal cortex, all patient groups failed to show a normal deactivation when verbal fluency was compared with word repetition. Again this result was interpreted to reflect abnormal functional connectivity between frontal and temporal cortex.

Friston and Frith (1995) performed an additional analysis of their data from the same three groups of schizophrenic patients according to the level of task performance: poverty (no words), odd (wrong words) and unimpaired. They used special analytic techniques to assess cortico-cortical interactions. Normal controls showed negative fronto-temporal interactions whereas all the schizophrenic patients showed positive interactions, mostly between the left PFC and infero-temporal cortex. Friston and Frith suggested that this might represent a failure of the PFC to inhibit the temporal lobes. They postulated that the temporal lobes may be required to recognize the consequences of actions initiated by the frontal lobes in order to integrate action and perception.

Verbal Self-Monitoring

Patients with schizophrenia with auditory hallucinations and delusions show impairments on tasks that require monitoring self-generated actions. This is true for motor actions (Frith and Done, 1989; Blakemore *et al.*, 2000; Frith *et al.*, 2000) and verbal self-monitoring tasks (Johns *et al.*, 2001). In particular, when patients

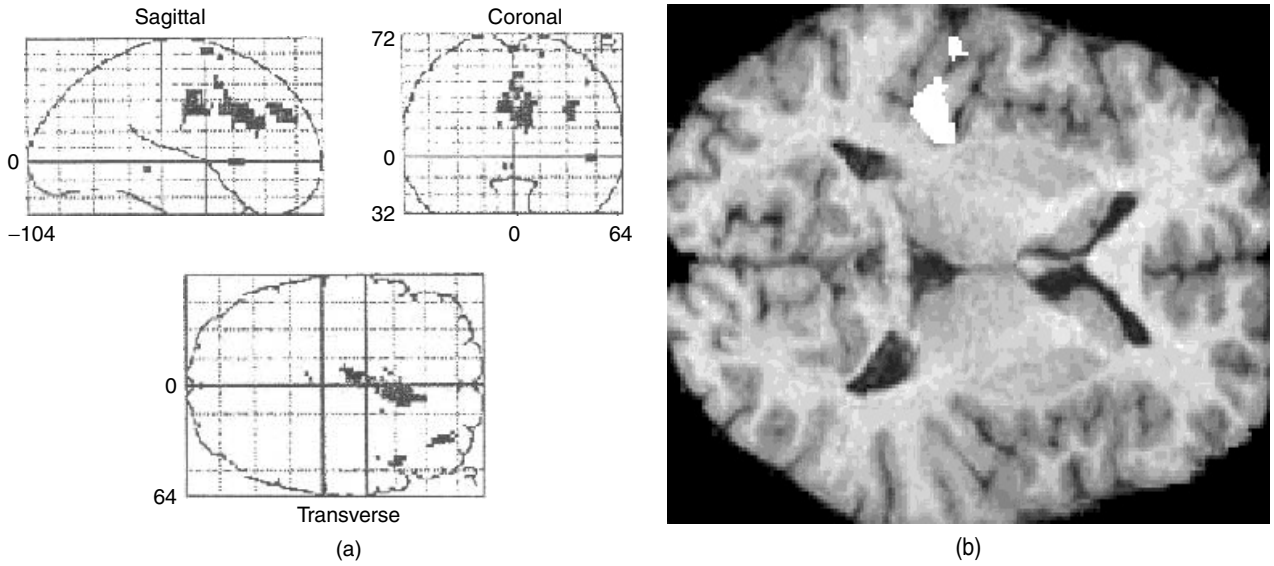

Figure XVII-8.2 (a) Statistical parametric maps (SPMs) showing brain regions where there was a significant ($p < 0.005$) difference in drug (apomorphine)–task (verbal fluency) interaction between the schizophrenic group and the control group. The area in which there was an augmenting effect of the drug on the task-related activity occurring in schizophrenics compared to controls was the anterior cingulate gyrus. In other words, the impaired cingulate activation seen during the verbal fluency task in schizophrenic patients was significantly reversed by apomorphine. (b) Transverse section showing the verbal fluency task-related deactivation of the superior temporal gyrus that was absent in schizophrenic relative to control subjects. The authors interpret the failure of task-related deactivation of the superior temporal gyrus in the schizophrenic group to suggest the presence of impaired functional temporo-frontal integration. Reprinted from Fletcher *et al.* (1996), with permission from the *Journal of Neuroscience*

speak aloud but hear a distorted version of their own voice, they report hearing someone else rather than themselves. Researchers have recently developed an event-related fMRI acquisition sequence to scan patients during verbal self-monitoring paradigms. Fu *et al.* (2001) compared brain activation patterns of healthy volunteers, schizophrenic patients with acute psychotic symptoms and schizophrenic patients in remission while they read adjectives aloud and heard their voice, which was either undistorted or distorted (in several ways). Words were read and heard during silent portions of the acquisition sequence to control for the problems of scanner noise. The hippocampus, cingulate and cerebellum were particularly activated when healthy controls heard their own distorted voice. Acutely psychotic patients tended to misidentify their own distorted voice as 'other' and did not engage these regions, while the pattern of activation in patients in remission was intermediate between the other two groups. This supports the notion that abnormal brain activity in schizophrenics may be associated with current symptomatology.

Memory Tasks

Memory impairments are especially enduring symptoms in schizophrenia (Green, 1996), with memory storage particularly affected (Feinstein *et al.*, 1998). The DLPFC and the hippocampal formation have been the subject of investigation in schizophrenia, as these are involved in various aspects of memory (Goldman-Rakic and Selemon, 1997; Arnold, 1997). The DLPFC is activated by semantic processing during encoding and retrieval, while hippocampal activation is associated with the detection of novelty and the creation of associations during encoding in normal individuals (Schacter *et al.*, 1996; Dolan and Fletcher, 1997).

Several functional neuroimaging studies have failed to find evidence for abnormal activation of temporal or frontal cortex in schizophrenia during memory tasks (Busatto *et al.*, 1994, using a verbal memory task with SPECT; Ragland *et al.*, 1998, using a Paired Associate Recognition Test with PET). Other studies have shown rCBF changes that overlapped in the schizophrenic and control groups, with a trend towards patients showing smaller activations than controls in frontal and superior temporal cortical regions (e.g. Ganguli *et al.*, 1997, using a verbal free-recall supraspan memory task). These differences may be due to the different type of memory tasks used by each group. Other groups have found evidence for hypofrontality during memory tasks. Carter *et al.* (1998) used PET to evaluate rCBF associated with the 'N-back' working memory (WM) task, during which subjects are presented with a sequential series of items and have to press a button when a presented item has already been presented a certain number (N) of items earlier. This task activates the PFC as a function of WM load in normal subjects. Under low WM load conditions, the accuracy of both groups in the N-back task was equal, but when the memory load increased the patients' performance deteriorated more than did that of control subjects. The rCBF response to increased WM load was significantly reduced in the patients' right DLPFC. Callicott *et al.* (1998) investigated blood oxygen level-dependent (BOLD) signal changes in 10 patients with schizophrenia and 10 controls performing a novel N-back WM task, using fMRI. After removing confounds and matching subjects for signal variance (voxel stability), decreased DLPFC activity and a tendency for overactivation of parietal cortex were seen. However, these findings are difficult to interpret in the context of abnormal task performance in patients. There may be nothing inherently abnormal about the physiology of the frontal cortex in schizophrenia, but patients may be failing to select frontally mediated cognitive strategies because of abnormal connectivity between otherwise normal regions.

Wiser *et al.* (1998) measured rCBF during a long-term recognition memory task for words in schizophrenic patients and in healthy subjects using PET. The task was designed so that performance scores were similar in the patient and control subjects. This memory retrieval task did not activate PFC, precuneus and cerebellum in patients as much as it did in the control group. This finding

suggests that there is a dysfunctional cortico-cerebellar circuit in schizophrenia.

Hypofrontality has not always been found in studies using modified tasks to optimize the performance of schizophrenic subjects. Hypofrontality was not found in a study by Heckers and colleagues (1998), in which they used PET to evaluate 13 schizophrenic patients and a group of normal control subjects during memory retrieval tasks. In this study activation of the PFC correlated with effort of retrieval, and hippocampal and parahippocampal activation occurred during successful retrieval in normal control subjects, a finding consistent with previous studies of memory in normals (Schacter *et al.*, 1996; Dolan and Fletcher, 1997). The schizophrenic patients recruited the PFC during the effort of retrieval but did not recruit the hippocampus during conscious recollection. In addition to the task-specific hippocampal underactivation, the authors observed a generally higher overall level of non-specific hippocampal activity, supporting previous metabolic studies (e.g. Liddle *et al.*, 1992). The authors suggested that high baseline hippocampal activity together with an absence of task-specific activation demonstrates abnormal cortico-hippocampal functional integration in schizophrenia. The schizophrenic patients showed a more widespread activation of prefrontal areas and parietal cortex during recollection than controls, and the authors propose that this overactivation represents an 'effort to compensate for the failed recruitment of the hippocampus'. This interpretation again moves away from the simple notion of dysfunction in isolated brain regions explaining the cognitive deficits in schizophrenia, and towards the idea that neural abnormality in schizophrenia reflects a disruption of integration between brain areas.

Fletcher *et al.* (1998) used PET to compare rCBF in memory-impaired and non-impaired schizophrenic patients with normal controls during a parametrically graded memory task. They found that DLPFC activity correlates with memory task difficulty and performance in the control group. In contrast, for both schizophrenic groups DLPFC activity levels plateaued as task difficulty increased, despite a significant difference in performance between the two schizophrenic groups. The authors therefore suggested that hypofrontality in schizophrenics correlates with *task difficulty* rather than task performance, since the memory-impaired schizophrenic group performed worse than the non-impaired, even though both groups showed no increase in PFC activity as task difficulty increased.

Unlike the control group, there was no inferior temporal/parietal deactivation in either schizophrenic group. The authors suggest that the lack of deactivation of these areas might represent a temporo-frontal disconnection in schizophrenics. Indeed they suggested that because temporal/parietal activations were not correlated with performance, they therefore might represent a core pathology of schizophrenia. This study improves on previous cognitive activation studies in which the confound of non-matched task performance occurs.

Further evidence for abnormal integration between brain areas in schizophrenia comes from a study that specifically investigated the functional integration (Fletcher *et al.*, 1999). Functional integration considers complex cognitive processes as emergent properties of interconnected brain area, building on the idea that simple cognitive processes can be localized in discrete anatomical modules (referred to as 'functional segregation'). Brain areas A and B may be functionally connected if it can be shown that an increase (or decrease) of activity in area A is associated with an increase (or decrease) in area B, which can be shown empirically by analysis of covariance. In this case, activity in A might cause activity in B, or activations in A and B might be caused by changes in another area (C), which projects to A and B. Alternatively, areas A and B may be effectively connected if their relationship can be shown to be causal. This requires a more complex approach in which the anatomical components of a cognitive system are defined. Connections between these regions are designated on the basis of empirical neuroanatomy and the connections are allocated weights or path strengths by an iterative least-squares approach in such a way that the resultant functional model of interregional influences best accounts for the observed variance–covariance structure generated by the functional neuroimaging observations (Friston *et al.*, 1993).

A simplified version of effective connectivity (Friston *et al.*, 1997) was employed by Fletcher *et al.* (1999) to evaluate effective connectivity between regions in the data from their PET study of a graded memory task in schizophrenia. They demonstrated that in control subjects, but not in the schizophrenic patients, the product of PFC and anterior cingulate gyrus (ACG) activity predicted a bilateral temporal and medial PFC deactivation. The authors interpreted these results as showing that in schizophrenia there is an abnormality in the way in which left PFC influences the left superior temporal cortex, and this abnormality is due to a failure of the ACG to modulate the prefronto-temporal relationship (see Figure XVII-8.3).

Conclusion

There is a body of evidence suggesting that schizophrenic patients show abnormal interactions and influences among brain regions (or functional integration) during cognitive tasks. There is currently little *direct* evidence in favour of this hypothesis, and several regions have been found to function abnormally, with no unequivocal evidence for any particular region being involved. However, the majority of positive findings suggest that a disruption of fronto-temporal integration is a core feature of schizophrenia. However, findings have been confounded by several factors, especially use of poor control tasks such as rest, and non-matched task performance in the schizophrenic and control groups. Future cognitive activation studies using improved methodologies should resolve issues such as whether abnormal frontal function causes or reflects poor task performance in schizophrenia. A question of clinical importance is whether different patterns of cortical interaction correlate with or predict schizophrenic symptoms or outcome.

Imaging Symptoms

Functional neuroimaging is also useful for evaluating neural activity in patients experiencing psychotic symptoms.

Hallucinations

Hallucinations, perceptions in the absence of external stimuli, are prominent among the core positive symptoms of schizophrenia. Although auditory hallucinations are more common than any other type, hallucinations in other sensory modalities occur in a proportion of patients. Hallucinations in all modalities tend to be associated with activity in the neural substrate associated with that particular sensory modality. However, there is evidence that activation of the sensory cortex particular to the false perception is not in itself sufficient for the perception. Instead, research suggests that the interaction of a distributed cortico-subcortical neural network might provide a biological basis for schizophrenic hallucinations.

Auditory Hallucinations

The most common type of hallucination in schizophrenia occurs in the auditory domain, and normally consists of spoken speech or voices (Hoffman, 1986). Functional neuroimaging studies of auditory hallucinations suggest that they involve neural systems dedicated to auditory speech perception as well as a distributed

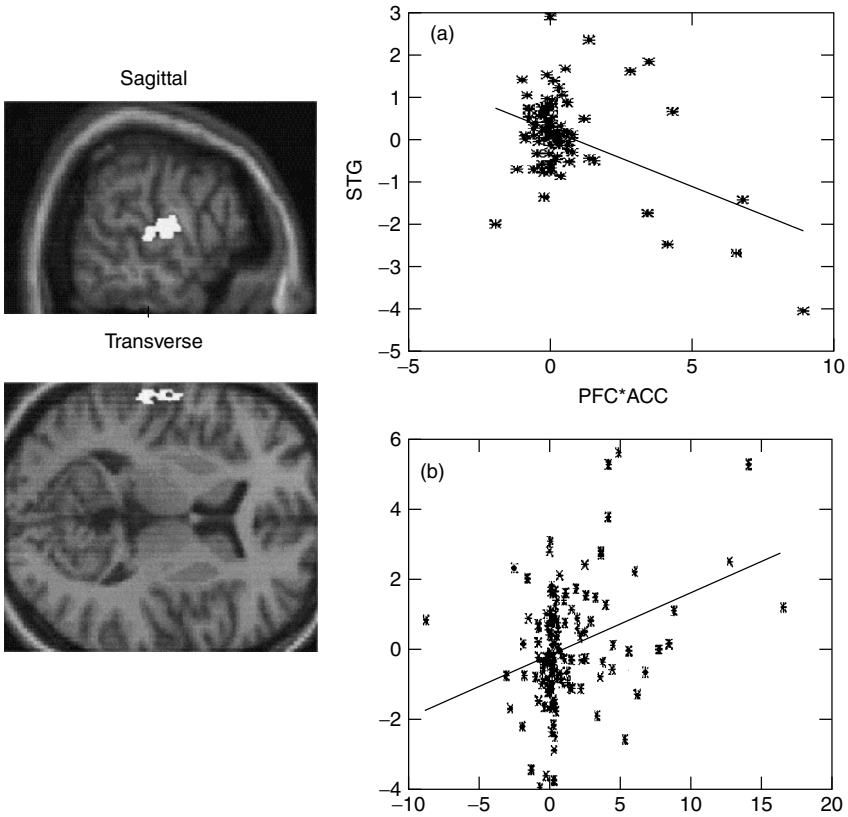

Figure XVII-8.3 Left panel: Region of left superior temporal cortex showing a significant difference between control and schizophrenic subjects ($p < 0.05$). An SPM rendered onto sagittal and transverse section of a stereotactically normalized structural MRI is shown. Right panel: Graphic representation of the relationship between activity in left superior temporal cortex (STG; *y*-axis) and the combination of cingulo-prefrontal activity (PFC × ACG; *x*-axis in (a) controls and (b) schizophrenic subjects. The linear best fit is shown for both groups. The regression coefficient of each of the two groups is considered to provide a measure of *contribution* of PFC × ACC to superior temporal cortical activity. Thus, in the controls an increase in PFC × ACC produces an inhibition of superior temporal activity. In the schizophrenic subjects the line slopes upward, indicating the opposite effect. Reprinted from Fletcher *et al.* (1999), with permission from Neuroimage

network of other cortical and subcortical areas. There are two distinct approaches to the study of the physiological basis of auditory hallucinations. The first, called the state approach, asks what changes in brain activity can be observed at the time hallucinations are occurring. The second, called the trait approach, asks whether there is a permanent abnormality of brain function present in patients who are prone to experience auditory hallucinations when they are ill. This abnormality will be observable even in the absence of current symptoms.

State Studies

Silbersweig *et al.* (1995) used PET to study brain activity associated with the occurrence of hallucinations in six schizophrenic patients. Five patients with classic auditory verbal hallucinations demonstrated activation in subcortical (thalamic and striatal) nuclei, limbic structures (especially hippocampus) and paralimbic regions (parahippocampal and cingulate gyri and orbito-frontal cortex). Temporo-parietal auditory–linguistic association cortex activation was present in each subject. One drug-naive patient had visual as well as auditory verbal hallucinations, and showed activations in visual and auditory/linguistic association cortices. The authors propose that activity in deep brain structures seen in all subjects may generate or modulate hallucinations, and the particular sensory cortical regions activated in individual patients may affect their specific perceptual content. Importantly this study pointed to the possibility that hallucinations coincide with activation of the sensory and association cortex specific to the modality of the experience, a notion that has received support from several further studies.

David *et al.* (1996) used fMRI to scan a schizophrenic patient while he was experiencing auditory hallucinations and again when hallucination free. The subject was scanned during presentation of exogenous auditory and visual stimuli, and while he was on and off antipsychotic drugs. The BOLD signal in the temporal cortex to exogenous auditory stimulation (speech) was significantly reduced when the patient was experiencing hallucinating voices, regardless of medication. Visual cortical activation to flashing lights remained the same over all four scans, whether the subject was experiencing auditory hallucinations or not.

A similar result was obtained by Woodruff *et al.* (1997b), who used fMRI to study seven schizophrenic patients while they were experiencing severe auditory verbal hallucinations and again after their hallucinations had subsided. On the former occasion, these patients had reduced responses in temporal cortex, especially the right middle temporal gyrus, to external speech, compared to when their hallucinations were mild. The authors thus proposed that auditory hallucinations are associated with reduced responsivity in temporal cortical regions that overlap with those that normally process external speech, possibly due to competition for common neurophysiological resources.

Recently, Dierks *et al.* (1999) used event-related fMRI to investigate three paranoid schizophrenics who were able to indicate the onset and offset of their hallucinations as in the study by Silbersweig *et al.* Using this design they found that primary auditory cortex, including Heschl's gyrus, was associated with the presence of auditory hallucinations. Secondary auditory cortex, temporal lobe and frontal operculum (Broca's area) were also activated during auditory hallucinations, supporting the notion that auditory hallucinations are related to inner speech. Finally hallucinations were also associated with increased activity in the hippocampus and amygdala. The authors suggested that these subcortical activations could be due to retrieval from memory of the hallucinated material and emotional reaction to the voices, respectively. A similarly elegant design was employed in a recent fMRI study (Shergill *et al.*, 2000). They found that frontal and temporal speech areas and several other cortical and subcortical regions were activated during auditory hallucinations.

Trait Studies

The finding that auditory hallucinations are associated with activation of auditory and language association areas is consistent with the proposal that auditory verbal hallucinations arise from a disorder in the experience of inner speech (Frith, 1992). This was investigated by McGuire *et al.* (1996). They used PET to evaluate the neural correlates of tasks that engaged inner speech and auditory verbal imagery in schizophrenic patients with a strong predisposition to auditory verbal hallucinations (hallucinators), schizophrenic patients with no history of hallucinations (non-hallucinators) and normal controls. There were no differences between hallucinators and controls in rCBF during thinking in sentences. However, when imagining sentences spoken in another person's voice, which entails both the generation and monitoring of inner speech, hallucinators showed reduced activation of the left middle temporal gyrus and the rostral supplementary motor area, regions activated by both normal subjects and non-hallucinators. Conversely, when non-hallucinators imagined speech, they differed from both hallucinators and controls in showing reduced activation in the right parietal operculum (see Figure XVII-8.4). McGuire and his colleagues suggest that the presence of verbal hallucinations is associated with a failure to activate areas concerned with the monitoring of inner speech.

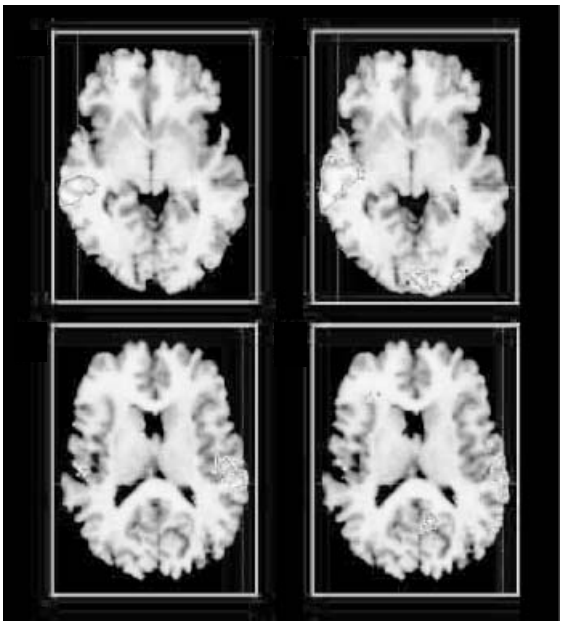

Figure XVII-8.4 Difference in rCBF between schizophrenic patients with a strong predisposition to auditory verbal hallucinations (hallucinators), schizophrenic patients with no history of hallucinations (non-hallucinators), and normal controls during tasks that engaged inner speech and auditory verbal imagery. When imagining sentences spoken in another person's voice, which entails both the generation and monitoring of inner speech, hallucinators showed reduced activation of the left middle temporal gyrus and the rostral supplementary motor area, regions activated by both normal subjects and non-hallucinators. Adapted from McGuire *et al.* (1996) (See Colour Plate XVII-8.4)

Visual Hallucinations

The neural correlates of visual hallucinations also seem to be located in the neural substrate of visual perception. At least part of the activity in the brain associated with the experience of visual hallucinations is located in the visual cortex. For example, using SPECT Hoksbergen *et al.* (1996) found that visual hallucinations were associated with hypoperfusion in the right occipito-temporal region, which showed partial normalization after the visual hallucinations had subsided. Howard *et al.* (1997) used fMRI to investigate the visual cortical response to photic stimulation during and in the absence of continuous visual hallucinations. When visual hallucinations were absent photic stimulation produced a normal bilateral activation in striate cortex. During hallucinations, very limited activation in striate cortex could be induced by exogenous visual stimulation. Similarly, ffytche *et al.* (1998) found activity in ventral extrastriate visual cortex in patients with the Charles Bonnet syndrome when they experienced visual hallucinations. Moreover, the content of the hallucinations reflected the functional specialization of the region, for example, hallucinations of colour activated V4 (the human colour centre; Zeki *et al.*, 1991).

In conclusion, functional neuroimaging studies suggest that hallucinations involve an interaction between the neural systems dedicated to the particular sensory modality in which the false perception occurs and a widely distributed cortico-subcortical system, including limbic, paralimbic and frontal areas. Intersubject variability in the specific location of the sensory activation associated with the hallucination could arise from differences between the patients in the sensory content and experience of their hallucinations.

Thought Disorder

McGuire *et al.* (1998) scanned six schizophrenic subjects with PET while they described a series of ambiguous pictures, which provoked different degrees of thought-disordered speech in each patient. The severity of thought disorder was correlated with rCBF across the 12 scans, controlling for differences in the total number of words articulated. Verbal disorganization (positive thought disorder) was inversely correlated with activity in the inferior frontal, cingulate and left superior temporal cortex, areas implicated in the regulation and monitoring of speech production. The authors propose that this reduced activity might contribute to the articulation of the linguistic anomalies that characterize positive thought disorder. Verbal disorganization was positively correlated with activity in the parahippocampal/anterior fusiform region bilaterally, which may reflect this region's role in the processing of linguistic anomalies.

Passivity Symptoms

Spence *et al.* (1997) performed a PET study in which subjects had to make voluntary joystick movements in the experimental

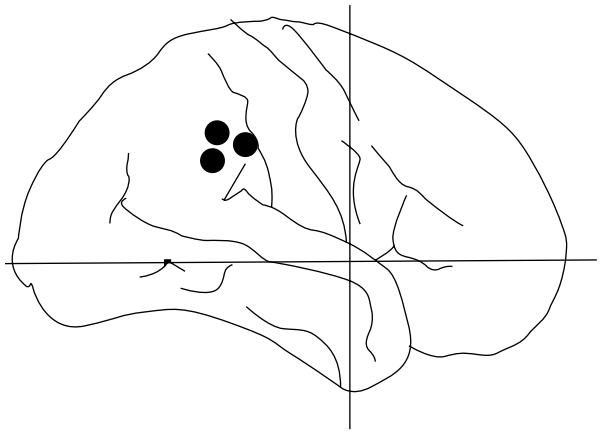

Figure XVII-8.5 Diagram of overactivity in right parietal lobe (Brodmann area 40) in patients with passivity while making voluntary joystick movements (in the study by Spence *et al.*, 1997). When patients were in remission, and no longer experienced passivity symptoms, a reversal of this hyperactivation of parietal lobe was seen. Hyperactivity in parietal cortex might reflect the patient's experiencing the movement as 'unexpected,' as if it were being caused by some external force

condition, and stereotyped (routine) movements in the baseline condition, and do nothing in the rest condition. They investigated a group of schizophrenic patients with passivity (delusions of control) and without (the same group in remission), and a group of normal controls. Schizophrenic patients with passivity showed hyperactivation of inferior parietal lobe (BA 40), the cerebellum and the cingulate cortex relative to schizophrenic patients without passivity (see Figure XVII-8.5). Similar results were found when schizophrenic patients with passivity were compared with normal controls. A comparison of all schizophrenics with normal controls revealed hypofrontality in the patients. When patients were in remission, and no longer experienced passivity symptoms, a reversal of the hyperactivation of parietal lobe and cingulate was seen. Hyperactivity in parietal cortex may reflect the 'unexpected' nature of the experienced movement in patients, as though it were being caused by an external force.

OBSTACLES TO FUNCTIONAL NEUROIMAGING AND SCHIZOPHRENIA STUDIES

Subject Matching

It is important that patients are matched to the control group on as many factors as possible, but the best way to match IQ and education level is uncertain. It is not clear whether the control group's education level should be matched to the patient's parental or premorbid education level. However, both of these are superior to matching controls to patients' current IQ level, which may be considerably impaired by illness.

Another question is whether the control group should be normal or psychiatric. Normal controls are important in order to establish a baseline model of the neural circuitry involved in an experimental task. However, there are several problems with using non-psychiatric controls. These include the fact that normal controls are not taking medication, hospitalized or affect-flattened, factors that might cause the patients to be more or less motivated, to attend or think more or less, or that might have a direct effect on rCBF. Since psychiatric patients will be more matched on these factors they may be a preferable control group. However, there are also problems with using psychiatric patients as controls. There is the question of which psychiatric population should be used. Should they be taking the same medication, or is hospitalization the more important factor? They might not be able to perform the experimental task for some reason that is different from that causing impairment in schizophrenic patients. Therefore, comparing schizophrenic with depressed patients, for example, may reveal activity specific to depression or to schizophrenia.

Experimental Tasks

As has been discussed at length throughout this chapter, there is a conceptual problem with applying the approach of cognitive activation studies to patient groups. It is difficult to interpret patterns of brain activity that differ between control and patient groups when the performance of the two groups on tasks differs in terms of degrees of efficiency and success. Any difference in brain activity between the two groups could represent a critical abnormality in schizophrenia and might cause poor task performance, or alternatively it might reflect poor performance. It is difficult to distinguish these two alternatives. Recent studies have employed tasks on which performance of the patient and control group is matched. However, there is also a problem with interpreting the results of activation studies in which the task performance of the patient and control group is matched. What do differences in brain activity mean in the context of normal task performance? If an area is activated more in the controls than in the patient group during such a task, the functional significance of that activation is difficult to understand — it is clearly not necessary for performing the task. The most obvious interpretation is that patients and controls are using different strategies to achieve similar task performance. Therefore, interpretational difficulties remain: what is the nature of the relationship between differences in brain activity and behaviour? How do these two variables relate to the schizophrenic state? These problems have not been resolved, and remain when interpreting data from studies in which task performance of patient and control groups is matched.

Symptom-Specific Groups

Schizophrenia is a heterogeneous illness, comprising a variety of different symptoms. Using groups of patients defined by diagnosis (schizophrenia) may explain the inconsistent and equivocal results of functional imaging studies, since each symptom may be associated with a different brain pattern or functional abnormality. Attempts have been made to correlate cognitive brain activity with specific symptoms or clinical signs. However, as reviewed here, the results are inconsistent.

A clear advantage of using symptom-specific schizophrenic groups is that the control group can comprise people with a diagnosis of schizophrenia, who are thus matched in terms of medication and hospitalization, but who do not have a particular symptom. Better still, the same group of schizophrenic patients can be used as their own control group if and when the symptom evaluated remits (see the study by Spence *et al.*, 1998). However, a clear shortcoming of using symptom-specific groups is that little can be discovered about schizophrenia as a syndrome.

OVERALL CONCLUSION

Although there has been no specific, replicated finding peculiar either to the syndrome of schizophrenia or to a particular symptom, there have been repeated findings of frontal and temporal abnormalities in schizophrenia, in both resting metabolism and cognitive activation studies. PET and SPECT studies have shown that the density

of DA (in particular D2) receptors is increased in schizophrenic brains, and that typical antipsychotic drugs bind mainly to D2 receptors whereas atypical antipsychotics bind mainly to 5-HT receptors. It is becoming increasingly evident from PET and fMRI studies that schizophrenic patients show abnormal interactions between brain regions during cognitive tasks. There is currently little *direct* evidence in favour of the hypothesis of functional disconnection, and several regions have been found to function abnormally, but with no unequivocal evidence for the consistent involvement of any particular region. However, the majority of positive findings suggest that a disruption of cortico-cortical (and cortico-subcortical) integration is a core feature of the schizophrenic syndrome. Future studies would do well to investigate this possibility, using methods of evaluating functional or effective connectivity to evaluate the influence of one brain region over another.

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XVII-9

Neurogenetics of Schizophrenia

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INTRODUCTION

The aetiology and pathophysiology of schizophrenia remain largely unknown although many hypotheses prevail. The one aspect of aetiology that has been strongly and repeatedly supported is the existence of an underlying genetic component. In this chapter we present an overview of the literature supporting a genetic basis for vulnerability to schizophrenia, and describe attempts to locate and identify susceptibility genes and the problems inherent with genetic studies of this disorder.

It is important to note that as there are no objective diagnostic tests for schizophrenia diagnosis is based upon patterns of clinical symptoms and signs. Within schizophrenia there is wide variation in the range of symptoms, illness course and outcome, but despite this it has not been possible to delineate aetiologically distinct subgroups. The use of structured and semi-structured interviews together with explicit operational diagnostic criteria means that it is often possible to achieve high degrees of diagnostic reliability within this disorder, and to define a syndrome with high heritability. However, the possibility remains that schizophrenia, as defined by current diagnostic criteria, includes a number of heterogeneous disease processes and that this will hinder any attempts at identifying genetic aetiological factors.

GENETIC RISK

Historical observations that schizophrenia runs in families has led to a number of family, twin and adoption studies which, despite some variation in results, overall provide strong evidence for a genetic component to this disorder with even conservative estimates suggesting a heritability of greater than 60% (McGuffin *et al.*, 1994). The methodology underlying the principles of quantitative genetics are described in detail in Chapter XII (Neurogenetics) and will therefore not be covered in this chapter.

The lifetime risk for schizophrenia in the general population worldwide is generally reported to be around 1%, with the risk increasing exponentially with the degree of relation to an affected individual. The lifetime expectancies of schizophrenia in the relatives of probands has been reviewed by Gottesman and Shields (1982), who looked at all Western European studies prior to the relatively recent introduction of operational diagnostic criteria. The risk to siblings was found to be 10%, offspring of affected probands 13%, parents of an affected proband 6% (somewhat lower than expected, probably reflecting the reproductive disadvantage conferred by schizophrenia), and risk to offspring where both parents were affected was 46%. Many of the studies in this analysis predate up-to-date methods, but in a review of seven methodologically modern studies the finding of an approximately 10-fold increase in risk of schizophrenia in first-degree relatives of schizophrenic probands remains (Kendler and Diehl, 1993).

More recent studies have shown a wider variation in lifetime expectancies for relatives, probably as a result of the use of different diagnostic criteria which vary with regard to strictness of case selection. Standardization of diagnoses is therefore an important factor in reducing bias of results. For this reason an approach used by many groups carrying out genetic studies of schizophrenia is to obtain data in a manner that allows different operational criteria to be applied to the same data set, thereby allowing direct comparisons between different studies. One example of this is the Operational Criteria Checklist (OPCRIT), which allows diagnoses according to seven different criteria to be made, including ICD-10 and DSM-III-R diagnoses (McGuffin *et al.*, 1991).

Twin and adoption literature on schizophrenia permit genetic factors to be differentiated from infective, cultural, socio-economic or other environmental factors potentially causing the familial clustering observed in family studies. Adoption studies of the three main study designs (adoptee studies, cross-fostering studies and adoptee family studies) provide evidence that there is an increased risk of schizophrenia in first-degree relatives of probands, though not in non-biologically related adopted or adoptive family members who share the same environment as probands. (Heston, 1966; Rosenthal *et al.*, 1971; Kety *et al.*, 1994; Wender *et al.*, 1974). The data of Kety *et al.* (1994) from Denmark have been reanalysed applying DSM-III criteria, with consistent results (Kendler *et al.*, 1994): 7.9% of first-degree biological relatives of proband adoptees had DSM-III schizophrenia, compared with 0.9% of first-degree relatives of control adoptees. A further Finnish study (Tienari *et al.*, 1994) found a higher prevalence of DSM-III-R schizophrenia in the adoptive offspring of mothers with schizophrenia or a related disorder, compared with control offspring (4.4% vs. 0.5%).

Twin studies compare the concordance rates between monozygotic (MZ) and dizygotic (DZ) twins, and the overwhelming evidence from these studies is that shared genes rather than shared environments underlie the increased risk of illness in relatives of probands (McGuffin *et al.*, 1994). Based upon the five most recent systematically ascertained twin studies, the proband-wise concordance rate for schizophrenia in MZ twin pairs is 41–65% compared with 0–28% for DZ twin pairs, corresponding to heritability estimates of approximately 80–85% (Cardno and Gottesman, 2000). Furthermore, a review of MZ twins reared apart (McGuffin *et al.*, 1994) found a concordance rate in this group of 58%, similar to that for MZ reared together.

It is clear therefore that although environmental factors play an important role in determining liability to schizophrenia (MZ concordance rates substantially less than 100%), the major determinant of individual variation in susceptibility seems to be genetic. Studies on the recurrence risk in various classes of relative allow us to exclude the possibility that schizophrenia is a single-gene disorder or collection of single-gene disorders even when incomplete penetrance is taken into account (O'Rourke *et al.*, 1982; McGue and Gottesman, 1989). Rather, the mode of transmission, like that

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of other complex disorders, is complex and non-Mendelian. The commonest mode of transmission is probably oligogenic (a small number of genes of moderate effect) or polygenic (many genes of small effect) or a mixture of the two (McGuffin *et al.*, 1995). However, the number of susceptibility loci, the disease risk and attributable risk conferred by each locus, the degree of epistasis (interaction between loci) and interaction with environmental factors all remain unknown. Complex inheritance implies that not all affected individuals will carry a given susceptibility variant and, conversely, many unaffected individuals will also carry these susceptibility variants, i.e. risk alleles may be neither sufficient nor always necessary to cause schizophrenia.

The contribution of individual genes to the familiality of a disorder can be expressed in terms of λ_s, which is the relative risk to siblings compared to the general population resulting from possession of the disease allele (Risch, 1990a). By comparing expected with observed recurrence rates for a range of classes of relative, Risch (1990a) has calculated that the data for recurrence risks in the relatives of probands with schizophrenia are incompatible with the existence of a single locus of $\lambda_s > 3$ and, unless extreme epistasis exists, models with two or three loci of $\lambda_s \leq 2$ are more plausible. It should be emphasized that these calculations are based upon the assumption that the effects of genes are distributed equally across the whole population and it is quite possible that genes of larger effect are operating in a subset of patients.

Although the weight of evidence from genetic epidemiology supports the view that genes play a substantial role in aetiology, the distinction between genetic and environmental factors is not always as straightforward as at first appears. There are a number of genetic mechanisms such as somatic mutation, genomic imprinting and mitochondrial inheritance that could cause phenotypic differences, including discordance, between MZ twins and which would therefore contribute to the so-called 'environmental' effects observed in twin studies (McGuffin *et al.*, 1995; Morgan *et al.*, 1999). It is also possible that stochastic factors could play a role in mediating the effects of genotype on phenotype, particularly where a complex process such as brain development is concerned (McGuffin *et al.*, 1995), and these too would inflate the contribution of environmental effects estimated from twin and adoption studies. On the other hand, interactions between genes and environment tend to be included in the 'genetic' component of models; for example, phenylketonuria is regarded very much as a genetic disorder (eliminating all the disease genes from the population would eradicate the disease), although it could also be thought of as totally environmental (since removing all phenylalanine from the environment would also eradicate the disease).

While it is clear therefore that there is a genetic contribution to schizophrenia, it is equally clear that what is inherited is not the certainty of disease accompanying a particular genotype but rather a predisposition or liability to develop the disorder. Moreover, twin and adoption studies have also shown that schizophrenia shares familial, and probably genetic, liability with a range of other psychotic illnesses (Kendler *et al.*, 1993a) and both schizotypal and paranoid personality disorder (Kendler *et al.*, 1993b), collectively known as the schizophrenia spectrum disorders.

At its most general, the implication is that spectrum disorders probably share at least some genetic risk factors in common with schizophrenia itself, although it is also possible that all these disorders share the same risk factors under a single liability model. The hypothesis that each disorder represents a separate threshold on a liability distribution was tested on family data (Kendler *et al.*, 1995b) and the patterns of familial aggregation were found to be consistent with a spectrum model, though the order in which the disorders occurred could not be specified. There has also been support for a continuum between schizophrenia and schizotypal personality disorder (Baron and Risch, 1987), but not for one between schizophrenia and a 'spectrum' comprising a range of non-affective psychotic, neurotic, personality and other psychiatric disorders (Tsuang *et al.*, 1983). This uncertainty regarding the limits of this spectrum of disorders and its relationship with other disorders further complicates the process of genetic analysis of schizophrenia (Kendler *et al.*, 1995a, 1998; Cardno *et al.*, 2002).

GENE-MAPPING STRATEGIES

The two main groups of strategies used to identify genes in complex disorders are linkage and association. In linkage studies the aim is to identify alleles that segregate with the disease in families with two or more affected individuals. This approach can detect the presence of genes over large chromosomal distances and thus is readily applicable to genome-wide studies. It is ideally suited to detecting genes in monogenic disorders, but its power to detect genes of moderate to small effect is limited.

In association studies, on the other hand, the unit of study is a sample of unrelated people usually in the form of a case–control design. Here the aim is to detect alleles that are more (or less) common in cases than controls. Association studies have considerable power to detect the genes of small effect that are most likely to be operating in complex disorders such as schizophrenia, but depend upon the marker studied either being the pathogenic variant itself or being so close as to be in linkage disequilibrium (LD) with it. LD refers to the non-random association in a population of alleles at two closely linked loci and can occur when most cases of a disease are caused by a mutation in a common ancestor, known as a founder mutation. The disequilibrium will be eroded by recombination during meioses and therefore for LD to be maintained over many generations marker alleles need to be much closer to susceptibility loci than they do for linkage-based studies. A systematic, genome-wide search for association would thus require many thousands of markers. Other strategies employed in the search for susceptibility genes for schizophrenia that will be covered in this chapter include the investigation of cytogenetic abnormalities and quantitative trait locus (QTL) mapping. For further detail regarding the principles and techniques underlying all these strategies please refer to Chapter XII.

Linkage Studies

The first wave of molecular genetic studies of schizophrenia effectively ignored the evidence for genetic complexity by focusing on large multiply affected pedigrees under the assumption that aetiological heterogeneity exists and that such families, or at least a proportion of them, are segregating genes of major effect. This approach has been successful in other complex disorders, particularly Alzheimer's disease, where mutations in three genes, APP, PS1 and PS2, are now known to cause rare forms of the disorder where disease of unusually early onset is inherited in an autosomal dominant fashion (Goate *et al.*, 1991; Sherrington *et al.*, 1995; Levy-Lahad *et al.*, 1995). Similar studies of large families segregating schizophrenia and related phenotypes also initially produced positive findings (Sherrington *et al.*, 1989) but unfortunately these could not be replicated. The reasons for this have become clear as data from systematic genome scans have accumulated. It seems likely that highly penetrant mutations causing schizophrenia are at best extremely rare and quite possibly non-existent (McGuffin and Owen, 1996; Craddock *et al.*, 1995), with type I (false positive) errors arising largely from a combination of multiple testing and the use of statistical methodology and significance levels derived from work on single-gene disorders.

In spite of the failure to identify genes of major effect in multiply affected families, moderately significant evidence for linkage has been found in more than one data set in several

chromosomal regions. Evidence for linkage on chromosomes 22q11–12, 6p24–22, 8p22–21 and 6q have received supportive evidence from international collaborative studies (Gill *et al.*, 1996; Schizophrenia Linkage Collaborative Group, 1996; Levinson *et al.*, 2000), while a number of other promising areas of putative linkage have been supported by other individual groups, although not from larger international consortia. These include 13q14.1–q32 (Lin *et al.*, 1995; Blouin *et al.*, 1998; Brzustowicz *et al.*, 1999), 5q21–q31 (Schwab *et al.*, 1997; Straub *et al.*, 1997) and 10p15–p11 (Schwab *et al.*, 2000; Faraone *et al.*, 1998; Straub *et al.*, 1998). One other region of note is 1q21–q22 following a report of strong linkage (maximum heterogeneity LOD score of 6.5) from one study (Brzustowicz *et al.*, 2000). This finding more than meets standard criteria for claiming 'significant' linkage as proposed by Lander and Kruglyak (1995), but the need for replication from other sources remains an important issue. There have been other reports of linkage to markers on chromosome 1 (Hovatta *et al.*, 2000; Ekelund *et al.*, 2000), but these are distal to 1q21–q22 and it is not yet clear that these findings are consistent with the existence of a single susceptibility locus on 1q.

While the linkage data based upon multiplex families are not definitive, it seems likely that one or more of the above loci are true positives. However, it should be noted that in every case there are negative as well as positive findings, and in only two cases, 13q14.1–q32 and 1q21–q22, did any single study achieve 'genome-wide significance' at $p < 0.05$. Genome-wide significance refers to a linkage value that is expected by chance less than once in 20 complete genome scans and which therefore takes account of the multiple testing inherent in systematic searches for linkage.

As an alternative to linkage based upon multiplex families, some groups have advocated the use of smaller families with two or more affected in a single sibship. This is in the belief that such families may be more suited for the analysis of complex traits, and being much more common may also be more representative of disease in the general population. This approach has been successful in detecting linkage to several complex diseases, including late-onset Alzheimer's disease (Myers *et al.*, 2000). Williams *et al.* (1999) conducted the largest systematic search for linkage in affected sibling pairs (ASPs) with schizophrenia published to date. This study of 196 ASPs was designed to have power >0.95 to detect a susceptibility locus of $\lambda_s = 3$ (the maximum effect size estimated by Risch, 1990b) with genome-wide significance of 0.05. However, no regions of the genome gave linkage results approaching genome-wide significance although modest evidence (at the level expected by chance once per genome scan) was found for loci on chromosomes 4p, 18p and the centromeric region of X.

The pattern of findings from linkage studies of schizophrenia demonstrates several features that are to be expected in the search for genes involved in disorders that are predisposed to by the combined action of several genes of moderate effect (Suarez *et al.*, 1994; Lander and Schork, 1994; Lander and Kruglyak, 1995). First, no finding replicates in all data sets; secondly, levels of statistical significance and estimated effect sizes are usually modest; and thirdly, chromosomal regions of interest are typically broad (often >20–30 centimorgans). Unfortunately, it is debatable whether at present the statistical support for linkage associated with any of the regions reported above is sufficiently strong to warrant large-scale and expensive efforts aimed at cloning putative linked loci, given that some or all of these findings could be type I errors. Linkage methods can detect smaller-sized genetic effects of the magnitude likely to be operating in schizophrenia (λ_s 1.5–3) in sample sizes that are realistic (600–800 ASPs) (Hauser *et al.*, 1996; Scott *et al.*, 1997) although this would be considerably larger than sample sizes used to date. It is of great importance therefore that in the future large enough samples are used to provide sufficient power to detect linkage, though if schizophrenia does just reflect the operation of many genes of small effect then even these large-scale studies may be unsuccessful.

Association Studies

Association studies offer a powerful means of identifying genes of small effect in realistically sized samples of unrelated cases. The requirement for markers to be themselves the susceptibility locus, or at least so close as to be in linkage disequilibrium with it, means that this approach is ideally suited to the study of polymorphisms within candidate genes. The main problem with this approach is that we have such limited knowledge about the pathogenesis of schizophrenia that the potential choice of candidate genes is limited largely by the number of genes in the human genome (estimated at around 40 000).

As a result of doubts concerning the robustness of linkage findings in schizophrenia, most studies to date have focused upon functional candidate genes (i.e. those involved in a biological process thought to be relevant to schizophrenia) rather than positional candidates in regions implicated by linkage studies. Genes involved in dopaminergic and serotonergic neurotransmission have received particular attention as candidate genes based upon neuropharmacological studies, which suggest that abnormalities of both these systems may play a role in the aetiology of schizophrenia. However, it is becoming increasingly clear that other neurotransmitter systems, and in particular glutamatergic and GABAergic systems, are probably involved in the pathogenesis of schizophrenia, while neuropathological and neuroimaging studies provide some support for the importance of neuromodulation and neurodevelopment as pathological processes. Our widening concept of the possible mechanisms underlying the aetiology of schizophrenia is reflected in the choice of functional candidates being studied (Malhotra, 2001).

Numerous candidate gene analyses have been reported in schizophrenia, but no positive finding has received sufficient support from more than one group to suggest a true association with even a modest degree of probability, except perhaps for the serotonin 5-HT2a and dopamine D3 receptor genes discussed below.

Serotonin 5-HT2a Receptor Gene

The serotonergic system is a therapeutic target for several antipsychotic drugs. The first genetic evidence for its involvement in schizophrenia was a report of association with a polymorphism at nucleotide 102 in the HTR2A gene, which encodes the 5-HT2a receptor (Inayama *et al.*, 1996), whereby a point mutation results in a thymine-to-cytosine base change. This association was based upon a small sample of Japanese subjects. Firmer evidence for association subsequently emerged from a large multi-centre European consortium (Williams *et al.*, 1996) and a meta-analysis of all the data available in 1997 based upon more than 3000 subjects (Williams *et al.*, 1997).

If we assume that schizophrenia is homogeneous and that the association is correct, the odds ratio (OR) for the C allele is approximately 1.2 (95% confidence interval 1.07, 1.31). Given the difficulties and expense inherent in these types of studies some may question whether any priority should be given to finding such genes of small effect, though of course if a risk factor is common in the population then it can be associated with a high attributable risk even though the relative risk may be low. For example, in schizophrenia the population attributable fraction associated with possession of allele C of the T102 C polymorphism in 5-HT2A is 0.35 (Williams *et al.*, 1997).

Since the meta-analysis was undertaken, a few further negative reports have followed, none with the sample sizes (and thereby power) required to detect an association. Sample sizes of 1000 subjects are required for 80% power to detect an effect of

this size even at a 5% significance level. However, while the negative studies do not refute the putative association, the evidence presented even in the meta-analysis ($p = 0.0009$) is well short of genome-wide significance (estimated at around $p = 5 \times 10^{-8}$ by Risch and Merikangas, 1996). Admittedly, demanding genome-wide significance is inappropriate for such a strong candidate gene because the prior probability that it is involved in schizophrenia is greater than a gene selected at random. However, as there is no way of quantifying how much greater the prior probability is, we have no compelling alternative to genome-wide significance. Consequently, we have to surmise that the evidence tends to indicate association between 5-HT2a and schizophrenia, but that the burden of proof has not yet been met.

Another reason to be sceptical about the putative association is that there are no variants yet detected in the gene that clearly alter receptor function or expression, although there is some indirect evidence for the existence of a sequence variation elsewhere that alters 5-HT2a expression in some regions of the brain (Bunzel *et al.*, 1998).

Dopamine D2 Receptor Gene

The dominant neurochemical hypothesis of schizophrenia involves dysregulation of the dopaminergic system, and increased levels of striatal dopamine D2 receptors has been repeatedly reported in post-mortem studies, although it remains unclear how much of this is secondary to treatment with antipsychotic medication. Neuroimaging studies have provided conflicting results regarding D2 receptor levels, but nevertheless the strength of evidence implicating this receptor makes the DRD2 gene a promising candidate for genetic studies (Kahn and Davidson, 1995; Harrison, 1999). However, association analyses of the DRD2 gene to date have essentially been negative, although recently three studies have found an association with a polymorphism (−141C Ins/Del) in the promoter region of this gene that alters DRD2 expression *in vitro* (Arinami *et al.*, 1997; Ohara *et al.*, 1998; Jonsson *et al.*, 1999). Unfortunately, the largest single study of this polymorphism found no evidence for association (Li *et al.*, 1998). Furthermore, when the UK data from this study were combined with a large data set from Scotland, the allele that was associated with schizophrenia in the other three studies was actually significantly less common in cases than controls (Breen *et al.*, 1999). This finding has been interpreted as suggestive that the polymorphism tested in these studies is simply a marker for the true susceptibility variant (i.e. that the association is due to linkage disequilibrium). While it is reasonable to attribute reversal of allelic association to LD differences between samples of different ethnic origins, the explanation would be more convincing if the same allele was associated in both UK and Swedish samples. Furthermore, if LD is the explanation for the association, then the fact that this particular polymorphism is functional is no longer relevant. Further studies of this polymorphism are therefore required to clarify the possible role of this variant in the aetiology of schizophrenia.

Dopamine D3 Receptor Gene

The dopamine hypothesis has received somewhat stronger, although not robust, support from studies of the DRD3 gene, which encodes the dopamine D3 receptor. Association has been reported between schizophrenia and homozygosity for a Ser9Gly polymorphism in exon 1 of this gene (Crocq *et al.*, 1992). As expected, both positive and negative reports of association with this polymorphism have emerged, but unlike the situation with the DRD2 gene, sufficient data are available for meta-analysis. Based upon all available data from over 5000 individuals, Williams *et al.* (1998) concluded in favour of an association although the effect size was extremely small ($OR = 1.23$, 95% CI 1.09, 1.38) but nominally significant ($p = 0.0002$). For the same reasons outlined above regarding the 5-HT2a gene, the level of statistical evidence here, while supportive of a significant association, is not conclusive.

Anticipation and Trinucleotide Repeats

One class of mutation rather than a particular gene that merits specific mention is that of expanded trinucleotide repeats, whose hallmark is the phenomenon of anticipation, whereby a disease becomes more severe or has an earlier age at onset in successive generations. Numerous studies have now suggested that the pattern of illness in multiplex pedigrees segregating schizophrenia and other psychotic illnesses is consistent with anticipation, although ascertainment biases in these studies is difficult to rule out (O'Donovan and Owen, 1996). This hypothesis has received support from a number of studies reporting that large but unidentified CAG/CTG trinucleotide repeats are more common in patients with schizophrenia and other psychoses than in unaffected controls (e.g. O'Donovan *et al.*, 1995; Morris *et al.*, 1995), although these early findings have been followed by numerous failures of replication. Also, the specific loci responsible for around 50% of the anonymous CAG/CTG repeats detected in the previous studies have now been identified, but are not associated with psychosis (e.g. Vincent *et al.*, 1999; Bowen *et al.*, 2000). These data have tempered much of the enthusiasm for the CAG/CTG hypothesis, but it is still premature to discard it. Indeed two independent groups have recently detected the presence of proteins in a small number of schizophrenics that react with an antibody against moderate–large polyglutamine sequences (Ross, 1999). This is of relevance here because polyglutamine sequences are often encoded in DNA by CAG repeats, though it remains to be seen whether these proteins are involved in the disease rather than simply chance findings.

Interpreting Association Studies of Schizophrenia

Unfortunately association studies are prone to type I errors, the most likely reasons being the low prior odds of association and the multiple testing involved (Owen *et al.*, 1997; Risch and Teng, 1998), a problem that will be even more significant when genome-wide scans are undertaken. Additionally, association studies are heavily influenced by population structure, and confounding may occur due to population stratification, resulting in different allele frequencies at the marker locus in the cases and controls. This problem can be addressed by family-based association methods (Schaid and Sommer, 1994), but large enough samples of individuals with schizophrenia with all relevant family members willing to participate are difficult to collect (O'Donovan and Owen, 1999).

A further problem is that samples are often ascertained in widely differing and unsystematic ways, and any associations reported might therefore be with other confounding variables such as co-morbidity, or with modifying factors such as those influencing disease severity or age at onset. For example, genes involved in the monoamine system implicated in the aetiology of schizophrenia may help to determine drug response rather than contribute to the aetiology in any way. The design of studies using only incident cases may be required before explanations of this sort can be excluded.

It is important to note, however, that the overwhelmingly negative reports from association studies of schizophrenia cannot be considered as definitive exclusions of small genetic effects due to the possibility of type II (false negative) errors resulting from insufficient power of most association studies to reliably detect a small effect size. It is therefore very important that association studies that aim to test putative associations should be based upon large enough samples (usually several hundred to a thousand cases

with as many, if not more, controls) to provide meaningful tests of association. Even with larger samples though, if aetiological heterogeneity does exist in schizophrenia, the lack of biologically meaningful diagnostic tests means it is possible that conflicting studies contain different balances of unknown subtypes of the disease which might dramatically alter the power for excluding association. Similarly, if there are differences in the contribution of a given allele in different ethnic groups (arising from different allele frequencies or different allele frequencies at interacting loci), aetiological heterogeneity will again alter the power to find a true association. At present, there are no simple ways to take such possibilities into account other than to recognize their potential existence. Basing future studies on sound epidemiological principles will be essential to avoid some of the problems inherent in studies to date, and to maximize the potential for finding true associations in this disorder.

Cytogenetic Abnormalities

A third approach by which investigators have sought to locate susceptibility genes for schizophrenia has been to identify chromosomal abnormalities in affected individuals. Cytogenetic anomalies, such as translocations and deletions, may be pathogenic through several mechanisms; direct disruption of a gene or genes, indirect disruption of the function of neighbouring genes by a so-called position effect or an alteration of gene dosage in the case of deletions, duplications and unbalanced translocations. It is also possible for an abnormality merely to be linked with a susceptibility variant within a particular family.

There have been numerous reports of associations between schizophrenia and chromosomal abnormalities (Bassett, 1992; Baron, 2001) but with two exceptions none has as yet provided convincing evidence to support the location of a gene conferring risk to schizophrenia. The first strongly suggestive finding is a t(1;11) balanced reciprocal translocation found to co-segregate with schizophrenia in a large Scottish family (St Clair *et al.*, 1990). This cytogenetic abnormality results in a portion of chromosome 11 being translocated to chromosome 1 and vice versa, and which has recently been reported to directly disrupt three genes, of unknown function, on chromosome 1 (Millar *et al.*, 2000). Interestingly the breakpoint appears to be located close to the markers implicated in the two Finnish studies mentioned previously in the linkage section of this chapter (Hovatta *et al.*, 2000; Ekelund *et al.*, 2000). However, until mutations have been identified in other families or convincing biological evidence implicating this locus has been obtained, the mechanism by which the translocation confers risk to mental illness remains obscure.

The second finding of interest is the association between velocardio-facial syndrome (VCFS) and schizophrenia. VCFS, also known as DiGeorge syndrome, is associated with small interstitial deletions of chromosome 22q11. The phenotype of VCFS is variable, but in addition to characteristic core features of dysmorphology and congenital heart disease there is strong evidence that individuals with VCFS have a dramatic increase in the risk of psychosis, and in particular schizophrenia and allied disorders (Shprintzen *et al.*, 1992; Pulver *et al.*, 1994; Papolos *et al.*, 1996; Murphy *et al.*, 1999). With an estimated prevalence of one in 4000 live births, one can estimate that VCFS cannot be responsible for more than a small fraction (~1%) of cases of schizophrenia, and this estimate is in keeping with empirical data (Karayiorgou *et al.*, 1995). However, from the perspective of genetic research, the high rate of psychosis in VCFS may provide a short-cut to a gene within the deleted region which is involved in susceptibility to schizophrenia even in cases without a deletion. As we have mentioned already, evidence for linkage of a general schizophrenia susceptibility locus on 22q has been found, and although most studies suggest that this maps outside the VCFS region, linkage mapping in complex diseases is imprecise and modest evidence for linkage within the VCFS region has also been reported (Blouin *et al.*, 1998; Lasseter *et al.*, 1995; Shaw *et al.*, 1998). Overall, the combination of these findings supports the view that a susceptibility gene for schizophrenia exists within this region and efforts are currently under way to try and identify this gene.

Refining the Phenotype

The effectiveness of molecular genetic studies will depend upon the genetic validity of the phenotype. If we were better at defining schizophrenia, would we be better at finding genes? The first thing to note at this point is that the commonly used diagnostic criteria define phenotypes with high heritability. In principle, therefore, it should be possible to identify the genes predisposing to schizophrenia if sufficiently large samples are studied. However, perhaps genetic validity could be improved by focusing upon aspects of clinical variation such as age of onset or symptom profiles, or by identifying biological markers that predict degree of genetic risk or define more homogeneous subgroups.

Despite much work, it has not been possible to identify genetically distinct subtypes of schizophrenia. Instead, clinical variation is likely to reflect in part at least a combination of quantitative variation in genetic risk for the disorder and the effect of modifying genes which influence illness expression rather than the risk of illness *per se*. Examples of this phenomenon relating to age at onset and symptom pattern in schizophrenia have been discussed elsewhere (Owen *et al.*, 2000).

The search for trait markers aims to move genetic studies beyond the clinical syndrome by identifying indices of genetic risk that can be measured in asymptomatic individuals and/or by identifying markers of pathophysiological processes that are closer to the primary effects of susceptibility genes than clinical symptoms. Indeed it may be that there are no genes 'for' schizophrenia as such, but only those for other factors such as personality and IQ that increase risk of the disorder. Candidate trait markers for schizophrenia to date include schizotypal personality traits, measures of cognitive processing, brain-imaging abnormalities, brain-evoked potentials and abnormalities in eye movements (Cannon *et al.*, 2001; Holzman, 2000; Turetsky *et al.*, 2000; Egan *et al.*, 2000; Adler *et al.*, 1999), and this area of research is discussed further in Chapter XVII-10. However, it seems unlikely that this search for trait markers will provide a rapid solution to the problem of identifying susceptibility genes for schizophrenia. First, we will need to ensure that the measures used are stable and determine the extent to which they are affected by state. Second, to be of use in gene mapping, such measures will have to be practicably applied to a sufficient number of families or unrelated patients. Third, we will need to ensure that the traits identified are highly heritable, which will itself require a return to classic genetic epidemiology and model fitting.

Efforts to improve the selection of phenotypes are also concerned with enhancing the traditional categorical approach to defining psychiatric disorders, by identifying genetically valid phenotypes that can be measured quantitatively. These can be used in quantitative trait locus (QTL) approaches to gene mapping. QTL linkage (Kruglyak and Lander, 1995) is generally carried out in sib-pairs and is based on the principle that at a locus influencing a trait or at a linked marker locus sibs with more similar phenotypic scores should share more alleles identical-by-descent (ibd), while QTL association (Page and Amos, 1999) is based on samples of unrelated individuals, and seeks evidence of differences in phenotypic values according to allelic or genotypic differences at a locus. This approach could be applied to measures of schizophrenia proneness, though again state effects may pose difficulties, or to quantitative features of the illness such as age at onset, symptom profile or outcome which could reflect the degree of genetic loading, modifying

loci or a combination of the two. For example, there is strong evidence for a genetic contribution to variation in age of onset, with correlations of 0.76 for MZ and 0.26 for DZ pairs reported by Cannon *et al.* (1998). Attempts to identify genes that modify age of onset and other clinical variation within schizophrenia are under way, although there have been no replicated positive findings as yet (Kendler *et al.*, 2000; Brzustowicz *et al.*, 1997; Cardno *et al.*, 2001).

FUTURE DIRECTIONS

Functional Studies

The most important and the most obvious implication of identifying genetic risk factors for schizophrenia is that it will inspire a new wave of neurobiological studies from which new and more effective therapies will hopefully emerge. However, while the unequivocal identification of associated genetic variants will represent a great advance, many years of work will still be required before this is likely to translate to the bedside. An early problem will be to determine exactly which genetic variation amongst several in LD in a given gene is actually responsible for the functional variation. Even where a specific variant within a gene can be identified as the one of functional importance, functional analysis, in terms of effect at the level of the organism, is likely to be particularly difficult for behavioural phenotypes in the absence of animal models. Disorders that predominantly involve higher cognitive functions such as schizophrenia are difficult to model in animals although some features of the human phenotype, including enlarged cerebral ventricles and information-processing abnormalities such as defects in pre-pulse inhibition, can be detected in animals. An extra level of complexity, however, is the need to produce model systems, both *in vivo* and *in vitro*, that allow gene–gene and gene–environment interaction to be studied. For further details regarding the use of animal models in research into schizophrenia please refer to Chapter XVII-1.

Pharmacogenetics

Another rapidly expanding field within the realm of research into schizophrenia is that of pharmacogenetics, where underlying genetic variation is investigated as a cause for individual variation in response to antipsychotic drug treatment. Antipsychotic pharmacogenetic studies have focused mainly on the atypical agent clozapine, with some evidence for an association between polymorphisms in the 5-HT2a receptor gene and clinical response (Arranz *et al.*, 1998). Additionally, Arranz *et al.* (2000) studied the role of 19 neurotransmitter gene polymorphisms, and reported that a combination of six polymorphisms had a 77% success rate at predicting clozapine response, with a sensitivity of 95% for satisfactory response, although replication by prospective studies is required before one can envisage a use for this sort of information in clinical practice. Studies of genetic variation predicting side effects of antipsychotics are also under way, and there is some evidence to date for an association between a DRD3 polymorphism and antipsychotic-induced tardive akathisia and dyskinesia (Eichhammer *et al.*, 2000; Basile *et al.*, 1999).

Nosology

While the development of new therapies will take time, it is likely that the identification of susceptibility genes will have an earlier impact on psychiatric nosology. By correlating genetic risk factors with clinical symptoms and syndromes it should be possible to study heterogeneity and co-morbidity in order to improve the diagnosis and classification of psychosis, which will clearly facilitate all avenues of research into these disorders. Improvements in diagnosis and classification should enhance our ability to detect further genetic and environmental risk factors and thus a positive feedback between nosology, epidemiology and molecular genetics can be envisaged.

Genome-Wide Association Studies

In recent years there has been increasing interest in the possibility of systematic, genome-wide association studies that have the potential of allowing systematic searches for genes of small effect in polygenic disorders (Risch and Merikangas, 1996; Collins *et al.*, 1997; Owen *et al.*, 2000). Optimism has been fuelled by the fact that the most abundant form of genetic variation, the single nucleotide polymorphism (SNP), is usually bi-allelic and potentially amenable to binary, high-throughput genotyping assays, the most promising of which are at present micro-arrays (so-called DNA chips). Moreover, as the amount of sequence data accumulates through the work of the Human Genome Project, it has become possible to contemplate the construction and application of very dense maps of hundreds of thousands of SNPs (Wang *et al.*, 1998). Association could then be sought between the disease and a comprehensive catalogue of every variant that can alter the structure, function or expression of every single gene. At present this is not feasible technically or economically, and studies in the next few years will probably utilize SNPs from coding sequences that actually alter protein structure in a wide range of functional and positional candidate genes. However, given the difficulty in identifying functional SNPs in regulatory, rather than coding, regions of the genome, as well as our ignorance of the pathophysiology of schizophrenia, the expectation should be that most reported associations will be false, and this will only be resolved by replication in large well-characterized samples.

An alternative approach is to seek associations between markers and disease that are due to linkage disequilibrium between the markers and susceptibility variants. This would allow the whole genome to be systematically screened if dense enough marker maps could be applied. Although this approach is, at present, not widely applicable at a genome-wide level, smaller-scale studies focusing upon specific regions indicated by the results of linkage studies may allow loci to be mapped. However, several practical difficulties as well as uncertainties about these approaches remain (Owen *et al.*, 2000) and the era of genome-wide association studies is not yet at hand.

Molecular Epidemiology

The identification of genetic risk factors can be expected to provide a new impetus to epidemiological studies of schizophrenia by allowing researchers to investigate the ways in which genes and environment interact. Studies of this kind will bring together methodologies from genetics and epidemiology that have traditionally adopted somewhat differing analytical approaches (Sham, 1998). Treating susceptibility alleles as risk factors in an epidemiological context will allow estimates of effect sizes within a population to be made. Accounting for specific genetic effects will also facilitate the search for independent environmental factors, and the investigation of potential gene–environment interactions. Epidemiologically based studies are likely to reduce the effects of confounding (for example, by using incident cases of schizophrenia to guard against confounding by illness chronicity), while the inclusion of a prospective element to studies will counteract the tendency of patients to forget historical details, and the difficulty of making observed ratings retrospectively from case records. However, the price of improved scientific rigour is likely to be considerably more expensive studies, due to the longer period of study and the

larger number of investigators that will be required to ascertain the detailed data on the thousands of subjects that will probably be required. Further details about the study of gene–environment interactions in schizophrenia can be found in Chapter XVII-10.

Genetic Testing

Finally, one other implication of genetic epidemiology studies concerns genetic testing. Given that susceptibility to schizophrenia almost certainly depends upon the combined effects of predisposing and protective alleles at a number of loci as well as interactions with various environmental factors, the predictive value of genetic tests is likely to be low (Owen *et al.*, 2000). Although determining exact risk may be impossible, investigation of gene–environment interactions may allow for the introduction of lifestyle changes to reduce risk in individuals identified as having increased susceptibility to the disorder, and encourage closer medical supervision for early diagnosis, repeatedly shown to be an important prognostic factor in schizophrenia. Genetic testing could also help to optimize treatment choices by testing genes that are found to influence treatment responses in schizophrenia, leading to a greater individualization of treatment.

CONCLUSION

Research to date has largely excluded the possibility that genes of major effect exist in schizophrenia, even in a subset of families, although there is some evidence to suggest that allelic association in at least two genes, those encoding the 5-HT2a and DRD3 receptors, confer a small degree of susceptibility to this disorder. It is clear that over the next few years the completion of the human genome project and the identification of polymorphisms that affect disease risk will have a significant impact on our understanding of pathogenic mechanisms and encourage the development of more specific treatments for schizophrenia. The study of how these genes interact with each other and with non-genetic environmental factors is likely to be fascinating and productive, and we envisage that genetic studies will contribute substantially towards reducing the population impact of this disorder in the future.

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XVII-10

Gene–Environment Interactions in Schizophrenia

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INTRODUCTION

Schizophrenia is a disorder with multiple phenotypes, in which clinical symptoms can manifest in a variety of combinations that differ from individual to individual. This phenotypic heterogeneity not only makes it difficult to determine the aetiology of the disorder, but also underscores the issue of whether schizophrenia has multiple genetic and environmental aetiologies. In the search for useful phenotypes, clinical symptoms can be misleading. For example, many disorders outside the schizophrenic spectrum share features of schizophrenia, including hallucinations and delusions, two core diagnostic staples. Moreover, psychotic symptoms can occur without an underlying psychiatric disorder present, for instance by exposure to various toxins or from head injury. Clinical symptoms, such as psychosis, may represent rather remote end states of the aetiological processes that produced them, and may actually confound the quest to characterize those processes. Between the initial liability for schizophrenia and the expression of clinical symptoms, multiple levels of biological and environmental modulation may alter the course and outcome of the disorder. In order to gain a clearer picture of the genetic and environmental events that produce both the liability to, and the actual manifestation of, schizophrenia, it is important to examine phenotypes that are closer to their genetic and biological aetiologies than are clinical symptoms. In this chapter, we will consider alternative phenotypic candidates for use in genetic studies, including physiological, anatomical and neuropsychological measures, as surrogates for classical clinical criteria. We will begin by reviewing environmental factors implicated in the aetiology of schizophrenia, followed by a discussion of how they might combine with genetic risk factors to produce 'schizotaxia', which is the predisposition to schizophrenia or schizophrenia-like conditions. Based on this conceptualization, phenotypic expressions of schizotaxia will be considered.

INFLUENCE OF ENVIRONMENTAL FACTORS

It is well established through family, twin and adoption studies that schizophrenia and schizophrenic spectrum disorders have underlying genetic aetiological components (see Chapter XVII-9). Although the risk of developing schizophrenia is associated strongly with the number of shared genes between a family member and an individual with schizophrenia, genetics are not the only determining factor in the aetiology of the disorder. Several lines of reasoning support this point. First, the degree of risk for family members does not approach that which would be predicted based entirely on the percentage of shared genes. For example, monozygotic twins are genetically identical and thus share 100% of their genes. If one twin has schizophrenia, however, the risk to the other twin is closer to 50%, rather than the 100% that would be expected if genetic factors accounted for the disorder completely. First-degree relatives, including dizygotic twins, share about 50% of their genes, yet their risk has been estimated to be between 9% and 16% (Gottesman, 1991; Gottesman, 1993). In addition, neither inbreeding nor reduced level of procreation appear to affect the incidence of schizophrenia, which conflicts with the pattern observed in primarily genetic disorders (Andreasen, 2000; Torrey and Yolken, 2000).

Second, although elevated risk in adopted-away offspring of schizophrenic parents points to genetic influence, mutual environmental factors still exist, including a shared uterine environment (in the case of a schizophrenic mother) and a shared post-natal environment before adoption. Moreover, even monozygotic twins, who share identical genes, experience different environments from the moment of conception. Prenatal environmental differences between identical twins can include blood supply and position in the womb; in addition, twins experience differences during delivery (e.g. birth order), regardless of whether complications arise. In the case of identical twins with one affected member, the genetic predisposition is present in both individuals but is expressed only in the twin who has also undergone certain environmental experiences.

Thus, the interaction between genetic liability and environmental factors plays an important role in determining outcome. This conclusion is also evident in studies of heritability, which vary across samples and methods of ascertainment. Kendler, for example, reported the results of twin studies with an average heritability of around 70% (Kendler and Diehl, 1993), although recent studies using *Diagnostic and Statistical Manual of Mental Disorders*, 3rd, 3rd revised and 4th editions (DSM-III, DSM-III-R, DSM-IV) (American Psychiatric Association, 1980, 1987, 1994) diagnostic criteria also showed heritability estimates between 80% and 86% (Cannon *et al.*, 1998; Onstad *et al.*, 1991). Although the value obtained in a specific study might be accurate, estimates vary because heritability is a function of the population studied (including its size), the environment, the phenotypic criteria used for diagnosis, the statistical model used, the effect sizes of relevant variables, and many other factors. This means that while differences between individuals can be understood largely in terms of genetic factors, estimates of heritability vary, and its contribution to the schizophrenia phenotype cannot be understood without taking into account the importance of environmental factors.

Environmental factors implicated in the development of schizophrenia range from biological to psychosocial in nature and include, among others, pregnancy and delivery complications, location of birth/residence, and family environment. A few representative examples of these are considered below.

During pregnancy, environmental factors that have a potential negative impact on the developing fetus and have been implicated as risk factors for schizophrenia include pre-eclampsia (resulting in reduced blood supply), exposure to toxins (e.g. alcohol, radiation), and exposure to viral infections, including measles, influenza, meningitis and the common cold (Tsuang and Faraone, 1995).

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The times of greatest vulnerability to the developing brain may include the second and third trimesters of pregnancy. During this period, environmental factors may disrupt neuronal migration of cells to the cortex, resulting in abnormal development of the prefrontal cortex, entorhinal cortex and hippocampus (Arnold and Trojanowski, 1996). Delivery complications associated with increased risk for schizophrenia include fetal hypoxia, ischaemia, extreme prematurity, low birth weight and dysmaturity (post-term birth). Seasonality of birth has also been implicated, with winter–spring births being associated with increased risk for schizophrenia (Jones and Cannon, 1998). Although it seems that this increase could be due to a higher incidence of maternal infection (e.g. influenza), there have been conflicting reports. For example, Battle *et al.* (1999) demonstrated in a large US statewide sample that the incidence of schizophrenia correlated significantly with winter births but not with statewide incidence of influenza or measles. However, they did not look specifically at rates of maternal infection.

Overall, pre-eclampsia has been implicated as the highest individual obstetric risk factor for schizophrenia (Jones and Cannon, 1998). Pre-eclampsia, leading to hypoxia during pregnancy, results in fetal malnutrition, including lack of oxygen, iodine, glucose and iron. Chronic hypoxia can result in restricted fetal growth and damage to brain regions, including the basal ganglia. Moreover, blood and oxygen deprivation due to pre-eclampsia during delivery can also result in injury to regions of the brain, including the hippocampus and cortex (Dalman *et al.*, 1999). Seidman *et al.* (2000a), utilizing the New England cohort of the National Collaborative Perinatal Project, demonstrated a relationship between obstetrical complications and neuropsychological deficits in children at 7 years of age. Low birth weight had the strongest association with neuropsychological impairments, followed by an index of inferred hypoxic insults, and then by maternal conditions suggesting chronic hypoxia. Jones and Cannon (1998) reviewed significant relationships between hypoxia during delivery and genetic risk factors in predicting enlarged ventricles in adult schizophrenics. Recently, Zornberg *et al.* (2000) reported results from a 19-year follow-up study of a large sample of individuals with a previously documented history of birth complications and matched controls. The individuals with a history of birth complications were classified according to whether the complications were related to hypoxic ischaemia. A significant relationship was reported between hypoxic ischaemia-related complications and increased risk for schizophrenia.

Zornberg *et al.* (2000) suggested that pregnancy and birth complications interact with genetic liability and increase the chance for developing schizophrenia in an already at-risk child. Consistent with this view, Cannon (1996) found a dose-dependent relationship between risk of schizophrenia and severity of perinatal hypoxia. In addition, birth complications were unrelated to the development of schizophrenia in a control low-risk group whose parents did not have schizophrenia. Pregnancy and birth complications themselves occur more frequently in schizophrenic mothers compared with normal controls (Geddes and Lawrie, 1995; Jablensky, 1995; Lewis and Murray, 1987; Tsuang and Faraone, 1995), which raises the level of risk for their (already vulnerable) children even further. Prenatal famine is another environmental contingency related to schizophrenia and other psychiatric disorders. Brown *et al.* (1999), for example, showed that conditions of famine at the time of conception were associated with subsequent elevations in rates of nonaffective psychoses, including schizophrenia, while famine in the second or third trimester was associated with subsequent elevation in rates of affective psychoses.

There is an ongoing discussion of urban birth as a potential risk factor for schizophrenia. Studies have reported an association between urban birth and later development of schizophrenia, and a correlation between population density and the incidence risk ratio (IRR) (Marcelis *et al.*, 1998; Torrey *et al.*, 1997). Potentially, the risk associated with urban birth could be due to complications during pregnancy or birth, although Eaton *et al.* (2000) demonstrated that increased risk due to urban birth was not associated with obstetric complications in individuals born in Copenhagen versus those born in rural areas of Denmark. It is possible that this effect could be one not of urban birth but of urban residence. In this case, the risk of developing schizophrenia could be due to environmental stressors inherent in city living that compound the risk to already genetically liable individuals (Marcelis *et al.*, 1998; Mortensen *et al.*, 1999).

Although the double-bind hypothesis of the schizophrenogenic mother whose contradictory messages produce schizophrenia in her child is no longer held widely, there are other factors within the family environment implicated as sources of potential risk. Kinney *et al.* (1997), using the Danish sample, found elevations of the thought disorder index (TDI) in biological relatives of schizophrenic patients compared with normal controls. In contrast, elevations of the TDI were not evident in the adoptive relatives of either schizophrenic or control subjects. Findings from the Finnish adoption studies (Tienari, 1991; Tienari *et al.*, 1994; Wahlberg *et al.*, 1997) were consistent with those reported by Kinney and colleagues. For instance, Wahlberg *et al.* (1997) demonstrated that symptoms of thought disorder in offspring of schizophrenic mothers were more probable when they had been raised by adoptive mothers who themselves showed elevated levels of 'communication deviance'. In contrast, offspring of schizophrenic mothers who were raised by adoptive parents with low communication deviance were less likely to show thought disorder. There was no relationship between thought disorder in control adoptees and communication deviance in the adoptive parents. In other words, these findings did not detect the presence of a schizophrenogenic environment for individuals who did not demonstrate a pre-existing genetic liability.

Additional family environmental factors that potentially interact with genetic risk include early absence of the father (Walker *et al.*, 1981), and biological factors within families, such as diet, frequently used medications and infectious agents (Torrey and Yolken, 2000). Malaspina *et al.* (2001) showed that increased paternal age was associated with elevated risk of schizophrenia and related disorders in offspring, but was not related to other psychiatric disorders. Walker *et al.* (1981) demonstrated in two groups of at-risk children that the group that experienced the loss of a parent had a higher rate of developing schizophrenia. Torrey and Yolken (2000) made a case for the transmission of infection to family members by house cats. In their view, viruses, bacteria, fungi, parasites, etc., carried by cats could have a greater impact on an already genetically vulnerable individual. This discussion underscores the point that genetic factors alone do not explain the development of schizophrenia, and that a multiplicity of interactions with the environment likely mediate the development and course of the disorder.

IMPACT OF GENE AND ENVIRONMENTAL INTERACTION: SCHIZOTAXIA

The mechanisms by which genetic and environmental factors interact to produce the liability for schizophrenia are not understood clearly, but they point to an interactive, neurodevelopmental process. For example, as discussed above, many genetic and environmental risk factors are apparent either before or around the time of birth (Jones and Cannon, 1998). In addition, children at risk for schizophrenia show difficulties in several areas, including motor, intellectual and social development (Erlenmeyer-Kimling, 2000). Severity of childhood deficits is also predictive of later schizophrenia. Furthermore, psychotic symptoms generally manifest after puberty, a time when ongoing brain development, hormonal changes and environmental stressors all co-occur.

Recently, we modified Meehl's use of the term 'schizotaxia' (Meehl, 1962) to describe this liability based on the theoretical premise that the neurobiological basis for schizophrenia is formed by the integrated effect of genes and adverse environmental risk factors. Meehl originally introduced the term 'schizotaxia' as a theoretical construct to describe the unexpressed genetic predisposition to schizophrenia. He proposed that individuals with schizotaxia would develop either schizotypy or schizophrenia, depending on the protection or liability afforded by environmental circumstances. Although he suggested later that schizotaxia need not progress into either of these more overt conditions in all cases (Meehl, 1989), he still hypothesized that most cases would progress to one of those outcomes. Although schizotypy eventually entered the psychiatric nomenclature in the form of schizotypal personality disorder, schizotaxia did not. Instead, it remained a term used by researchers to describe — in a generic sense — the liability to schizophrenia. Four decades of subsequent research with nonpsychotic relatives of schizophrenic patients, however, have provided a preponderance of evidence that schizotaxia is a clinically meaningful condition with distinct psychiatric and neurobiological features.

Our reformulation of schizotaxia (Faraone *et al.*, 2001) describes genetically vulnerable individuals who are probably exposed to early adverse events (e.g. obstetrical complications) that result in abnormal development of certain brain structures. As presented above, this liability presents from childhood as schizotaxia, which is expressed through a combination of cognitive, neurobiological and social skill deficits that vary in severity. For most individuals, the condition remains stable throughout their lifespan, but for some, a combination of the liability with later adverse environmental events (e.g. substance abuse or stressful psychosocial circumstances) may produce schizotypal personality disorder or psychosis. The effects of psychosis may then lead to chronic schizophrenia. That is, schizotaxia plus psychosis results in the DSM or *International Classification of Diseases* (World Health Organization, 1992) (ICD) syndrome of schizophrenia. In this view, however, psychosis itself may not necessarily relate to the specific aetiology of schizophrenia (see also Schizotaxia and alternative phenotypes for schizophrenia below). If this model is substantially accurate, then it follows that psychosis and the subsequent diagnosis of schizophrenia are events that occur well after the expression of the genetic liability to schizophrenia was first manifested, rather than at its onset. This view is consistent with the notion of an underlying continuum of genetic liability that has schizophrenia as only one of its possible outcomes.

Schizotaxia is a broader construct than schizophrenia. Our empirical studies suggest that the basic symptoms of schizotaxia occur in 20–50% of first-degree relatives of schizophrenic patients (Faraone *et al.*, 1995a; Faraone *et al.*, 1995b). In comparison, only about 10% of relatives will become psychotic, and less than 10% will develop schizotypal personality disorder (Battaglia *et al.*, 1995; Battaglia and Torgersen, 1996). These figures suggest that schizotaxia does not lead inevitably to schizotypal personality or to schizophrenia, but in most cases is a stable, long-term condition.

Recently, we described preliminary research diagnostic criteria for schizotaxia (Tsuang *et al.*, 1999) based on two features that occur frequently in nonpsychotic relatives of patients with schizophrenia: negative symptoms and neuropsychological deficits (Faraone *et al.*, 2001; Seidman, 1997; Tsuang *et al.*, 1991). All subjects were first-degree biological relatives of schizophrenic patients who met predetermined inclusion criteria (Tsuang *et al.*, 1999). The next step is to validate the proposed syndrome of schizotaxia. Consistent with guidelines advanced by Robins and Guze (1970), it will be necessary to assess the validity of the syndrome with converging evidence from multiple domains. Thus far, two lines of evidence support the proposed syndrome. First, concurrent validation of schizotaxia was obtained by comparing subjects who met our clinical criteria for schizotaxia with those who did not on independent measures of clinical function (Stone *et al.*, 2001). On each of these scales, irrespective of whether they were rated by the subjects or the investigators (blindly), the schizotaxic subjects showed poorer clinical or social function than did subjects who did not meet criteria for schizotaxia. These differences were not attributable to age, IQ, education, parental education, family genetic loading, gender or comorbid psychiatric disorders.

The second line of support for the validity of schizotaxia involves the response to treatment (Tsuang *et al.*, 1999). Six of the eight pilot subjects who met our criteria for schizotaxia agreed to receive a brief 6-week trial of low-dose risperidone (up to 2.0 mg per day) (see Tsuang *et al.*, 1999 for a report on the first four cases). During the risperidone trial, five of the six individuals reported increased cognitive abilities, and three of the six individuals reported greater levels of interest in, and enjoyment of, social activities. Objective assessments showed that five of six subjects demonstrated improvements in measures of anhedonia and asociality, and also showed substantial improvements in attention and working memory. The cognitive and clinical improvements in these individuals are encouraging, and a larger, double-blind study is under way to determine whether our initial findings can be replicated.

Schizotaxia and Alternative Phenotypes for Schizophrenia

Schizotaxia is an evolving concept. While our validation studies suggest that our current definition may provide a useful foundation for investigations into this condition, we assume that schizotaxia will eventually encompass additional clinical and neurobiological domains. One implication of this conceptualization is that schizotaxia may provide a more specific expression of the aetiological processes underlying schizophrenia than do clinical symptoms (e.g. psychosis), which appear later in the course of the disorder and which may be more general indicators of severe mental illness than of a particular condition (Tsuang *et al.*, 2000). In this context, several types of alternative phenotypic expressions merit consideration as candidate symptoms for schizotaxia. Examples of these are considered below.

Neuropsychological Deficits

Nonpsychotic relatives show a range of neuropsychological deficits (Cannon *et al.*, 1994; Erlenmeyer-Kimling, 2000; Faraone *et al.*, 2001; Green *et al.*, 1997; Park *et al.*, 1995). As in schizophrenia, deficits in executive functions, attention and working memory, and long-term declarative memory occur consistently (Faraone *et al.*, 2001; Faraone *et al.*, 1995a; Faraone *et al.*, 1995b), which is the reason for their inclusion in our criteria for schizotaxia. These cognitive deficits coexist with each other in relatives but not in control subjects (Toomey *et al.*, 1998), are stable over a 4-year follow-up period (Faraone *et al.*, 1999), are more prominent (e.g. in verbal declarative memory) in 'multiplex' relatives (i.e. more than one close biological relative with schizophrenia) than 'simplex' relatives (i.e. one affected relative) (Faraone *et al.*, 2000), and when present in childhood are predictive of schizophrenia-related psychosis in adulthood (Erlenmeyer-Kimling *et al.*, 2000).

Social Function

Poor social adjustment has been demonstrated in relatives of schizophrenic patients. Problems in social adjustment included poor peer engagement (especially with members of the opposite gender), immaturity and unpopularity with peers (Hans *et al.*, 2000). Moreover, Toomey *et al.* (1999) showed that compared with controls, relatives of schizophrenic patients exhibited deficits in perception

of nonverbal social cues when assessed with the profile of nonverbal sensitivity test (PONS). These deficits correlated significantly with slower reaction times on vigilance tasks, supporting earlier findings that suggest impairments in attention contribute to development of social deficits (Dworkin *et al.*, 1993; Erlenmeyer-Kimling and Cornblatt, 1992).

Psychophysiological Function

Nonpsychotic adult relatives of schizophrenic individuals demonstrate a diminished ability to filter sensory input from the environment. For example, relatives show difficulties in smooth pursuit eye-tracking (Levy *et al.*, 1994; Park *et al.*, 1995; Ross *et al.*, 1998a; Ross *et al.*, 1998b), pre-pulse inhibition (Braff *et al.*, 1999) and P50 suppression (Adler *et al.*, 1998), which are similar to deficits observed in schizophrenic patients (Clementz *et al.*, 1998; Friedman and Squires-Wheeler, 1994; Waldo *et al.*, 1994). Moreover, the likelihood of having a deficit in P50 suppression increases as the degree of biological relatedness to an individual with schizophrenia increases (Waldo *et al.*, 1995; Waldo *et al.*, 1991).

Neuroimaging

Imaging techniques also show structural brain abnormalities in nonpsychotic adult relatives that are similar to those found in schizophrenic patients. For example, structural magnetic resonance imaging (MRI) studies indicate that nonpsychotic relatives of schizophrenic patients have significant volume reductions in the hippocampus, right amygdala, putamen, left thalamus, cerebellum and brainstem, and significantly increased volumes in the pallidum, as compared with matched controls (Seidman *et al.*, 1997b).

Moreover, structural and functional abnormalities are associated with the psychophysiological and neuropsychological deficits often found in relatives. For example, degree of perfusions in the left inferior prefrontal and anterior cingulate cortex in relatives of schizophrenic patients showed significant correlations with verbal memory and P300 amplitude in the left inferior prefrontal cortex and with P300 latency in the anterior cingulate cortex (Blackwood *et al.*, 1999). Recently, Seidman *et al.* (2000b) showed that deficits in verbal declarative memory were associated with smaller left hippocampal volumes and with the degree of genetic loading for schizophrenia (i.e. it was greater in multiplex than in simplex relatives). In a study utilizing functional MRI, Seidman *et al.* (1997a) demonstrated that in comparison to matched controls, nonpsychotic relatives of schizophrenic patients were impaired significantly on working memory tasks with interference. The tasks produced activation in the lateral and medial frontal cortex, posterior parietal and prefrontal cortex, and thalamus in both groups, but compared with controls the relatives had a greater number of extraneous, bilateral activations during the tasks they performed worse on.

CONCLUSIONS AND FUTURE DIRECTIONS

A century of research shows that schizophrenia has a significant genetic component, but that environmental factors are also critically important in the expression and course of the disorder. Where do we go from here? Certainly, technological advances in molecular biology and other fields will facilitate the search for susceptibility genes for schizophrenia, which will clarify its genetic basis further. But it is also critically important that conceptual progress on the nature of the disorder keep pace. This is true in the area of diagnosis, and especially so in the attempt to understand how clinical symptoms are related to underlying aetiology.

Thus far, clinical diagnosis has predominated as the method of identifying and classifying schizophrenic individuals and their affected versus unaffected family members for research studies and clinical treatment. Although diagnosis has improved substantially in the last two decades, it is far from precise due to the variety and range of clinical expression of schizophrenia and related disorders. Moreover, attempting to classify relatives who carry the liability to schizophrenia but exhibit few, if any, clinical symptoms is even more challenging. Yet this is a goal that must be attained in order to progress in our understanding of schizophrenia's genetic and environmental determinants.

In this context, the conceptualization, validation and study of schizotaxia have important implications for future research and clinical treatment. First, as schizotaxia may provide a more specific expression of the aetiological processes underlying schizophrenia than do clinical symptoms (e.g. psychosis), this model could aid in the facilitation of future research. For example, family studies (e.g. linkage studies) could benefit from a more sensitive method of identifying relatives who carry the liability for schizophrenia. More generally, the use of alternative clinical symptoms, and of neuropsychological and neurobiological measures, underscores their potential importance in parsing out genetic and environmental influences on schizophrenia. Moreover, as susceptibility genes for schizophrenia are identified in schizotaxic individuals, specific biological products (e.g. proteins) of those genes factors will also become identifiable. Consequently, the identification of more specific genetic-environmental interactions will become possible, which may also open up new avenues for understanding how environmental factors affect the liability (in positive or negative directions) for schizophrenia.

Second, the components of schizotaxia provide new potential targets for the development of treatments aimed at modifying fundamental phenotypical expressions of schizophrenia. The treatment of schizotaxia in nonpsychotic relatives could serve to alleviate symptoms that are clinically meaningful but are overlooked in the absence of a valid diagnosis. Perhaps even more importantly, the successful treatment of schizotaxia might serve to facilitate longer-term strategies aimed at the prevention of schizophrenia itself. This would be the ultimate environmental modification of the liability to the disorder, and the one that will attract increasing attention in the coming years.

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XVII-11

Gender Issues in Schizophrenia

David J. Castle and Robin M. Murray

This chapter concerns gender differences in schizophrenia. We start with an overview of the general differences between men and women that may be relevant to gender differences in schizophrenia. Differences between males and females with schizophrenia are then detailed, including epidemiology and clinical features, as well as aetiological factors. Finally, we offer an integration of these findings.

There are, of course, myriad differences between males and females in psychological and social characteristics. Here, we will concentrate on biological domains rather than psychosocial differences. Our justification for this is that the gender differences in schizophrenia seem to operate across cultures; therefore, specific cultural influences are unlikely to be of major effect in explaining such gender differences. Having said this, some psychosocial factors may be of importance in mediating certain differences between men and women with schizophrenia, and these are addressed at the end of the chapter.

GENDER DIFFERENCES IN THE HUMAN BRAIN

The brains of males and females differ from each other in subtle but important ways. The roots of such differences lie ultimately in genetic make-up; indeed, neural cultures from male and female brains show sex-specific morphological and functional characteristics even in the absence of sex hormones (Reisert and Pilgrim, 1991). Nevertheless, hormonal factors are of undoubted importance in mediating the effects of genes, particularly the masculinizing effects of testosterone, which, in humans, is secreted from the male fetal testes at around week 9 of intrauterine development. One of the effects of testosterone is, in essence, to slow the rate of development of the male brain. Thus, Taylor (1969) hypothesized that the male brain lags behind the female brain in rate of myelinization and establishment of neural connections; it appears that the most profound impact is on the left hemisphere.

Differential development of male and female brains also results in structural sex differences. Not only is the male brain generally larger than the female brain, but there are more specific differences. For example, the size of the preoptic nucleus of the hypothalamus is relatively larger in males than females (Swaab and Fliers, 1985). There also appears to be a difference in lateralization, with the male brain being more asymmetrical than the female brain. Thus, Wada *et al.* (1975) reported left–right asymmetry in the planum temorale more commonly in males then females. In rats, there are sex differences in lateralization of the hippocampal-dentate complex (Diamond, 1989). Witelson (1989), after extensive investigations of gender effects in size and shape of the corpus callosum, concluded that the isthmus of this bridge between the hemispheres tends to be larger in females; she went on to speculate that this was a reflection of greater bilateral representation of cortical functions in females. These differences translate into functional differences between the sexes in performance on lateralized neurocognitive tasks, with females tending to show less lateralization of function (McGlone, 1980).

The relatively slow development of the male brain may result in it being vulnerable for a longer period than the female brain to adverse neurodevelopmental insults. For example, Amato *et al.* (1987) showed that among low-birthweight infants, males are more likely to suffer from peri-intraventricular haemorrhage. Furthermore, males are more likely to suffer longer-term adverse consequences from such early insult, perhaps the greater laterality of functioning leaving the male brain less able to compensate for lateralized brain damage. Brothwood *et al.* (1986) found that low-birthweight males were more likely than their female counterparts to be impaired developmentally at the age of 2 years. It was also reported that children exhibiting minor neurological dysfunction at age 12 years were most likely to be males and to have suffered from preterm birth or other early environmental adversity, notably hypoxia. Grimm and Frieder (1985) found in rats that prenatal brain insult resulted in impaired performance on complex learning tasks in males but not in females.

Perhaps as a consequence of this relative vulnerability of the male brain, males are far more likely than females to suffer from a range of neurodevelopmental disorders, e.g. severe mental retardation, autism and Asperger's syndrome. Such disorders have the following as their most consistent characteristics (see Goodman, 1990):

- left-sided brain dysfunction, evidenced by increased rates of non-right-handedness;
- association with early brain insult;
- over-representation among individuals with brain abnormalities.

THE AGEING BRAIN

Not only do male and female brains develop differently, but they also age differently. For example, Murphy *et al.* (1996) showed that in comparison with female brains, the male brain exhibits greater loss of whole-brain and frontal and temporal volume with age, while female brains show more rapid loss in the parietal lobe and hippocampus. Also, female brains exhibit relatively more age-related decline in the thalamus and hippocampus. There is also some evidence that women are more prone to Alzheimer's disease, even once the longer survival of women is taken into account; this has been related to the effect on the female brain of withdrawal of oestrogen (Henderson, 1997). A similar explanation has been put forward to explain the excess of women among late-onset schizophrenia patients (see below).

Of possible relevance to schizophrenia is the evidence that rates of decline of brain dopamine D2 receptors differ between men and

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women. Thus, males start life with a relative excess of dopamine D2 receptors but lose them more rapidly, such that by mid-life females have a relative excess (Wong *et al.*, 1994; Pohjalainen, 1998). Given that schizophrenia has often been regarded as a state of functional dopamine excess, this has potential clinical implications.

BEHAVIOURAL DIFFERENCES BETWEEN MALES AND FEMALES

Differences in behaviour between boys and girls are evident from early life. Boys are more likely than girls to exhibit overactivity and difficulties in concentration, while girls are more likely to exhibit fearfulness (Earls, 1987). Sex differences in socialization have also been noted, with girls being overall more sociable and interested in others, and boys being more likely to be content with solitary play (Garai and Scheinfeld, 1968). Some authors believe that these gender differences continue into adult life and form the basis for the greater frequency of negative symptoms in males, relative to females, with schizophrenia (see below). This line of reasoning implies that these characteristics of psychotic patients are merely a reflection of the behavioural characteristics of males and females in the general population.

GENDER DIFFERENCES IN THE EPIDEMIOLOGY OF SCHIZOPHRENIA

It is generally considered that the lifetime risk of schizophrenia is roughly equal between men and women. However, this belies the fact that male : female ratios are affected markedly by the diagnostic criteria that are applied. For example, Castle *et al.* (1993) showed, in a representative case register sample, that although the application of broad International Classification of Diseases, 9th edition (ICD-9) (World Health Organization, 1978) criteria result in a near even male : female ratio (1.08 : 1), application of the somewhat more stringent Research Diagnostic Criteria (RDC) criteria enhanced the sex differential (male : female ratio 1.15 : 1), and the even more stringent *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised (DSM-IIIR) (American Psychiatric Association, 1987) criteria produced a male : female ratio of 1.35 : 1. Furthermore, age at onset affected the gender ratios profoundly. For example, whilst applying ICD-9 criteria generated a male : female ratio of 1.41 : 1 in subjects with an onset before the age of 45 years, applying DSM-IIIR criteria produced a ratio of 2.13 : 1 in this group. In subjects with an onset of illness after age 45 years, females predominated, irrespective of the diagnostic criteria applied (e.g. female : male ratios of 1 : 0.61 for ICD-9 criteria and 1 : 0.58 for DSM-IIIR criteria).

Numerous studies have demonstrated that the mean age of onset is earlier in males than females with schizophrenia; this has been reported in samples ascertained by widely differing methodologies and across a number of different sociocultural settings (see Castle, 2000). However, many studies report only mean age-at-onset differences between the sexes, and reliance on means can be criticized on the basis that male and female onset distributions are not distributed parametrically. Indeed, disparate studies from a number of different geographical settings have shown that the pattern of onset distribution is a complex one, and that the distributions for men and women are not isomorphic. For example, in a case register sample, Castle *et al.* (1998) showed that the male distribution encompasses at least two peaks, the predominant peak being of early onset (mode 21.4 years) and the other (lesser) peak manifesting in mid-life (mode 39.2 years). In females, the early peak (mode 22.4 years) is less dramatic, but the mid-life peak (mode 36.6 years) exceeds that for males. Furthermore, females show a further peak in late life (mode 61.5 years). These results have, in large part, been replicated independently (see McLachlan *et al.*, 1998).

Almost universally in the west, age of onset of schizophrenia is significantly earlier in males than females. This is also mostly the case for samples from the developing world, but there are several studies from India in which this gender difference disappears (Murthy *et al.*, 1998). The reason for this is unclear, but one suggestion has been that schizophrenia in India includes a greater proportion of brief psychotic conditions than in the west. A further exception to the earlier onset in males is in those cases of schizophrenia from families that are multiply affected by the disorder. Here, the gender difference in onset is diminished markedly and, in several studies, disappears (Walsh *et al.*, 1996). This might imply that the earlier age of onset is an environmental effect caused, for example, by the greater frequency of drug abuse in males or the effect of pregnancy and birth complications (Kirov *et al.*, 1999).

There has been considerable interest in gender ratios for schizophrenia in the very young and the very old. Since age at onset tends to be earlier in males across adult life, one might anticipate that very-early-onset (adolescent and preadolescent) schizophrenia should be more common in males. However, there remains some doubt about this. This may reflect in part the rarity of the disorder at this age and the difficulty in reaching a definitive diagnosis at this age. Furthermore, many of the larger case series are from specialist tertiary referral units and might well be biased and unrepresentative. In reviewing this area, Orr and Castle (in press) concluded that it is probable that in adolescent cases, males and females are roughly equally prone but that males predominate in cases of prepubertal onset.

There is no doubt about gender differences in prevalence of very-late-onset schizophrenia (i.e. onset after age 60 years). Castle (1999), in reviewing the epidemiology of late-onset schizophrenia, established male : female ratios for very-late-onset schizophrenia from a number of studies from different treatment settings and that had used different diagnostic criteria. All studies that had included both sexes had an excess of females, with male : female ratios ranging from 3.1 : 1 to 22.5 : 1. These findings are not simply a reflection of the relative longevity of females.

CLINICAL DIFFERENCES BETWEEN MALES AND FEMALES WITH SCHIZOPHRENIA

Overall, premorbid adjustment appears to be better in females than males who later develop schizophrenia. This superiority covers social and school and work functioning (reviewed by Seeman and Fitzgerald, 2000). Of course, such conclusions are based on grouped data and belie the heterogeneity in premorbid functioning in schizophrenia; indeed, some females with schizophrenia show marked developmental deviance. Partly, gender effects are confounded by age at onset, with an earlier onset being (in general) associated with worse premorbid functioning, but this does not explain away the entire gender effect (Preston *et al.*, in press).

In terms of symptoms, gender differences are confounded by the diagnostic issues alluded to above. In particular, females are more likely than males to exhibit affective symptoms and to be excluded when stringent diagnostic criteria for schizophrenia are applied. However, even after controlling for such factors, females with schizophrenia tend to show more affective features and fewer negative symptoms than their male counterparts (see Seeman and Fitzgerald, 2000).

The longitudinal course of schizophrenia tends to be more benign in females than males; this applies to hospitalization, social functioning and occupational outcome. Females also tend to show a better response to antipsychotic treatment, and require lower doses

of such medication, at least premenopausally (reviewed by Seeman and Fitzgerald, 2000).

AETIOLOGICAL PARAMETERS

Genetics

The most important and best established aetiological factor for schizophrenia is genetic predisposition. Although the mode of inheritance has not been established absolutely, biometrical and molecular genetic studies have all but ruled out the existence of major genes of large effect. Thus, the current consensus is that a number of genes of small effect interact with each other and with a number of small environmental effects to project the individual across the threshold for the expression of schizophrenia.

In terms of gender, a number of studies have assessed family loading for schizophrenia in males and females with schizophrenia, and found that the morbid risk of schizophrenia is greater in the relatives of females than males with schizophrenia (see Goldstein and Lewine, 2000). One study reported an excess of schizophrenia amongst relatives of female probands, but it also found that schizotypal personality disorder was over-represented in the families of males with schizophrenia. These relatives were also more likely to exhibit flat effect, putatively an expression of subsyndromal phenomenology of the disorder (see Goldstein, 1995).

Early Developmental Insult

Considerable evidence points to early developmental insult being important in the aetiology of at least some cases of schizophrenia. The putative mechanism is a disruption of the normal development of the brain at certain crucial phases, leaving the individual with aberrant neural networks at risk for the later manifestation of psychosis as the brain matures.

Apart from genetically mediated developmental mechanisms, the most robust findings in this area are those implicating pregnancy and birth complications. Several meta-analyses (Verdoux *et al.*, 1997; Geddes *et al.*, 1999) have shown that exposure to obstetric complications approximately doubles the risk of the disorder, and that such complications appear to be associated especially with schizophrenia of relatively early onset. In terms of gender, a number of studies suggest that pregnancy and birth complications are more common in males (Hultman *et al.*, 1999), but this is not a consistent finding (Verdoux *et al.*, 1997).

Intrauterine infections have been another putative candidate for early neurodevelopmental insult that raise the risk of later schizophrenia. Most of the evidence is based on population samples exposed to epidemics of influenza viruses. Such studies inevitably suffer from ecological bias, but nevertheless they are of interest. Few of these studies have examined gender differences explicitly, but those that have tend to show that the effect is more potent in females than males (reviewed by McGrath and Castle, 1995).

Early Head Injury

Another early developmental event of putative importance in the risk of schizophrenia is childhood head injury. Here, the literature is scant and confounded by the potential for preschizophrenic children to be more clumsy than their counterparts not at risk for later illness. One study (Nasrallah and Wilcox, 1989) reported early head injury to be a feature of males rather than females with later schizophrenia, but we are not aware of replication of this finding, and indeed the result needs to be interpreted in the context of an overall male predisposition to such injury. Recently, Neilsen *et al.* (submitted) examined the rates of head injury in 8288 Danes suffering their first episode of schizophrenia. After adjusting for the greater risk of other fractures in males, both concussion and head injury were found to be increased significantly in male but not female, subjects.

Illicit Substance Use

It is well established that people with schizophrenia show a high rate of use of illicit substances, particularly substances with a dopaminergic effect such as amphetamines, cocaine and cannabis, and that this is more the case for males than females with schizophrenia (Jablensky *et al.*, 2000). The latter is, at least in part, a reflection of the fact that in the general population, males are more likely than females to abuse drugs. However, it may also be that males with more severe illness are more likely to use substances to alleviate negative symptoms or to reduce side effects of antipsychotic medications. Furthermore, males with early-onset illness are likely to be particularly socially impaired and might use illicit substances to aid socialization. It is also generally accepted that such substances can exacerbate psychotic symptoms and worsen the longitudinal course of the disorder. However, cause and effect are difficult to disentangle.

There remains controversy as to whether illicit substances such as cannabis actually increase the risk of development of schizophrenia rather than simply precipitate relapse in those with an extant vulnerability. The only published study of which we are aware that can begin to answer this is the Swedish conscript cohort study of Andreasson *et al.* (1987). This study suggested cannabis use at conscription to predict later schizophrenia. No conclusions can be drawn about any gender effects because the cohort was exclusively male.

NEUROCOGNITION AND NEUROIMAGING

Males and females exhibit fairly consistent group differences on tests of neuropsychological functioning (see Kimura, 1992). Thus, males tend to perform better on tests requiring imaginal rotation of objects, navigating through a route, and target-directed motor skills; they also tend to excel in mathematical reasoning. Females, on the other hand, show relatively greater aptitude for test of perceptual speed, arithmetical calculation, recalling landmarks form a route, and certain precision manual tasks. Girls also generally outperform age-matched boys on tests of verbal fluency (McGee, 1982). These parameters underline the differences in organization and function of male and female brains, and are relevant to gender differences in schizophrenia in terms of both aetiological parameters and interpretation of neuropsychological findings in schizophrenia patients.

There is long-standing evidence that preschizophrenic boys show lower IQ than preschizophrenic girls (Aylward *et al.*, 1984), a difference reflective of the poorer performance of the former in a range of psychological and social functions. A number of studies of adult schizophrenia patients have also found that males show more severe neuropsychological impairment than females (Goldstein *et al.*, 1998). However, when schizophrenia is defined narrowly, neuropsychological differences between the sexes tend to disappear (Hill *et al.*, 2001).

Ames (1991) points out that in the normal population, adult females tend to be superior to their male counterparts on tests of verbal memory while males are superior on certain visuospatial tests. Since verbal memory is impaired in schizophrenia, it is possible to speculate that one reason for the greater susceptibility of males to severe schizophrenia (see below) could be that their lesser competence in verbal memory places them nearer the threshold for expressing the disorder.

There is a large and inconsistent literature on brain imaging in males and females with schizophrenia (Goldstein and Lewine,

2000). In general, it appears that male patients show more severe brain structural abnormalities; indeed, some studies have failed to show any differences in these parameters between female patients and controls. Thus, McDonald and Murray (2001), in a recent study of families where several members were affected with schizophrenia, found that the brain imaging differences between patients and controls, and relatives and controls, were accounted for wholly by the male patients and relatives, respectively.

INTEGRATION

We have reviewed gender differences in schizophrenia, pointing out those parameters that appear most consistently to differentiate the sexes. These include:

- an excess of males in strictly defined samples of schizophrenia patients;
- a difference in onset distributions, with males greatly over-represented amongst patients with an onset in early adult life, and females showing an excess in mid-life, and a massive female preponderance in very-late-onset schizophrenia;
- poorer premorbid functioning in males;
- more negative symptoms in males;
- more affective symptoms in females;
- a worse longitudinal course in males;
- a more favourable response to antipsychotic medication in females, at least before menopause;
- more impairment of neurocognitive functioning in males;
- possibly an excess of brain abnormalities in males;
- probably a higher morbid risk of schizophrenia in the relatives of females with schizophrenia;
- possibly an excess of early developmental insult in males.

How can we integrate these findings? As we noted in the introduction, we concentrate here on biological explanations for these gender differences, as most of the differences appear to operate across cultures, and thus psychosocial parameters are likely to exert only a modest modifying role in their determination. We consider the biological factors in two main domains, namely hormonal influences, and brain organizational and structural differences.

HORMONAL INFLUENCES

Pubertal and postpubertal hormonal differences between the sexes bear consideration in trying to explain gender differences in schizophrenia. For example, testosterone production in the nonpsychotic adolescent male probably accounts at least in part for the upsurge of aggressive, impulsive and risk-taking behaviours at that age. Arguably, this could result in males with schizophrenia causing more domestic and peer-related disruption, and cause them to come into contact with psychiatric services earlier than their less aggressive female counterparts. However, this is unlikely to be a major effect, given that the lag between first symptom onset (either prepsychotic or psychotic) and first psychiatric contact is much the same for both genders (see Hafner *et al.*, 1989).

Of more compelling interest in terms of hormonal effects is the putative protective effect of oestrogens in females. Oestrogens have been shown in animal and laboratory studies to block dopamine D2 receptors and to reduce haloperidol-induced cataplexy (Hafner *et al.*, 1991; Kulkarni and Fink, 2000). Clinical studies suggest that women with schizophrenia are more vulnerable to relapse at times in their menstrual cycle when endogenous oestrogen levels are low, whilst pregnancy, associated with high oestrogen levels, is believed to be a time during which women with schizophrenia enjoy relative wellness in terms of psychotic symptomatology (see Lewine and Seeman, 1995). The postpartum period, during which oestrogen levels plummet, is associated with a particular vulnerability to psychotic illness, even in women with no prior history of psychosis (Kendell *et al.*, 1987). Furthermore, Hafner *et al.* (1989) have suggested that the second peak in schizophrenia in females in mid-life (see above) is a consequence of menopausal fall in oestrogen levels and the removal of the protective effect on the dopamine system.

Seeman and Lang (1990) have therefore implicated the protective effect of oestrogen in explaining some of the gender differences in schizophrenia outlined above. This hypothesis gains some support from *in vivo* studies of the effects of oestrogen on schizophrenia symptoms. For example, Kulkarni *et al.* (1996) claim that the administration of oestradiol to psychotic women as an adjunct to antipsychotic medication results in an accelerated amelioration of psychotic symptoms.

There are, however, some shortcomings to the oestrogen hypothesis. These include the fact that childhood premorbid function is characteristically poorer in males than females who later develop schizophrenia, and this obviously cannot be accounted for by direct hormonal influences as it antedates puberty. The mid-life peak in onset of schizophrenia has never been shown to be correlated directly with oestrogen levels and, indeed, there is also a mid-life peak (admittedly less pronounced) in males. Also, the very-late-onset peak in females occurs many years after the menopause. It has also been found that in families with a high genetic loading for schizophrenia, the females do not show the usual later onset of the disorder relative to males (see above). Furthermore, the data pertaining to pregnancy being a time of psychiatric well-being for women with schizophrenia is equivocal, whilst the postpartum peak in psychotic disorders is due largely to affective psychosis rather than schizophrenia (reviewed by Barkla and McGrath, 2000).

It is probably reasonable to conclude, then, that oestrogenic effects may go some way towards explaining some of the clinical and epidemiological gender differences in schizophrenia, but they cannot account for the complexity of these differences and should be seen in conjunction with explanations based on differences in male and female brains, as outlined below.

BRAIN DIFFERENCES AS AN EXPLANATION FOR GENDER DIFFERENCES IN SCHIZOPHRENIA

We have expressed our belief that gender differences in schizophrenia can be understood, at least in part, by an appreciation of brain differences between the sexes (see Castle and Murray, 1991). Thus, the differential maturation of the male and female brain, and in particular the slowed left hemisphere maturation in males, renders the male brain prone to neurodevelopmental insult, as outlined above. The considerable evidence pointing to at least some forms of schizophrenia being consequent upon neurodevelopmental deviance (see Murray *et al.*, 1992) is thus integrated into a typology of schizophrenia based on gender.

Testing this hypothesis, Castle *et al.* (1994) and Sham *et al.* (1996) reported a latent class typology of schizophrenia where males were particularly vulnerable to an early-onset dementia praecox type of illness associated with poor premorbid functioning and negative symptoms, and putatively of neurodevelopmental aetiology. A later-onset schizoaffective subtype, with prominent mood symptoms, affected almost exclusively females, and was interpreted as being related aetiologically to the mood disorders (there was a family loading for depression in this subtype). A further later-onset subtype, with predominant paranoid ideation, affected men and women in roughly equal proportions.

This typology does not encompass very-late-onset schizophrenia-like disorder (late paraphrenia), whose vast female preponderance could be interpreted in the context of degenerative differences between male and female brains, as outlined above (see Castle, 1999).

Thus, a typological approach has some utility in understanding gender differences in schizophrenia, but any such explanatory model needs to be sufficiently adaptable to accommodate hormonal and psychosocial influences, which differentiate the sexes and which play a part in the determination of vulnerability to, and expression of, psychosis in men and women.

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Schizophrenia and Other Psychotic Disorders: Therapeutic Armamentarium

Michael J. Travis

INTRODUCTION

Since the discovery of the antipsychotic effects of chlorpromazine in the early 1950s, we have entered an era of effective pharmacological treatment for schizophrenia and related psychoses. Since the initial 1952 report of reduction in agitation, aggression and delusional states in agitated psychotic patients, a wealth of placebo-controlled trials have established the efficacy of typical antipsychotic (neuroleptic) medication for acute schizophrenia (Davis and Androukis, 1986) and for maintenance in chronic schizophrenia (Gilbert *et al.*, 1995; Viguera *et al.*, 1997).

Antipsychotics have therefore been the mainstay of treatment in schizophrenia and related psychoses for four decades. The knowledge of the chemistry and pharmacology of these medications has led to a greater understanding of the neurochemical basis of psychosis.

In the last decade, the reintroduction of clozapine has led to a new optimism in the treatment of schizophrenia and related psychoses, and the development and introduction of a new generation of antipsychotics, the 'atypicals', which bring the possibilities of fewer side effects and greater efficacy in comparison with the original antipsychotics (typicals). Nevertheless, a proportion of psychotic patients will not respond to standard treatments.

This chapter will selectively review the available literature that can inform our rational use of medication to treat psychosis in the twenty-first century.

SCHIZOPHRENIA AND PSYCHOTIC DISORDERS

Just as schizophrenia is heterogeneous, so are the disorders in which psychotic symptoms can occur. In the absence of large-scale clinical trials in related psychotic disorders, such as persistent delusional disorder, this chapter will focus on the treatment of schizophrenia. There is compelling evidence that medications used to treat schizophrenia are truly antipsychotic rather than antischizophrenic and thus may be used to treat psychotic symptoms in any psychiatric disorder in which they occur.

CLASSIFICATION OF ANTIPSYCHOTICS

Antipsychotic drugs are not a homogeneous group, and there are various classes. The phenothiazines include those with aliphatic side chains, e.g. chlorpromazine, those with piperidine side chains, e.g. thioridazine and pipothiazine, and those with piperazine side chains, e.g. trifluoperazine and fluphenazine. Other classes include the butyrophenones (haloperidol and droperidol), the thioxanthenes (flupenthixol and zuclopenthixol), the diphenylbutylpiperidines (pimozide), and the substituted benzamides (sulpiride and amisulpride). The relevance of grouping the medications by chemical class comes when switching antipsychotics. Patients who do not respond to a medication belonging to one particular chemical class should be switched to a medication of a different chemical class. It is interesting to note from Table XVII-12.1 that each of the newer

Table XVII-12.1 Chemical classification of antipsychotic drugs

Type	Class	Example
Typical antipsychotics	Phenothiazines	
	Aliphatic side chain	Chlorpromazine
	Piperidine side chain	(Thioridazine), pipothiazine
	Piperazine side chain	Trifluoperazine, fluphenazine
	Butyrophenones	Haloperidol (droperidol)
	Thioxanthenes	Flupenthixol, zuclopenthixol
	Diphenylbutylpiperidines	Pimozide, fluspirilene
Atypical antipsychotics	Dibenzodiazepines	Clozapine
	Benzixasoles	Risperidone
	Thienobenzodiazepines	Olanzapine
	Dibenzothiazepines	Quetiapine
	Imidazolidinones	(Sertindole)
	Benzothiazolylpiperazines	Ziprasidone
	Substituted benzamides	Sulpiride, amisulpride (sulpiride is considered by some to be a typical antipsychotic)

Medications in brackets have been withdrawn in the UK.

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antipsychotic medications belongs to a different chemical class, such as dibenzodiazepine (clozapine) and the benzixasole (risperidone). In light of this, the emphasis is now changing to pharmacological rather than chemical classes when considering switching medications.

MECHANISM OF ACTION OF ANTIPSYCHOTICS

Dopamine

The observation from the early use of chlorpromazine that clinical improvement was often accompanied by parkinsonian-like symptoms led to a focus on dopaminergic mechanisms in the neurochemistry of schizophrenia and mechanisms of action for antipsychotics. In 1963, Carlsson and Lindqvist reported that antipsychotics increase turnover of brain dopamine and suggested that this was in response to a functional 'blockade' of the dopaminergic system. Creese *et al.* (1976) demonstrated that the affinity of a wide range of antipsychotics to dopamine D_2 receptors was proportionate to their clinical potency (Figure XVII-12.1).

Emission tomography has reinforced the accepted theory that antipsychotic efficacy is related to D_2 receptor antagonism, with 65% blockade a putative threshold for antipsychotic response (Kapur *et al.*, 2000). Nonetheless, the situation appears very complex in that non-responders to antipsychotics may still show high levels of striatal D_2 blockade (Farde *et al.*, 1992; Pilowsky *et al.*, 1992). Furthermore, the highly effective atypical antipsychotic clozapine produces far less striatal D_2 blockade than typical antipsychotics (Farde *et al.*, 1992; Pilowsky *et al.*, 1992; Broich *et al.*, 1998), often below the putative 65% threshold. These findings have yet to be explained fully.

Extrapyramidal side effects (EPS) have also been studied; a threshold of 80% D_2 occupancy has been shown repeatedly to be necessary to produce EPS (Farde *et al.*, 1995; Kapur *et al.*, 2000). It may be that high levels of serotonin 5-HT_{2A} receptor blockade, as seen with most of the newer antipsychotics, may be protective against EPS by altering this threshold (Kapur and Remington, 1996).

Recent neurochemical imaging studies have indicated that people with schizophrenia have an increased sensitivity of their dopaminergic neurons to amphetamine challenge (Laruelle *et al.*, 1999) (Figure XVII-12.2). Thus, it may be that in response to stress, such people will over-release dopamine, which may drive psychosis.

There are currently five types of dopamine receptors identified in the human nervous system (D_1 to D_5). D_1 and D_5 receptors are similar in that they both stimulate the formation of cyclic adenosine monophosphate (cAMP) by activation of a stimulatory G-coupled protein. D_2 to D_4 receptors act by activating an inhibitory G-protein, thereby inhibiting the formation of cAMP. D_2 receptors are more ubiquitous than D_3 or D_4 receptors. D_3 receptors are situated differentially in the nucleus accumbens, one of the septal nuclei in the limbic system and D_4 receptors are concentrated especially in the frontal cortex.

There are a number of different dopaminergic pathways or tracts (Figure XVII-12.3). The nigrostriatal tract projects from the substantia nigra in the midbrain to the corpus striatum. This tract has a role primarily in motor control, although the ventral striatum has a role in reward- and goal-directed behaviours. It is by blocking dopamine receptors at the termination of this pathway in the dorsal striatum that the parkinsonian side effects of antipsychotics arise. The mesolimbic/mesocortical tract has its cell bodies in the ventral tegmental area adjacent to the substantia nigra. This tract projects to the limbic system and neocortex, as well as the striatum. This dopaminergic innervation supplies fibres to the medial surface of the frontal lobes and to the parahippocampus and cingulate cortex, the latter two being part of the limbic system. It is thought that this tract is where antipsychotic medication exerts its beneficial effect. The third major pathway is the tuberoinfundibular tract. The cell bodies for this tract reside in the arcuate nucleus and periventricular

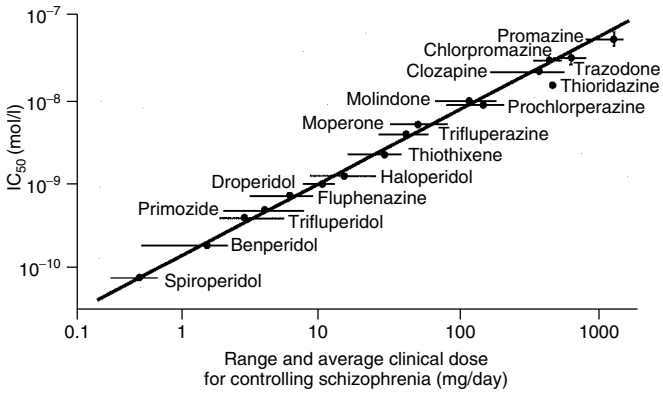


Figure XVII-12.1 Plot of the affinity of a wide variety of antipsychotic medications for the dopamine D_2 receptor against the average clinical daily dose used for controlling schizophrenia, an estimate of clinical potency. There is a direct relationship between these two indices. This replicated finding contributed to the use of high-dose antipsychotics on the assumption that giving higher doses would make an antipsychotic more potent by 'blocking' more receptors

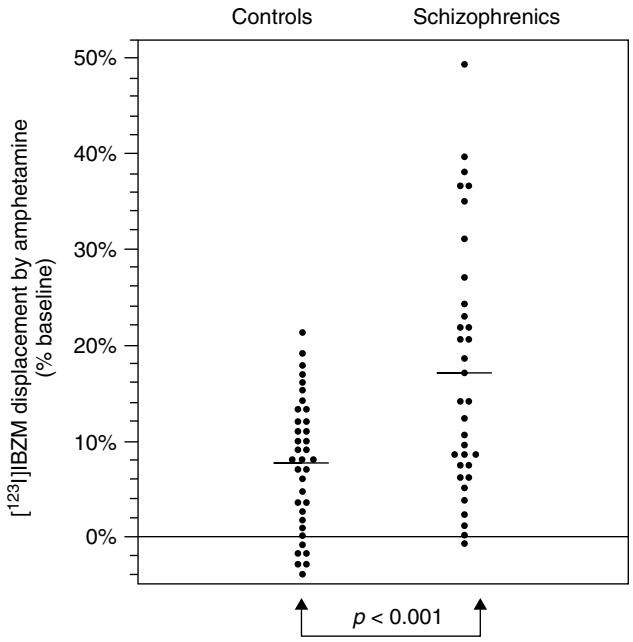
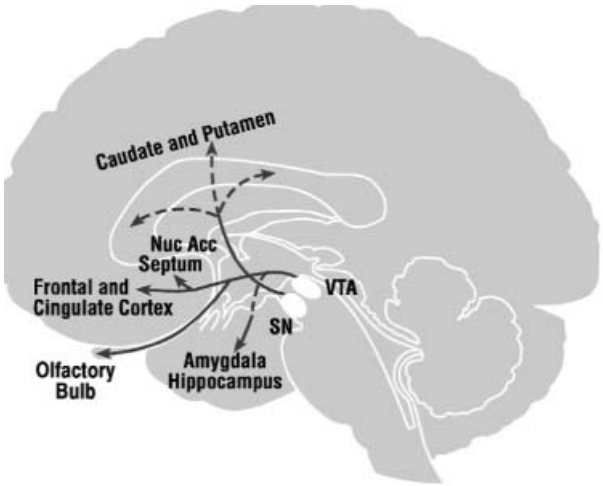


Figure XVII-12.2 Effect of amphetamine ($0.3\,mg\,kg^{-1}$) on [^{123}I]iodobenzamide ([^{123}I]IBZM) binding in healthy control subjects and untreated patients with schizophrenia. [^{123}I]IBZM is a tracer for D_2 receptors, which allows measurement of D_2 receptor availability (or binding potential) in humans *in vivo* using single photon emission tomography (SPET). The *y* axis shows the percentage decrease in [^{123}I]IBZM binding potential induced by amphetamine, which is a measure of the increased occupancy of D_2 receptors by dopamine following the challenge. Thus, these results indicate that when challenged with amphetamine, patients with schizophrenia release more dopamine than healthy controls



Manter and Gatz's Essentials of Clinical Neuroanatomy and Neurophysiology. Edition 8, F.A. Davis Co.: Philadelphia; 1992.

Nuc Acc = nucleus accumbens
SN = substantia nigra
VTA = ventral tegmental area

Figure XVII-12.3 Main dopaminergic tracts in the human brain. The nigrostriatal tract subserves motor control and the tuberoinfundibular tract prolactin secretion. The mesolimbic and mesocortical tracts innervate areas associated with the positive and negative symptoms of schizophrenia

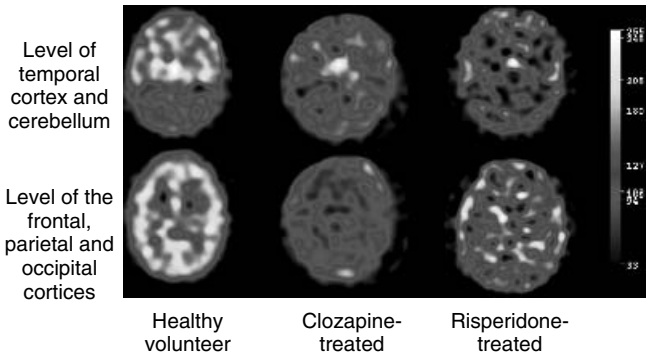
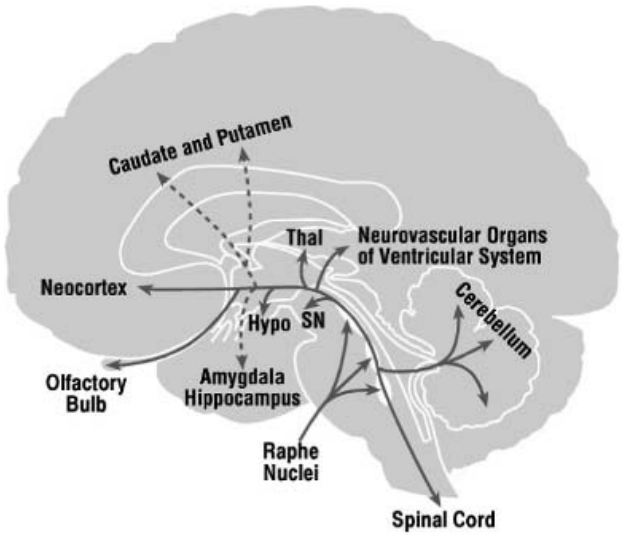


Figure XVII-12.4 ^{123}I-5-I-R91150 SPET brain scan of 5-HT_{2A} receptors in a healthy volunteer on no medication and volunteers with schizophrenia treated with clozapine 450 mg per day or risperidone 6 mg per day indicating a high saturation level of 5-HT_{2A} receptor occupancy with these medications



Manter and Gatz's Essentials of Clinical Neuroanatomy and Neurophysiology. Edition 8, F.A. Davis Co.: Philadelphia; 1992.

Hypo = hypothalamus
SN = substantia nigra
Thal = thalamus

Figure XVII-12.5 Major serotonergic pathways in the human brain. Serotoninergic connections arising from the raphe nuclei are distributed ubiquitously throughout the cortical grey matter

area of the hypothalamus, and project to the infundibulum and, via the portal venous system, to the anterior pituitary. Dopamine acts within this tract to inhibit the release of prolactin. The blockade of these receptors by antipsychotics removes the inhibitory drive from prolactin release and leads to prolactinaemia.

Serotonin

The serotonergic hypothesis of schizophrenia predates the dopaminergic hypothesis and stems from the finding by Woolley and Shaw in 1954 that the hallucinogen lysergic acid diethylamide (LSD) acts via serotonin (5-hydroxytryptamine, 5-HT). Additionally, there is a neuroanatomical and functional interaction of 5-HT and dopaminergic systems such that blocking 5-HT_{2A} receptors enhances dopaminergic transmission (reviewed by Busatto and Kerwin, 1997). The newer atypical antipsychotics, in contrast with the typical antipsychotics, all have a higher affinity for the 5-HT_{2A} receptor than for the D_2 receptor. In terms of treatment response, there is a correlation of serotonergic neuroendocrine responses with symptomatic improvement on clozapine (Curtis *et al.*, 1995), and preliminary data suggest that allelic variations in the 5-HT_{2A} receptor gene vary with, and may predict, treatment response (Arranz *et al.*, 1995).

Previous studies with positron emission tomography (PET) and single positron emission tomography (SPET) have indicated that the atypical and newer antipsychotic medications, such as clozapine, risperidone, olanzapine and sertindole, all lead to almost complete occupancy of cortical 5-HT_{2A} receptors at clinically relevant doses (Farde *et al.*, 1994; Travis *et al.*, 1998; Kapur *et al.*, 1999) (Figure XVII-12.4). Full characterization of the effects of the older typical antipsychotics on 5-HT_{2A} receptors remains to be completed; however, preliminary data indicate that broad-spectrum typical antipsychotics, such as phenothiazines and thioxanthines, also lead to a significant occupancy (or reduction in receptor availability) of cortical 5-HT_{2A} receptors, but the level of occupancy is still significantly lower than that seen with the newer medications (Travis *et al.*, 1998).

More than nine distinct 5-HT receptors have been identified. The 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 subgroups have been studied most extensively. The major site of serotonergic cell bodies is in the area of the upper pons and midbrain. The classic areas for 5-HT-containing neurons are the median and dorsal raphe nuclei. The neurons from the raphe nuclei project to the basal ganglia and various parts of the limbic system, and have a wide distribution throughout the cerebral cortices in addition to cerebellar connections (Figure XVII-12.5). All the 5-HT receptors identified so far are G-protein-coupled receptors, except the 5-HT_3 receptor, which is a ligand-gated Na^+/K^+ channel.

Other Neurotransmitters that may be Involved in Antipsychotic Mechanisms of Action

Recent efforts have been directed towards finding an alternative neurochemical target in schizophrenia. The first of these that

should be considered is gamma aminobutyric acid (GABA). GABA appears to have a regulatory role on dopaminergic function. The balance of evidence would tend to suggest that GABA decreases dopaminergic firing. This links with human post-mortem data indicating that GABAergic reductions correlate with increased dopamine concentrations (reviewed by Benes, 1997 and Wassef *et al.*, 1999). Thus, it is possible that in schizophrenia there is a reduction in GABAergic function, which leads to a dysregulation of dopamine and the production of psychotic symptoms.

A more likely candidate, however, appears to be the glutamatergic system. Glutamatergic dysfunction, particularly at the level of the *N*-methyl-D-aspartate (NMDA) receptor, has also been implicated in the pathophysiology of schizophrenia. Drugs that are antagonistic at the NMDA receptor, such as ketamine and phencyclidine, produce in healthy volunteers the positive, negative and neurocognitive symptoms that are characteristic of schizophrenia (Krystal *et al.*, 1994). There is evidence that the propsychotic effects of these drugs may be mediated via an increase in the release of glutamate acting on non-NMDA receptors (Moghaddam *et al.*, 1997). If the function of NMDA receptors is decreased, this may remove the drive to inhibitory GABAergic neurons, which regulate the excitatory neurons acting on areas such as the frontal cortex and the limbic regions. Thus, with decreased inhibitory control, these neurons may increase firing in these areas and produce psychotic symptoms (Farber *et al.*, 1998). Reducing glutamate release at all glutamate receptors may also have a role in improving symptoms in schizophrenia and related psychosess.

SIDE EFFECTS OF ANTIPSYCHOTICS

Acute neurological side effects secondary to dopamine D_2 receptor blockade with typical antipsychotics include acute dystonia. This is characterized by fixed muscle postures with spasm, e.g. clenched jaw muscles, protruding tongue, opisthotonos, torticollis and oculogyric crisis (mouth open, head back, eyes staring upwards). Acute dystonia appears within hours to days; young males are most at risk. This side effect should be treated immediately with anticholinergic drugs (procyclidine 5–10 mg, benzotropine 50–100 mg i.m. or i.v.). The response is dramatic.

Medium-term neurological side effects due to D_2 blockade include akathisia and parkinsonism. Akathisia is an inner and motor (generally lower limb) restlessness. It is usually very distressing by the patient and can lead to increased disturbance. Treatment is by reducing the neuroleptic dose and/or giving propranolol, *not* with anticholinergics. Akathisia usually appears within hours to days.

Parkinsonism is due to blockade of D_2 receptors in the basal ganglia. The classical features are a mask-like facies, tremor, rigidity, festinant gait and akathisia. It appears after a few days to weeks, and treatment involves anticholinergic drugs (procyclidine, orphenadrine), reducing the neuroleptic dose, or switching to an atypical antipsychotic that is less likely to produce such extrapyramidal symptoms.

The chronic neurological side effects due to D_2 blockade are tardive dyskinesia and tardive dystonia. Tardive dyskinesia is usually manifested as orofacial dyskinesia, and the patient exhibits lip smacking and tongue rotating. Tardive dystonia appears as choreoathetoid movements of the head, neck and trunk. It appears after months to years. There is an increased risk of tardive dyskinesia in older patients, females, edentulous patients, and patients with organic brain damage. With chronic use of antipsychotics, 20% of patients will develop tardive dyskinesia. There is no clear relationship to duration, total dose of treatment, or class of antipsychotic used. Increasing the dose may alleviate symptoms temporarily, while reducing the dose may exacerbate symptoms. Clozapine, olanzapine and quetiapine have been shown to improve symptoms, while risperidone has a lower propensity to cause tardive dyskinesia (Beasley *et al.*, 1999).

The effect of D_2 blockade on the neuroendocrine system produces hyperprolactinaemia by reducing the negative feedback on the anterior pituitary. High serum levels of prolactin produce galactorrhoea, amenorrhoea and infertility.

The most life-threatening side effect of neuroleptic use is neuroleptic malignant syndrome (NMS). This is thought to be due to derangement of dopaminergic function, but the precise pathophysiology is unknown. Symptoms include hyperthermia, muscle rigidity, autonomic instability and fluctuating consciousness. It is an idiosyncratic reaction. The diagnosis is often missed in the early stages, but a raised creatine phosphokinase level is often seen. It can occur at any time. Mortality is 20% if untreated, and immediate medical management is required. Bromocriptine (a D_1/D_2 receptor agonist) and dantrolene (a skeletal muscle relaxant) are used to reverse dopamine blockade and for muscular rigidity, respectively. Management includes supportive treatment for dehydration and high temperature. Renal failure from rhabdomyolysis is the major complication and cause of mortality. NMS can recur on reintroduction of antipsychotics, therefore it is recommended to wait at least 2 months and then introduce a drug of a different class at the lowest effective dose.

Anticholinergic side effects of antipsychotics include dry mouth (hypersalivation with clozapine), difficulty in urinating or urinary retention, constipation, blurred vision and ejaculatory failure. Profound muscarinic blockade may produce a toxic confusional state.

The sedative effects of antipsychotics are, in part, produced by the blockade of histamine-1 receptors. Side effects secondary to alpha-adrenergic blockade include postural hypotension, cardiac arrhythmias and impotence.

Some side effects may be due to autoimmune reactions, such as urticaria, dermatitis and rashes. Dermal photosensitivity and a grey/blue/purple skin tinge are more commonly seen with the phenothiazines, as are the conjunctival, corneolenticular and retinal pigmentation sometimes reported. Cholestatic jaundice due to a hypersensitivity reaction is now seen rarely with chlorpromazine.

Weight gain is also seen frequently with a wide variety of antipsychotics. This may be due to increased appetite. Although the mechanism is unclear, it may be due to a combination of histamine-H_1 and 5-HT_{2C} receptor antagonism (reviewed by Malhotra *et al.*, 1993).

There are also idiosyncratic side effects related to individual drugs, which are discussed in the relevant sections below.

Cardiac Side Effects

Recently, concern has grown over the ability of antipsychotic medications to produce changes in cardiac conduction. QT_c interval prolongation is the most widely reported conduction deficit. This first came to attention after sudden deaths secondary to arrhythmias with pimozide were noted. The UK Committee for the Safety of Medicine's (CSM) advice regarding pimozide is that all patients should have an electrocardiogram (ECG) before starting treatment, and patients with a known arrhythmia or prolonged QT interval should not receive the drug. Sertindole, an atypical antipsychotic, was suspended voluntarily from sale by its manufacturers in 1997 after similar concerns. Most recently, the CSM has advised on restrictions regarding the use of thioridazine and droperidol as these medications produce the most profound QT_c prolongations (Reilly *et al.*, 2000).

The QT interval on the standard ECG represents the interval between the end of ventricular depolarization and the end of cardiac repolarization. The 'c' in QT_c indicates that the QT value quoted has been corrected for cardiac rate. It is thought that prolongation of this interval increases the risk of a potentially fatal ventricular arrhythmia known as torsade de pointes. The

mechanism of this is becoming clearer and implicates the blockade of the delayed rectifier potassium channel (Ikr). Blockade of this receptor in the heart prolongs cardiac repolarization and thus the QT_c interval. It is known that drugs associated most specifically with QT_c interval prolongation bind specifically to the Ikr (Yap and Camm, 2002).

Although there is little consensus as to what represents a 'normal' QT_c, it is generally accepted that a QT_c over 500 ms increases the likelihood of an arrhythmia. When interpreting data on medication-related QT_c prolongation, it is important to note that the mean daily QT_c intrasubject variability is 76 ms (Morganroth, 2002).

Other risks factors that increase the likelihood of QT_c prolongation include age over 65 years and coadministration of other drugs associated with cardiac arrhythmias, such as tricyclic antidepressants. Safety studies are ongoing with both the newer and the older medications, and preliminary data suggest that the newer medications do not differ significantly in their likelihood to prolong the QT_c interval.

EFFICACY OF ANTIPSYCHOTICS IN THE ACUTE PHASE OF TREATMENT

The best known large-scale clinical trial, which gives a good idea of the effect size to be expected, was carried out by the National Institute of Mental Health in the USA (National Institutes of Health, 1964). This study involved four treatment groups — chlorpromazine, thioridazine, fluphenazine and placebo — with 90 randomly allocated subjects in each group. The subjects were treated for 6 weeks. In this study, 75% of subjects in the chlorpromazine-, thioridazine- and fluphenazine-treated groups showed significant improvement, 5% failed to be helped, and 2% deteriorated. In the placebo group, only 25% of patients showed significant improvement, and over 50% were unchanged or worse.

As we mentioned earlier, the medications used to treat schizophrenia are effective against psychotic symptoms *per se*. This is illustrated by the study of Johnstone *et al.* (1988), who showed that pimozide was antipsychotic (i.e. reduced the positive symptoms of psychosis) in patients with 'functional' psychosis regardless of whether the patients had prominent manic or depressive symptoms or were euthymic. This proved that 'neuroleptics', as they were then called, were truly antipsychotic rather than just antischizophrenic, providing the rationale for their use to treat psychotic symptoms even in the absence of a clear-cut diagnosis of schizophrenia.

Davis and Andriukaitis (1986) performed a meta-analysis using the trials involving chlorpromazine, and investigated the relationship between dose and clinical effect. They noted that a threshold of 400 mg of chlorpromazine is required. This was based on the fact that in 31 trials using a dose of 400 mg or more of chlorpromazine per day, only one trial failed to show that chlorpromazine was less effective than the reference treatment, whereas in the 31 trials using a dose of less than 400 mg of chlorpromazine, 19 failed to show a significant effect.

PHARMACOTHERAPY AS MAINTENANCE TREATMENT IN SCHIZOPHRENIA

Although it is accepted widely that antipsychotic medication is the mainstay of treatment in acute schizophrenia, its role in long-term maintenance has been more contentious. Nevertheless, the importance of maintenance drug therapy in the treatment of chronic schizophrenia has been evident since the early 1960s.

Initial studies indicated that between one-half and two-thirds of patients with schizophrenia who were stable on medication relapsed following cessation of maintenance pharmacological therapy compared with 5–30% of patients maintained on medication (Caffey *et al.*, 1964; Hogarty *et al.*, 1974; Davis, 1975). Two more recent reviews have collated the information from trials in which antipsychotics were withdrawn from populations of patients with schizophrenia who had responded to medication (Gilbert *et al.*, 1995; Viguera *et al.*, 1997).

In a review of 66 studies from 1958 through to 1993, Gilbert *et al.* (1995) noted that the relapse rate in the medication withdrawal groups was 53.2% (follow-up 6.3–9.7 months) compared with 15.6% (follow-up 7.9 months) in the maintenance groups. There was also a positive relationship between risk of relapse and length of follow-up. Viguera *et al.* (1997) investigated the relationship between gradual medication discontinuation (from last depot injection or tailing off oral medication over 3 weeks or more) and abrupt oral medication discontinuation. They noted a cumulative relapse rate of about 46% at 6 months and 56.2% at 24 months follow-up in patients whose medication was stopped abruptly. They calculated that in patients whose medication was stopped gradually, the relapse rate at 6 months was halved. Fifty per cent of inpatients had relapsed by 5 months after cessation of medication, whilst in the outpatient group, relapse rates remained at less than 50% to 4 years follow-up.

Thus, findings from medication discontinuation studies have shown conclusively that, as a group, patients with schizophrenia fare better if they receive antipsychotic medication. However, prolonged use of antipsychotic medication, particularly the older typical antipsychotics, carries a high risk of adverse effects, particularly tardive dyskinesia. In order to attempt to minimize the risk of these events, much work has focused on the use of low-dose medication regimes.

Low-Dose Antipsychotics

The rationale underlying the use of low-dose strategies is that significantly lower doses of medication are required for maintenance as opposed to the acute treatment of schizophrenia. This assumes that all major treatment goals have been met by the time of dose reduction. The two major aims are to ensure that the stability of symptomatic improvement is at least maintained and to minimize the risk of neurological side effects and secondary negative symptoms caused by higher doses of, in particular, typical antipsychotics.

A number of trials have investigated the use of standard doses of depot antipsychotics (250–500 mg chlorpromazine equivalents) in comparison with continuous low-dose regimes (usually at least 50% less) (reviewed by Schooler, 1993 and Barbui *et al.*, 1996). On the whole, these studies have indicated that patients treated with lower doses of antipsychotics have a higher rate of exacerbation of psychotic symptoms and higher rates of relapse.

Intermittent or Targeted Medication

This treatment strategy is based on the assumption that patients can be maintained on intermittently administered low doses of antipsychotics. The antipsychotics are given either for only a few days per week, or only when the patient is exhibiting signs of relapse and then ceased on resolution of that relapse. To summarize the results from the main published studies (Herz *et al.*, 1991; Carpenter *et al.*, 1990; Jolley *et al.*, 1990; Gaebel *et al.*, 1993), it appears that patients receiving intermittent targeted therapy have a higher rate of relapse than those on continuous therapy, and may have a higher rate of rehospitalization. Interestingly, at 2 years there is little difference in social functioning or psychopathology between the two groups.

In light of the increased risk of relapse and hospitalization, neither low-dose nor intermittent targeted treatment is generally recommended.

NEWER ATYPICAL ANTIPSYCHOTICS

The reintroduction of clozapine in the early 1990s, and the subsequent release of several new, atypical antipsychotics, has increased optimism in the treatment of schizophrenia. The term atypical has become controversial, as the definition of what makes an antipsychotic atypical is contentious. The simple definition is that an atypical antipsychotic is one that when given to laboratory rats in doses equivalent to those used to treat psychoses in humans will not produce catalepsy (a dystonic reaction). However, a more useful definition is that an atypical antipsychotic is one that has a wide therapeutic range. The therapeutic range of an antipsychotic can be conceptualized as lying between two doses. The lower dose is the dose at which 50% of people receiving that medication will respond to treatment. The higher dose is the dose at which 50% of people receiving that medication will begin to develop mild EPS. Thus, this allows us to give the medication at a dose where response can be predicted but at which there is a low liability for EPS. A critical review of the clinical trial literature on older typical antipsychotics used at low doses found no convincing evidence that there is a low dose of typical antipsychotics that is effective but does not produce EPS, therefore they have a very narrow and unpredictable therapeutic range (Taylor, 2000). All of the newer antipsychotics reviewed below have, to a greater or lesser extent, a predictable, wide therapeutic range. As these newer antipsychotics are likely to be the mainstay of treatment for schizophrenia in the future, it is worthwhile considering them individually.

Clozapine

Clozapine, the prototypical third-generation antipsychotic, has been used since the 1960s for treatment of schizophrenia. After reports of several deaths from neutropoenia, it was withdrawn and then reintroduced with restrictions. In most countries, clozapine can be used only for patients unresponsive to two other antipsychotics given at an adequate dose for an adequate duration, or those with tardive dyskinesia or severe EPS, and then only with blood monitoring. Each patient has to be registered, and the drug is only dispensed after a normal white cell count. In the UK, a blood count should be performed every week for 18 weeks, then every 2 weeks for the next year, and thereafter monthly. Clozapine is contraindicated in patients with previous neutropoenia.

Important aspects of clozapine's pharmacology include its low affinity for the D_2 receptor in comparison with older antipsychotics. Clozapine has higher affinity at the D_1 and D_4 receptors, and also binds to the extrastriatal D_2-like receptor, the D_3 receptor. It is thought that the low incidence of extrapyramidal side effects is due to the low activity at the D_2 receptor. Clozapine also has antagonistic activity at the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors. Clozapine is also an antagonist at the alpha$_1$ (less so at the alpha$_2$) receptor, resulting in sedation and hypotension. Clozapine's antagonism of the histamine-H_1 receptor adds to its sedative effects and may be responsible for weight gain. Weight gain may also be attributed to clozapine's relatively high affinity for the 5-HT_{2C} receptor. Other side effects include hypersalivation, tachycardia, sedation and hypotension. More rarely, clozapine can produce seizures (approximately 1%) and blood dyscrasias (<1–2%). The risk of neutropoenia is 1–2% and, in most cases, is reversible. The majority of cases (83%) of neutropoenia occur within the first 20 weeks of treatment. Risk of agranulocytosis decreases to 0.07% after the first year of treatment. Agranulocytosis probably results from toxic and immunologic factors (reviewed by Travis, 1997). It is this latter, potentially fatal side effect that has led to the limits on the use of clozapine, and the requirement for blood monitoring in patients receiving the drug. Clozapine does not increase serum prolactin.

Clozapine is the most effective treatment for schizophrenic patients refractory to other therapies, and improves both positive and negative symptoms. In non-comparative studies, clozapine leads to an >15% improvement in baseline ratings in 30–70% of previously treatment-refractory patients after 2–6 months treatment (reviewed by Travis, 1997). In comparison with chlorpromazine (Kane *et al.*, 1988; Claghorn *et al.*, 1987; Conley *et al.*, 1988), haloperidol (Breier *et al.*, 1994) or fluphenazine (Pickar *et al.*, 1992), clozapine shows a 30–100% response rate over a 4–17% rate for the comparator when administered to patients resistant to previous treatment with classical antipsychotics (see Treatment Resistance in Schizophrenia below). A recent systematic review and meta-analysis of randomized trials of clozapine has confirmed the superiority of clozapine over typical antipsychotics in the management of schizophrenia (Wahlbeck *et al.*, 1999). Clozapine has been investigated in few randomized clinical trials of maintenance therapy due to the restrictions imposed on its use. In one of the few studies published, Essock *et al.* (1996) followed up a sample of 227 patients randomized to either clozapine or treatment as usual. They reported that those patients treated with clozapine had significantly greater reductions in side effects, disruptiveness, hospitalization and readmission after discharge. Furthermore, clozapine's clinical efficacy in relapse prevention is well established, naturalistically, at 1–2 years of treatment, and there have been reports of good maintenance efficacy for up to 17 years of treatment (for review, see Travis, 1997).

Risperidone

Risperidone has high affinity for the 5-HT_{2A} receptor, with a similar affinity at the D_2 receptor as most typical antipsychotics. In the acute phase of treatment, risperidone appears to be as effective as haloperidol in terms of improvement in positive and negative symptom scores (Marder *et al.*, 1997).

The optimal dose of risperidone appears to be 4–6 mg per day. At doses above 8–12 mg per day, risperidone can cause extrapyramidal side effects of tremor, rigidity and restlessness, with a similar frequency to classical antipsychotics. Risperidone increases serum prolactin, which may lead to sexual dysfunction.

Risperidone has been assessed for long-term efficacy and safety in a number of long-term, open-label studies. The earlier data of Mertens (1991), Bressa *et al.* (1991) and Lindstrom *et al.* (1995) suggested that long-term therapy with risperidone was associated with meaningful reduction in psychopathology, amelioration of EPS, and improved social functioning from baseline measures or against placebo.

More recently, a meta-analysis of 11 risperidone/conventional antipsychotic comparator randomized controlled trials was performed (Song, 1997). The author reports that slightly more patients on risperidone showed clinical improvement than with comparison antipsychotics (57% *v*. 52%) and used significantly less medication for EPS (29.1% *v*. 33.9%).

Olanzapine

A more broad-spectrum atypical antipsychotic, olanzapine has a side-effect profile similar to that of clozapine but with a higher incidence of EPS at doses above 20 mg per day. Olanzapine also demonstrates antagonistic effects at a wide range of receptors, but

has a higher affinity for D_2 and 5-HT_{2A} receptors than clozapine and a lower affinity at the D_1 receptor. In acute-phase studies, olanzapine is efficacious for positive and secondary negative symptoms (Tollefson *et al.*, 1997).

Standard-dose olanzapine (5–15 mg per day) is effective maintenance treatment for schizophrenia (Dellva *et al.*, 1997). Initial data from a meta-analysis of three studies using haloperidol-treated patients as a test group indicate that 80.3% of patients receiving olanzapine maintain their response at 1 year in comparison with 72% for haloperidol-treated patients (Tran *et al.*, 1998).

Quetiapine

Another broader-spectrum atypical antipsychotic, quetiapine has a similar receptor-binding profile to clozapine but with relatively lower affinity for all receptors and virtually no affinity for muscarinic receptors. Quetiapine is effective in acute-phase studies for the treatment of positive and secondary negative symptoms. Initial randomized controlled trials indicated that quetiapine (250–750 mg per day, $n = 96$) was more effective than placebo ($n = 96$) and that this efficacy was not seen at doses of less than 250 mg per day (Small *et al.*, 1997). In comparison with chlorpromazine, response rates to quetiapine were similar across all symptom domains (mean dose 407 mg quetiapine *v*. 384 mg chlorpromazine) (Peuskens and Link, 1997). Response rates between haloperidol and quetiapine-treated groups were also similar (Arvanitis and Miller, 1997). Further studies have replicated these findings (Copolov *et al.*, 2000).

In all of these studies, the rates of EPS with quetiapine are similar to those seen in placebo-treated groups and are significantly lower than those seen in conventional antipsychotic comparator groups. Most common side effects are somnolence and dry mouth. Quetiapine demonstrates a lower potential to cause weight gain than clozapine and olanzapine, and similarly does not increase serum prolactin (Rak *et al.*, 2000).

In the open-label extensions of the acute trials, 265 responders to quetiapine were considered suitable for 1 year's treatment. Of these, 33% were still on quetiapine at 12 months with a sustained level of symptomatic improvement (Rak and Raniwalla, 2000). This is similar to the continuation rates seen in similar trials with olanzapine.

Amisulpride

In contrast with the other novel antipsychotics, amisulpride only has effects on the dopamine D_2 and D_3 receptors, where it is a potent antagonist. In animal models at lower doses, amisulpride appears to bind preferentially to presynaptic D_2 receptors, and at projected therapeutic levels it also appears to be selective for limbic D_2 and D_3 receptors (Schoemaker *et al.*, 1997). It has a similar efficacy to haloperidol for positive symptoms in acute exacerbations of schizophrenia (Delcker *et al.*, 1990; Moller *et al.*, 1997b; Puech *et al.*, 1998; Carriere *et al.*, 2000), with a projected optimum dose in this group of 400–800 mg per day (Puech *et al.*, 1998). Some studies at this dose range have reported a significantly greater efficacy for amisulpride in comparison with placebo for treating the negative symptoms of schizophrenia (Moller *et al.*, 1997b; Carriere *et al.*, 2000).

In the studies mentioned above, amisulpride has a significantly lower incidence of EPS at doses below 1200 mg per day than haloperidol. Amisulpride may cause less weight gain than other atypical antipsychotics, but it increases plasma prolactin (Coulouvrat and Dondey-Nouvel, 1999; Rein *et al.*, 2000).

In a 12-month trial of amisulpride 200–800 mg per day ($n = 370$) versus haloperidol 5–20 mg per day ($n = 118$), amisulpride showed enhanced efficacy for positive and negative symptoms. Importantly, those patients treated with amisulpride had significantly greater improvement in quality of life and significantly fewer EPS. Long-term efficacy and relapse prevention were similar in the amisulpride- and haloperidol-treated groups (Colonna *et al.*, 2000).

Ziprasidone

Ziprasidone has a high 5-HT_{2A}/D_2 receptor blockade ratio and a similarly high affinity for the 5-HT_{2A} receptor as risperidone. It is an agonist at the 5-HT_{1A} receptor. Ziprasidone also has potent affinity for D_3 and moderate affinity for D_4 receptors. It exhibits weak serotonin and noradrenergic reuptake inhibition. Ziprasidone appears to have relatively low levels of side effects. These may include somnolence and headache, but results of full clinical studies remain to be published.

An initial clinical trial of ziprasidone versus haloperidol 15 mg per day over 4 weeks suggested that ziprasidone 160 mg per day was as effective as haloperidol at reducing positive symptom scores but produced fewer side effects (Goff *et al.*, 1998). In two placebo-controlled trials involving 139 and 301 patients over 4 and 6 weeks, respectively (Keck *et al.*, 1998; Daniel *et al.*, 1999), the pooled data indicated that ziprasidone 80–160 mg was consistently significantly more effective than placebo and lower doses of ziprasidone. Improvements in positive and negative symptoms were similar in magnitude to those seen in patients treated with risperidone, olanzapine or quetiapine. Interestingly, 160 mg of ziprasidone was associated with a greater than 30% decrease in depressive symptoms in the subgroup of patients with significant depression at the outset of the trials.

Ziprasidone has also been tested in a 1-year placebo-controlled trial in order to assess its utility for relapse prevention. A total of 294 patients were studied and randomized to placebo treatment or ziprasidone 40–160 mg per day. Of the 117 patients who were maintained on ziprasidone, at 6 months only 6% had experienced exacerbation of their symptoms compared with 35% of the 23 patients remaining on placebo; this compares favourably with the available data on the older antipsychotics (reviewed by Keck *et al.*, 2001).

Adverse Events with Atypical Antipsychotics

The issue of side effects or adverse events is linked closely to tolerability and acceptability and therefore to both compliance and relapse prevention. The most debilitating and obvious side effects of conventional antipsychotics are often motor effects. As described above, the newer antipsychotics demonstrate a lower propensity to cause EPS at clinically effective doses. These drugs may provide benefits for patients who suffer from akathisia and tardive dyskinesia.

Akathisia develops in 18–75% of patients treated with typical antipsychotics. Studies of patients treated with clozapine have indicated that over a 12-week period, there is a striking reduction in akathisia (Safferman *et al.*, 1992; Chengappa *et al.*, 1994), with rates of less than 10%. Moller *et al.* (1997a) have reported that risperidone at 4–8 mg per day produces a significantly lower incidence than haloperidol. Olanzapine-treated groups have improved akathisia ratings from baseline (Beasley *et al.*, 1997), and quetiapine shows similar akathisia rates to placebo and leads to improvements in akathisia ratings in switch-over studies (Small *et al.*, 1997; Arvanitis *et al.*, 1997). Similarly, in long-term studies, amisulpride shows a significantly lower rate of akathisia than haloperidol (8% *v*. 21%) (Colonna *et al.*, 2000), although in short term studies rates are similar, (Rein *et al.*, 2000).

It is in the area of tardive dyskinesias that clozapine appears to have its most marked effect. Liebermann *et al.* (1991) reported

a 50% reduction of symptoms over 28 months of treatment in 43% of patients, and Gerlach and Peacock (1994) reported a resolution of tardive dyskinesia in 54% of patients after 5 years of clozapine treatment. Furthermore, Tamminga *et al.* (1994) reported a significant difference in reduction of tardive dyskinesia scores in a clozapine-treated group versus a haloperidol-treated group; this difference began after about 4 months of treatment. There have also been case reports that show that switching to clozapine may be effective in reducing tardive dystonia (Shapleske *et al.*, 1996).

In risperidone-treated groups at <8 mg per day, there are EPS benefits that increase over time with a rate of tardive dyskinesia at approximately 0.3% per year (Lindstrom *et al.*, 1995). Additional evidence comes from the large-scale controlled studies of olanzapine, which has a 0.52% 1-year risk of tardive dyskinesia versus 7.45% for haloperidol (13.5 mg *v.* 13.9 mg) (Beasley *et al.*, 1999). These findings tie in with early studies on quetiapine versus haloperidol and ziprasidone versus placebo, which both show placebo rates of tardive dyskinesia (Arvanitis *et al.*, 1997; Keck *et al.*, 2001).

As has been described above, the rate of other side effects, such as weight gain and prolactinaemia, may vary between each of the newer drugs, although fully adequate comparison studies remain to be published.

NEGATIVE SYMPTOMS

Negative symptoms are usually separated into primary and secondary negative symptoms of schizophrenia. Primary negative symptoms are usually classified as the those that are due directly to, or are 'core' to, the psychotic illness process. Secondary negative symptoms are negative-type symptoms that mimic the core symptoms but that are due to enduring positive symptoms or are secondary to drug treatment induced EPS.

It is claimed that clozapine has an almost unique action against the negative symptoms of schizophrenia out of proportion to its effect on positive symptoms (Meltzer, 1990; Meltzer, 1991), but the evidence for this is by no means clear. Tandon *et al.* (1993) found that the improvement in negative symptoms covaried with the improvement in positive symptoms, and Hagger *et al.* (1993) found no improvement in negative symptoms. A more recent finding is that in comparison with haloperidol, clozapine has a significant effect on negative symptoms in patients with non-deficit schizophrenia but not in those with deficit schizophrenia, i.e. those with enduring negative symptoms (Breier *et al.*, 1994; Conley *et al.*, 1994). It has therefore been suggested that clozapine's seemingly beneficial effect on negative symptoms may simply be a reflection of its reduced tendency to cause extrapyramidal side effects (Kane *et al.*, 1994). The weight of current evidence suggests that clozapine has an excellent effect on the secondary but not primary negative symptoms (Carpenter, 1996). Indeed, all of the newer antipsychotics appear to have an effect on secondary negative symptoms, and all of the available trials have indicated a modest but significant advantage for the newer medications over conventional antipsychotics in negative symptom improvement on standard rating scales (Song, 1997; Tollefson *et al.*, 1997; Small *et al.*, 1997; Tandon *et al.*, 1997).

Additional to the effects at 400–800 mg per day, amisulpride at low doses (50–300 mg per day) may have a unique effect in patients with only negative symptoms. In comparison with placebo, these low doses of amisulpride produce significant reductions in negative symptoms scores with little change in positive symptom scores (Danion *et al.*, 1999; Boyer *et al.*, 1995; Paillere-Martinot *et al.*, 1995). However, in a study utilizing a different design but similar patient population, low-dose amisulpride was similar to low-dose haloperidol treatment in terms of negative symptom improvement (Speller *et al.*, 1997).

COGNITION

The older antipsychotics have limited impact on the neurocognitive deficits, which are a core feature of schizophrenia, are apparent at the onset of illness, and may deteriorate during the first few years of illness, although inconsistent long-term improvements have been noted (Meltzer *et al.*, 1996; Bilder, 1997). There has been increasing interest in the possible role that the newer antipsychotics may have in ameliorating these problems, which are linked to poor outcome and future unemployment. Clozapine may lead to improvements in attention, memory and executive function over 6–12 months (Lee *et al.*, 1994). Risperidone in comparison with haloperidol appears to improve frontal function and spatial working memory (Gallhofer *et al.*, 1996; Green *et al.*, 1997). Olanzapine improves a variety of measures of function, including psychomotor speed, verbal fluency and memory (McGurk and Meltzer, 1998). A preliminary study comparing quetiapine and haloperidol in a randomized double-blind trial demonstrated that patients treated with quetiapine showed significant improvements in overall cognitive performance, verbal fluency and verbal contextual memory (Velligan *et al.*, 1999). It is still not clear how relevant the modest improvements or impairments reported in these studies are to long-term outcome.

DEPRESSION AND BIPOLAR AFFECTIVE DISORDER

Patients with schizophrenia are significantly more likely than the general population to suffer from other psychiatric disorders, such as depression, and all antipsychotics are used to treat other psychotic disorders outside of schizophrenia. Clozapine has been reported to be effective in patients with treatment-resistant schizoaffective or manic illnesses (Zarate *et al.*, 1995; reviewed by Kimmel *et al.*, 1994). In one study, clozapine reduced baseline mania ratings by more than 50% in 72% of a group of patients suffering from either mania or schizoaffective disorder, and 32% had a significant improvement in brief psychiatric rating scale (BPRS) scores. The latter finding was more frequent in the bipolar patients and the non-rapid cycling patients (Calabrese *et al.*, 1996). In depressive disorders, clozapine has a more equivocal response. Although seemingly effective against depressive symptoms occurring comorbidly with schizophrenia (Meltzer and Okayli, 1995), there has been little work showing a particular use for clozapine in treatment-refractory depression (Banov *et al.*, 1994; Rothschild, 1996).

Conventional antipsychotics may both improve and contribute towards depressive symptoms. Clozapine reduces depressive features and suicidality (Meltzer and Okayli, 1995). Risperidone produces significantly greater reduction in anxiety/depression subscales in comparison with haloperidol (Marder *et al.*, 1997). Olanzapine has significant antidepressant effects in comparison with haloperidol (Tollefson *et al.*, 1998). Amisulpride (50 mg per day) has been compared with imipramine (100 mg per day) and placebo in patients with dysthymia and major depressive disorder. In this study ($n = 219$), both imipramine and amisulpride produced similar and significant improvements on all rating scales; the implications of this in patients with schizophrenia have yet to be elucidated (Lecrubier *et al.*, 1997).

TREATMENT RESISTANCE IN SCHIZOPHRENIA

Treatment resistance in schizophrenia is usually defined as a failure to show a clinical response to two antipsychotics from different chemical and pharmacological classes after each has been given for an adequate period of time at an adequate dose (reviewed by Conley and Buchanan, 1997 and Peuskens, 1999). It is estimated that at least 25% (Brenner *et al.*, 1990) and possibly up to 50%

Table XVII-12.2 Definition of treatment resistance in schizophrenia (Kane criteria)

Clinical history	• No good level of functioning over the last 5 years
(previous treatment)	• Received three periods of treatment in the preceding 5 years with antipsychotics of at least two different chemical classes for at least 6 weeks, with an equivalent of at least 1000 mg chlorpromazine daily without significant relief
Cross-sectional	• BPRS total score ≥45 (18 items, rated from 1 to 7 [absent to severe])
	• Rating ≥4 on at least two of the following BPRS items: conceptual disorganization, unusual thoughts, hallucinatory behaviour, suspiciousness
	• CG1 score ≥4
Prospective	• Failure to reduce BPRS score by ≥20%, plus either a post-treatment BPRS score of ≥35 or CGI ≥3 with haloperidol 60 mg daily for 6 weeks

BPRS, brief psychiatric rating scale; CGI, clinical global impressions scale.
From Kane *et al.* (1988) and Peuskens (1999).

(Peuskens, 1999) or more of patients with chronic schizophrenia would, at least partially, fulfil the criteria given below for treatment resistance.

The criteria used in the seminal clozapine versus chlorpromazine trial (Kane *et al.*, 1988) are often quoted as the gold standard in the definition of treatment resistance. An adequate dose of a typical antipsychotic is generally considered to be up to about 600–750 mg of chlorpromazine equivalent (Barnes and McEvedy, 1996), although it must be noted that most of the early trials in treatment-resistant populations adopted the criteria used in the Kane *et al.* (1988) seminal clozapine trial of >1000 mg of chlorpromazine equivalent. For the newer medications, the optimum or maximum therapeutic doses would appear to be about 6 mg per day for risperidone, 20 mg per day for olanzapine, 750 mg per day for quetiapine and 800 mg per day for amisulpride. Treatment non-response has been defined variously, one possible definition being a rating above 45 on the BPRS (18-item scoring 1 to 7) and/or a failure for the BPRS total score to improve by 20% during a treatment trial (Kane *et al.*, 1988). These criteria, however, emphasize positive symptoms, thus a broader definition has been applied more recently that also includes functional disability (Brenner *et al.*, 1990). An adequate duration of treatment is usually considered to be about 4–6 weeks (Kane and Marder, 1993) (Table XVII-12.2).

The most effective medication for treatment-resistant schizophrenia is clozapine; evidence for its effectiveness compared with typical antipsychotics is given above. If a patient fulfils the criteria for treatment resistance, they should be encouraged to take clozapine.

It is timely, however, to consider therapeutic options for patients who do not respond adequately to clozapine monotherapy and indeed for those treatment-resistant patients who are unwilling or unable to take clozapine.

Patients Who do not Respond Adequately to Clozapine

Between 30% and 60% of patients do not respond adequately to clozapine (Kane, 1992). Some patients gain benefit from clozapine but are still symptomatically or functionally impaired because of their schizophrenic illness. In these patients, it is worthwhile considering ways in which clozapine use can be optimized. This issue has been reviewed by Barnes and McEvedy (1996) and Chong and Remington (2000).

Dose and Duration

While there is still little evidence to suggest a linear relationship between clozapine plasma levels and response, more recent work (reviewed by Wagstaff and Bryson, 1995) has indicated that there is a minimum therapeutic plasma concentration, ranging between $350\,\mu g\,l^{-1}$ and $420\,\mu g\,l^{-1}$. Below these levels, 8–38% of patients respond, while above these levels 55–75% of patients respond (Miller 1996). Thus, although patients may respond at lower plasma levels and therefore lower doses of clozapine, there is evidence to suggest that a patient should not be considered to be a non-responder to clozapine unless they have had a period of treatment with plasma levels above $420\,\mu g\,l^{-1}$ for an adequate duration of time.

The optimal duration of a trial of clozapine remains controversial. The American Psychiatric Association recommends a minimal trial of 12 weeks; however, improvement and response have been noted as needing 6 months to occur (Conley and Buchanan, 1997). In the majority of the literature, 6 months of treatment is considered adequate to deem a patient treatment resistant, with the caveats in regard to plasma levels of clozapine.

Augmentation Strategies

The combination of clozapine with other medications is common. Between 30% and 40% of patients on clozapine receive an additional psychotropic medication (Peacock and Gerlach, 1994; Joffe *et al.*, 1996; Naber, 1992). There is little evidence, however, from controlled trials to guide rational combinations of medications.

Antipsychotics

The apparent low occupancy of dopamine D_2 receptors by clozapine (38–65%) (Farde *et al.*, 1992), even at high doses, may be below a putative threshold of occupancy required for full antipsychotic response. This threshold may be between 60% and 70% for haloperidol (Kapur *et al.*, 1999). It has therefore been speculated that for at least some patients, in addition to the broad pharmacology of clozapine, there may be a need for a higher degree of D_2 receptor occupancy to ensure successful treatment.

Friedman *et al.* (1997) augmented the therapy of seven clozapine-resistant schizophrenia patients with the selective, high-affinity D_2 receptor antagonist pimozide. They reported a significant mean BPRS score reduction of 27, and six of the seven patients had a marked response.

A double-blind, placebo-controlled, randomized study by Shiloh *et al.* (1997) augmented clozapine monotherapy with sulpiride 600 mg per day in 28 clozapine-resistant schizophrenia patients over 10 weeks. Although the clozapine–sulpiride group exhibited significantly greater improvements in positive and negative psychotic symptoms, about half of this group of patients exhibited a mean reduction of 42.4% and 50.4% in BPRS and SAPS, respectively whereas half showed almost no improvement at all (<5%).

McCarthy and Terkelsen (1995) described two patients with schizophrenia who improved when risperidone was added to clozapine. This was reinforced by an open trial of augmentation with up to 6 mg per day of risperidone in 12 patients (Henderson and Goff, 1996). In this study, ten of the patients showed a greater than 20% fall in their BPRS total scores. Pharmacologically, it is likely that any effect seen was secondary to enhanced D_2 receptor occupancy by risperidone, as both clozapine and risperidone produce high and equal occupancy of 5-HT_{2A} receptors at a range of clinical doses (Travis *et al.*, 1998).

Although slight increases in EPS were noted in some of these studies, these strategies were generally well tolerated, and although more evidence is needed, the addition of selective D_2 antagonists, such as sulpiride, pimozide and potentially amisulpride, to clozapine will be beneficial to some patients.

Antidepressants

The case report literature would tend to suggest that there is little benefit, and an increased risk of toxicity, if tricyclic antidepressants are added to clozapine (Chong and Remington, 2000). The evidence from the case report literature is more in favour of clozapine/selective serotonin reuptake inhibitor (SSRI) combinations, particularly fluvoxamine, although not without some increased risk of toxicity (reviewed by Chong and Remington, 2000). Unfortunately, the one extant controlled trial comparing fluoxetine ($n = 18$) with placebo ($n = 15$) failed to show any effect on psychosis or mood rating scales over 8 weeks (Buchanan *et al.*, 1996).

It is highly possible that both the beneficial and adverse effects of SSRIs in clozapine augmentation are simply the effects of increasing clozapine plasma levels (Hiemke *et al.*, 1994), as with the exception of citalopram all of the currently available SSRIs inhibit the metabolism of clozapine (Wetzel *et al.*, 1998a).

Mood Stabilizers

Lithium and clozapine combinations were reported to be effective in an 11-patient case series (Bryois and Ferrero, 1993) and in 23 of 27 patients with schizophrenia or schizoaffective disorder in a retrospective study (Bender *et al.*, 1996). There is also evidence from the case report literature that the combination is effective in treatment-resistant bipolar disorder (Puri *et al.*, 1995). However, there are no data from any prospective controlled trials. Adjunctive lithium has, anecdotally, been used in conjunction with clozapine to increase white cell counts in patients who have a white cell count below the threshold for clozapine administration (Blier *et al.*, 1998). There is no evidence, however, that this is safe in the longer term, and it is possible that in such patients lithium may mask a fulminant neutropoenia or agranulocytosis. Furthermore, there is some evidence that the combination may be neurotoxic (Blake *et al.*, 1992), although the literature on this is by no means clear (Naber *et al.*, 1992).

A combination of clozapine and carbamazepine has a potential increased risk of neutropoenia and agranulocytosis (Gerson and Meltzer, 1992), and there is evidence that carbamazepine may reduce plasma clozapine levels (Ereshefsky, 1996). There is also little evidence to suggest any therapeutic benefit, therefore there is little to recommend carbamazepine as an augmentation strategy in clozapine non-responders.

The combination of clozapine and valproate is more promising. Valproate has long been suggested to be the antiepileptic of choice in clozapine-related seizure disorders (Devinsky and Pacia 1994), and it appears to have little effect on plasma clozapine concentrations (Facciola *et al.*, 1999). There is also preliminary evidence of some clinical effectiveness. Kando *et al.* (1994), in a review of 55 patients, 17 of whom had schizophrenia and 23 had schizoaffective disorder, reported efficacy in 87% of their patients. However, these data would need to be replicated in a prospective controlled study before a recommendation for this drug combination could be given.

Although evidence for the effectiveness of lamotrigine augmentation is sparse, in a recent open-label, non-randomized case series of six outpatients with a partial response to clozapine, and in whom lamotrigine was prescribed (up to a maximum dose of 250 mg per day), there was a consistent, highly significant improvement in baseline BPRS scores (Dursun *et al.*, 1999).

Other Strategies

Electroconvulsive therapy (ECT) and clozapine combinations have not been the subject of any controlled studies. There is some case report literature indicating a beneficial effect in some patients with a low degree of toxicity (reviewed by Chong and Remington, 2000), but there is insufficient evidence to make a clear judgement.

Benzodiazepines are used relatively frequently in combination with clozapine, with one study reporting their use in up to 11% of patients (Faisal *et al.*, 1997) and another in up to 30% of patients (Peacock and Gerlach, 1994). There has been no assessment of the clinical effect of this combination, however. There has been concern about the toxicity, particularly in terms of the incidence of respiratory depression or collapse. Larger studies tend to show that this combination is no more toxic than clozapine monotherapy, but smaller case reports and case report series indicate that there is a risk of respiratory depression with this combination, thus doses should be titrated slowly (reviewed by Chong and Remington, 2000).

Treatment-Resistant Patients Unwilling or Unable to Take Clozapine

Changing Antipsychotic

There is little evidence to support changing a treatment-resistant patient who is unable to take clozapine to a typical antipsychotic, as response rates to these medications in this group are uniformly low. An exception to this is the possibility of reviewing the patient's treatment history and restarting them on the medication to which they have consistently shown a beneficial response in the past. Failing this, there are some data to support the potential use of some of the atypical antipsychotics in treatment resistance.

Risperidone

In an early, naturalistic, open-label study, 25 chronically hospitalized patients who were partial non-responders were switched to risperidone 6–16 mg per day from their previous treatment (mean 1265 mg per day chlorpromazine equivalent). In this cohort, who could also receive adjunctive medication, 36% had a reduction in total BPRS of 20% or more after 6 months of treatment (Smith *et al.*, 1996).

Using the Kane (1988) criteria with three previously ineffective trials of medication, Wirshing *et al.* (1999) recruited 67 subjects and randomized them to receive 6 mg per day of risperidone or 15 mg per day of haloperidol preceded by a 7–9-day placebo washout phase. Patients were held at this fixed dose for 4 weeks and then had 4 weeks flexible-dose medication (risperidone mean 7.5 mg per day, haloperidol mean 19.4 mg per day). At the end of the fixed-dose period, risperidone had produced significantly more improvement in the total BPRS score (24%) than haloperidol (10%) ($P = 0.03$). Interestingly, this difference had disappeared by the end of the flexible-dose period. Throughout the study, risperidone-treated patients had fewer EPS than haloperidol-treated patients. This evidence is supported partially by a trial comparing risperidone and clozapine (Bondolfi *et al.*, 1998). Although not employing strict Kane criteria, this group randomized 86 patients who had failed to respond adequately to at least two trials of conventional antipsychotics to either risperidone (mean 6.4 mg per day) or clozapine (mean 291.2 mg per day) for 8 weeks. This cohort did not have psychopathology ratings as high as patients in trials using Kane criteria and were thus relatively treatment resistant. The results of this study indicated that both risperidone and clozapine led to clinical improvement (defined as a 20% reduction from baseline in positive and negative syndrome scale (PANSS) total) in roughly 60% of patients, with no difference between groups but possibly a slightly faster onset of action in the risperidone-treated group. EPS did not differ between groups. Similar results were noted in a smaller trial using Kane criteria with treatment over 12 weeks, although in this study the authors noted that the clozapine-treated group appeared to be continuing to improve at endpoint while the risperidone-treated group was not (Lindemayer *et al.*, 1998). Other smaller, less rigorously conducted, retrospective trials have either had similar results (Wahlbeck *et al.*, 2000) or shown a superiority

for clozapine over risperidone (Sharif *et al.*, 2000). Thus, there is some evidence that risperidone may be useful in some patients resistant to treatment with conventional antipsychotic medication, but the dose of risperidone may be critical to this response and should probably be no higher than 6 mg per day.

Olanzapine

Breier and Hamilton (1999) analysed a subgroup of 526 patients with partial non-response (in this case, failure to respond to at least one rather than two antipsychotics) drawn from a larger clinical trial comparing olanzapine and haloperidol. In this group, the mean olanzapine dose was 11.1 mg per day and that of haloperidol was 10 mg per day. Both the last observation carried forward and the completer analysis showed superiority for olanzapine over haloperidol in improvements in negative symptoms and depression ratings. The analysis of those patients who completed the trial revealed a superiority for olanzapine over haloperidol in all symptom domains, with a response rate for olanzapine of 45% compared with 36% for haloperidol. Unfortunately, as the population studied does not fulfil traditional treatment-resistance criteria, these data are more supportive of the use of olanzapine as a second-line medication once one antipsychotic has failed.

In a study using traditional Kane criteria, Conley *et al.* (1998) compared olanzapine 25 mg per day with chlorpromazine 1200 mg per day. They also excluded patients who had previously not responded to clozapine. Furthermore, the patients underwent 6 weeks treatment with haloperidol (10–40 mg per day) before randomization to olanzapine or chlorpromazine (a 'run-in' phase). A total of 103 patients started the haloperidol phase, with one subject responding to haloperidol. Eighty-four subjects were randomized to one of the treatment arms for 8 weeks of treatment. In this study, 7% of patients responded to olanzapine and 0% responded to chlorpromazine, with no difference between groups in mean BPRS score at any point during the trial. This contrasts with the results from a study comparing directly olanzapine and clozapine (Tollefson *et al.*, 2001). The criteria for treatment resistance were similar to the Conley *et al.* (1998) trial, except that patients had to have received the two antipsychotics at doses of greater than 500 mg (rather than 1000 mg) of chlorpromazine equivalents, and patients who had previously not responded to clozapine or olanzapine were excluded. There was no run-in phase. Patients received 15–25 mg per day of olanzapine (mean 20.5 mg) or 200–600 mg per day of clozapine (mean 303.6 mg). Over 18 weeks of treatment, there was no difference between the two groups in terms of symptom reduction or clinical response. Both medications produced a significant improvement from baseline ratings, with a response rate for olanzapine of 38.2% and for clozapine of 34.5%. Clozapine produced significantly more constipation and sialorrhoea than olanzapine. The authors concluded that olanzapine was not inferior to clozapine in treatment-resistant schizophrenia. The contrasting results of these two studies may be explained by the Conley *et al.* patient group being more treatment resistant by virtue of having received a higher dose of typical medication before inclusion in the study. The absence of a clozapine wing in the Conley *et al.* study also makes it difficult to comment on the clozapine responsiveness of the patient cohort, although a subgroup from the study was subsequently treated with clozapine and 11 of 21 patients did respond (Conley *et al.*, 1998; Conley *et al.*, 1999). Another possibility is the duration of the studies. The Conley *et al.* study took place over 8 weeks rather than the 18 weeks in the Tollefson *et al.* study, thus potentially the olanzapine-treated subjects in the former were not given adequate time to respond. It must be noted, however, that the majority of responses in both groups in the Tollefson *et al.* study had already taken place by 8 weeks.

Further preliminary evidence for the use of olanzapine in treatment resistance is provided by two open-label studies. In the first study, 48 patients, the majority of whom in addition to traditionally defined treatment resistance had also failed to respond to clozapine (91.1%), or had been intolerant of it, were prescribed olanzapine 5–25 mg (mean 22 mg per day) for 18 weeks. Treatment was well tolerated, and it is reported that 55.6% of patients had a 20% or greater reduction in BPRS from baseline (Dossenbach *et al.*, 2000).

There is therefore some evidence to suggest that a trial of olanzapine would be useful for treatment-resistant patients with schizophrenia, particularly for those who are unwilling or unable to take clozapine, and perhaps for those who have previously received lower doses of typical antipsychotics.

Quetiapine

In a study of 288 patients with a partial response to 4 weeks of 20 mg per day fluphenazine, quetiapine at 600 mg per day showed benefits over haloperidol at 20 mg per day over 8 weeks. For inclusion in the fluphenazine run-in, patients had to score at least 15 on the positive subscale of the PANSS and were excluded if they were clozapine non-responders. The subjects had a total PANSS score of 88 at randomization. Fifty-two percent of patients responded to quetiapine (>20% reduction in PANSS total) versus 38% to haloperidol ($P < 0.05$). Again, the patients receiving quetiapine had significantly fewer EPS (Emsley *et al.*, 2000). Even though this study does not address the use of quetiapine in classical treatment resistance, it does provide evidence that quetiapine may be useful in patients who are unable to start clozapine.

Other Atypical Antipsychotics

As the time of writing, there is no evidence to recommend the use of other atypical antipsychotics in this group of patients.

Antidepressants

Depression is a common comorbid disorder with schizophrenia, although it is difficult to separate it from the EPS side effects of older antipsychotics or indeed from the negative symptoms of schizophrenia (Hirsch *et al.*, 1989; Siris *et al.*, 1988; Kibel *et al.*, 1993). This is reflected in the wide range of prevalence figures quoted (from 7% to 75%), although there is a generally accepted prevalence of about 25% (Siris, 2000). As we have seen, the newer atypical antipsychotics may have beneficial effects on mood in comparison with the older antipsychotics. However, studies before the introduction of these medications indicated that the majority of depressive symptoms associated with schizophrenia resolved with successful resolution of the psychosis (Koreen *et al.*, 1993). Nevertheless, it is likely that a substantial proportion of patients with schizophrenia will remain with depressive symptoms hampering their recovery.

Whilst there is no evidence that antidepressants have any antipsychotic efficacy, there is evidence from a number of studies that antidepressants may alleviate comorbid depressive symptoms in schizophrenia (Hogarty *et al.*, 1995; Siris *et al.*, 1994; Siris *et al.*, 1987; Prusoff *et al.*, 1979). There is little evidence to suggest which antidepressant should be used. Although the majority of studies have investigated the effects of tricyclic antidepressants (reviewed by Levinson *et al.*, 1999), recent studies have suggested that SSRIs may also have a beneficial effect (reviewed by Evins and Goff, 1996). It has been suggested by Siris (2000) that in the absence of florid psychosis, if a patient with schizophrenia is experiencing persistent episodes of depression, and the possible contribution of EPS to these symptoms has been addressed, then there is sufficient evidence to suggest that a 6-week trial with an antidepressant at normal therapeutic doses would be worthwhile.

Mood Stabilizers

Lithium

Early studies suggested that lithium might have a role in the treatment of schizophrenia (Alexander *et al.*, 1979; Zemlan *et al.*, 1984). Lithium as monotherapy, however, does not appear to be an effective antipsychotic in schizophrenia, and in comparison with pimozide in the pivotal Northwick Park Functional Psychosis Study (Johnstone *et al.*, 1988), it only had the effect of reducing elevated mood. Similarly, lithium protects against the euphoriant effects secondary to dexamphetamine administration in subjects with schizophrenia, but does not protect against its propsychotic effects (Van Kammen *et al.*, 1985). This lack of efficacy for lithium in schizophrenia is reinforced by the findings of Wilson (1993), who reported that lithium was an ineffective augmenter of haloperidol if patients with affective symptomatology were excluded. Schulz *et al.* (1999) studied a group of 41 patients with schizophrenia who had failed to respond to fluphenazine decanoate. The patients were then randomized to receive either lithium or placebo for 8 weeks in addition to their stable dose of fluphenazine. The BPRS total scores at baseline were in the region of 46. Lithium levels were maintained between 0.8 and 1.0 $mEq\,l^{-1}$. Interestingly, although both groups showed a significant reduction in BPRS total during the trial ($P = 0.0135$), there was no difference between the placebo- and lithium-treated groups. Furthermore, there was no reduction in measures of depressed mood. Thus, whilst there may be a role for lithium in patients with schizoaffective disorder, there is little evidence for its usefulness in schizophrenia *per se*.

Carbamazepine

Carbamazepine has similarly not been shown to be an adequate monotherapy in schizophrenia (Sramek *et al.*, 1988; Carpenter *et al.*, 1991). Its use as an adjunctive therapy to antipsychotics may, however, be justified. In a review by Schulz *et al.* (1990) of nine studies of carbamazepine adjunctive treatment, there was a general but nonspecific trend in favour of the effectiveness of carbamazepine. As half of the studies focused on patients with violent behaviour, this may have biased the use of carbamazepine for this group. However, a meta-analysis of all of the extant carbamazepine trials has suggested that there is no evidence that this medication is useful as an adjunct in schizophrenia (Leucht *et al.*, 2000). The authors suggest that a trial of carbamazepine could only be recommended for patients who had previously shown a response or who had confirmed abnormalities on EEG. It is important to note that carbamazepine is a powerful enzyme inducer and may reduce the plasma levels of coadministered antipsychotics by as much as 50–80% (Jann *et al.*, 1989). This implies that there is a potential for carbamazepine to cause patients with schizophrenia to relapse.

Valproate

Valproate (sodium valproate, divalproex, valproate semisodium) appears to be used increasingly frequently in the treatment of schizophrenia. From 1994 to 1998, for example, the adjunctive use of valproate nearly tripled in people with schizophrenia treated in New York state hospitals (Citrome *et al.*, 2000). Despite this, there has been no systematic evaluation of valproate's role in treatment-refractory schizophrenia. There is some evidence from case reports for the potential role of valproate in the control of aggression in schizophrenia (Wassef *et al.*, 1989; Morinigo *et al.*, 1989), and also, when used as an adjunct to haloperidol, in reducing the doses of sedative medication prescribed and the hostile belligerence demonstrated by patients with schizophrenia (Dose *et al.*, 1998). Recent data from two small, placebo-controlled trials have indicated that valproate enhances global antipsychotic response both in acute exacerbations of schizophrenia and in partially responsive patients not on clozapine (Wassef *et al.*, 1999; Wassef *et al.*, 2000).

Lamotrigine

Lamotrigine may be an effective augmenter in partially resistant patients as there is preliminary evidence for its efficacy in clozapine non-responders (Dursun *et al.*, 1999) (see above).

THE FUTURE

Despite the advances in schizophrenia pharmacotherapy since the early 1950s, there are still many limitations. EPS make the use of high doses of the older typical antipsychotics problematic and often unpleasant for those taking them. Lower doses of typicals and the newer atypical antipsychotics offer benefit the patients in terms of reductions in EPS, but the latter medications are not without their own unpleasant side effects.

Another failing in the pharmacotherapy of schizophrenia is that there are still 40–50% of patients with schizophrenia who do not have an optimal response to medication and some 20% who are resistant to all forms of treatment, including clozapine.

Future developments may include a new generation of antipsychotic drugs that are partial agonists (rather than antagonists) at D_2 and D_2-like receptors, such as aripiprazole (Lawler *et al.*, 1999). Early clinical trials with this medication show good efficacy with placebo rates of EPS (Carson *et al.*, 2001).

In conjunction with developments in trial methodology, combinations of neurochemical and functional imaging may help to elucidate the neural correlates of treatment response and resistance and allow more rational therapeutic decisions.

Pharmacogenetics may help to define further the parameters that predict response or non-response to particular medications by analysing the allelic variations for individual receptors that correlate with response (Arranz *et al.*, 2000).

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XVIII

Mood Disorders

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XVIII-1

Animal Models of Depression: A Diathesis/Stress Approach

Paul Willner and Paul J. Mitchell

INTRODUCTION

Animal models of depression are used both as screening tests to discover and develop novel antidepressant drug therapies, and as simulations for investigating various aspects of the neurobiology of depressive illness, including the neuropharmacological mechanisms mediating the effects of antidepressant treatments. These different functions of animal models have different and, to some extent, conflicting requirements.

A simulation of depression aims to mimic aspects of the clinical situation, and should embody a degree of complexity, to permit investigation of the validity of the model. In addition, if a model is to be used to investigate antidepressant actions, a slow onset comparable to the clinical time course is highly desirable, and the model should therefore exhibit differences (either in direction of response, or in response magnitude) between single (acute) and repeated (chronic or continuous) treatment regimes. By contrast, the only essential requirement for antidepressant screening tests is that they make accurate predictions of antidepressant activity. For practical reasons, they should also be cheap, robust, reliable and easy to use (Danysz *et al.*, 1991; Willner, 1991a), and for all of these subsidiary reasons, a screening test should in principle be as simple as possible. However, the view that such screening tests should also respond acutely has changed during the past decade, in line with the drive from the clinic to identify rapid-onset antidepressant treatments. By necessity this approach involves the assessment of drug action associated with chronic/continuous drug treatment regimes and an acute response is thus of little value. Appropriate screening tests to be used relatively early during drug development should therefore have the ability to identify the time course of drug action associated with repeated treatment schedules.

The present chapter is concerned primarily to evaluate the suitability of the available animal models as research tools, and we therefore focus initially on issues relating to the validity of the available models as simulations of depression.

VALIDITY

In line with current thinking, our assessment of the validity of animal models of depression addresses the three dimensions of predictive, face and construct validity (Willner, 1984, 1991a). The concept of predictive validity implies that manipulations known to influence the pathological state should have similar effects in the model. Face validity refers to a phenomenological similarity between the model and the disorder being modelled. Construct validity implies that the model has a sound theoretical rationale. Some reviewers have advocated the primacy of one of these approaches (predictive validity: Geyer and Markou, 1995; face validity: Weiss and Kilts, 1998; construct validity, Sarter and Bruno, Chapter III). In principle, we share Sarter and Bruno's position, that construct validity is the most important of the three dimensions. In practice, however, the construct validity of animal models of depression is difficult to determine, and therefore we favour a balanced approach in which a view of the validity of a model is formed only after considering all three sources of evidence. We therefore begin by reviewing briefly the three sets of validation criteria, as they apply to animal models of depression.

Predictive Validity

In practice, the predictive validity of animal models of depression is determined solely by their response to antidepressant drugs. A valid test should be sensitive and specific: it should respond to effective antidepressant treatments ('true positive' effects), including electroconvulsive shock (ECS), and should fail to respond to ineffective agents ('true negative' effects). A model with high predictive validity should therefore maximize identification of both 'true positives' and 'true negatives', but should minimize identification of 'false positives' and 'false negatives'. Furthermore, positive responses should occur at behaviourally selective doses (i.e. those which do not generally disrupt behaviour, or induce motor impairment) that are within or close to the clinical range, and should be demonstrable with a range of structurally diverse compounds. It should be recognized that no animal model has a 100% prediction rate although some complex experimental paradigms have approached this level of predictive ability. Part of the problem lies not so much with the preclinical model but with the fact that there are several grey areas in the clinical literature where it is not known whether certain drugs (e.g. anticholinergics) possess antidepressant activity or not. It is generally agreed that the most effective treatment for depressive illness is electroconvulsive therapy (ECT). A suitable starting point to test the validity of an animal model should therefore be to demonstrate a positive response to repeated ECS. Failure to respond appropriately to ECS would severely question the predictive validity of the model.

About 30% of depressed patients fail to respond to antidepressant drug treatment, while the response rate for ECT is slightly higher. The occurrence of refractory patients probably reflects the heterogeneity of depressive illness. Nevertheless, a model that did not respond to the benchmark tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), or the recently developed serotonin–noradrenaline reuptake inhibitors (SNRIs)

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would not be taken seriously. This situation is unlikely to change in the absence of well-established therapies for tricyclic-resistant depression.

Beyond the clinically established antidepressant treatments (TCAs, monoamine oxidase inhibitors (MAOIs), SSRIs, SNRIs and some atypical antidepressants, such as mianserin) are a wide range of newer compounds for which antidepressant activity has been claimed, ranging from pharmacologically-selective compounds that are probably effective (e.g. α_2-adrenoceptor antagonists; ligands for serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes including $5HT_{1A}$ receptor ligands that possess agonist activity (and partial agonists), ligands for 5-HT_{2A} and 5-HT_{2C} receptor subtypes, and *N*-methyl-D-aspartate (NMDA) receptor ligands), through preparations of mixed pharmacological activity (e.g. SSRI with selective 5-HT_{1A} receptor antagonist activity) to compounds of uncertain status (e.g. phosphodiesterase inhibitors, calcium antagonists, anticonvulsants). Even in the case of the SSRIs, whose antidepressant efficacy is beyond doubt, it is still uncertain whether their spectrum of activity is identical to that of the TCAs. Therefore, while a well-rounded description of an animal model of depression should include an account of its pharmacological profile broader than that provided by its response to traditional antidepressants, the contribution of these newer compounds to validation is limited.

To some extent, similar uncertainties exist in relation to drugs which are ineffective as antidepressants. The 'false positives' most commonly encountered in animal models of depression are psychomotor stimulants, anticholinergics and opiates. However, prior to the development of antidepressants, drugs from all three of these classes were regularly prescribed for the relief of depression (see Willner, 1985). Indeed, certain opiates (e.g. buprenorphine) have antidepressant activity (Emrich *et al.*, 1983), but anticholinergics and stimulants have never been properly assessed, as their use was discontinued prior to the introduction of blind clinical trials. Again, the uncertain status of these compounds to some extent undermines their value as definitive standards of negative control.

Finally, the response to antidepressant drugs is insufficient to define an animal model of depression. Some antidepressants are active in some animal models of anxiety and panic, following chronic treatment (Bodnoff *et al.*, 1988; Fontana *et al.*, 1989), and indeed, are increasingly seen as the drugs of choice in many forms of anxiety (see Den Boer, Chapter XIX-13). It is therefore crucial for establishing the predictive validity of an animal model of depression to demonstrate that the model does not respond to benzodiazepines.

Face Validity

To assess face validity, the extent of similarity between the model and the disorder is examined, on as wide as possible a range of symptoms and signs. However, not all symptoms of a psychiatric condition carry equal weight and for an animal model to be valid, a resemblance to the clinically defined core symptoms of the disorder carries more weight than a resemblance to any subsidiary symptoms (Abramson and Seligman, 1978). The most recent edition of the *Diagnostic and Statistical Manual* (DSM-IV) provides a framework within which to assess these similarities. However, not all of the clinical symptoms of depression can be modelled in animals; symptoms conveyed by subjective verbal report (e.g. excessive guilt, feelings of worthlessness, suicide ideation) are in principle excluded (Willner, 1984, 1990).

A DSM-IV diagnosis of major depression requires the presence of at least one of two core symptoms: loss of interest or pleasure (anhedonia) and depressed mood. Of these two core symptoms, anhedonia can be modelled in animals, but depressed mood cannot. In assessing the face validity of animal models of depression, anhedonia therefore assumes a central position. The problem here, however, is that anhedonia is also a core symptom of schizophrenia. Drug-induced reversal of anhedonia in animal models, while highly encouraging, should thus be considered in relation to DSM-IV criteria for both depressive illness and schizophrenia.

The subsidiary symptoms of depression in DSM-IV that are amenable to modelling in animals include psychomotor changes, fatigue or loss of energy (which might be modelled as decreased persistence), and disturbances of sleep or food intake. Interestingly, psychomotor activity, sleep and appetite may be increased or decreased, and diametrically opposite changes in locomotion have both been cited in support for the validity of a model. This lack of precision, together with the fact that the clinical phenomena of psychomotor retardation and agitation (which are considerably more complex than gross changes in locomotor activity) may even coexist (Nelson and Charney, 1981), suggests that simulations in which a change in locomotor activity is the major, or only, behavioural feature should not be taken too seriously. Unfortunately, it is precisely these behaviours that feature most prominently in many animal models of depression (Willner, 1991b).

Because the pharmacotherapy of depression typically requires chronic drug treatment, the validity of an animal model is called into question by an acute response to conventional (e.g. TCA) antidepressant treatment. There is some evidence of very early antidepressant responses in clinical studies designed explicitly to detect them (e.g. Frazer *et al.*, 1985; see also Willner, 1989a) but even so, repeated treatment is required for a full response. Acute treatment refers to a single bolus dose of drug and any acute treatment effects are usually observed within minutes or hours following the bolus dose. Multiple acute doses may be administered, over a short period, simply to increase levels of drug in the biophase without evoking secondary adaptive changes in neurotransmitter mechanisms. In contrast, chronic treatment refers to repeated bolus doses administered for extended periods (days, weeks, months). Chronic treatment effects may be associated with drug-induced adaptive changes in neurotransmitter-receptor-mediated systems, rather than increased drug levels in the plasma. Thus, in comparison to chronic treatment effects, an acute response in an animal model may be opposite, orthogonal (due to side effects, e.g. sedation) or absent. Irrespective of how it responds to acute antidepressant treatment, to be valid, an animal model of depression must respond to chronic treatment. Furthermore, since tolerance does not occur to the clinical effects of antidepressants, the response in the model must be maintained until the cessation of antidepressant treatment. This test has not been universally applied to animal models of depression, but in general, those models to which the test has been applied have passed it, though tolerance to antidepressant effects has been reported in some behavioural paradigms (e.g. Cuomo *et al.*, 1983; Niesink and van Ree, 1982).

Construct Validity

An evaluation of the theoretical rationale of an animal model requires a means of bringing the theoretical accounts of both the disorder itself and the disordered behaviour exhibited by the model into alignment. However, any evaluation of animal models of depression is intrinsically limited by the rudimentary state of theories of the pathology of depression. Indeed, there is little in the extensive literature describing neurochemical abnormalities or biochemical markers reportedly associated with depression that can be usefully employed to provide a theoretical standard against which to validate animal models. Even the most basic questions of whether the level of activity in monoaminergic systems is elevated or decreased in depression were and remain controversial (Willner, 1985).

Similar problems arise in relation to modelling the aetiology of depression. It is now clear that a variety of different factors

are implicated in the aetiology of depression: 'psychological' factors include undesirable life events, chronic mild stress, adverse childhood experiences, and personality traits such as introversion and impulsiveness; 'biological' factors include genetic influences, and a variety of physical illnesses and medications (see Akiskal, 1985, 1986; Willner, 1985 for reviews). However, for most of these factors, there is little theoretical understanding of the processes by which they influence the physiological processes underlying mood. In certain cases, the immediate precipitant of a depression may be clearly identified: for example, seasonal affective disorder (SAD) and post-partum depression. More usually, the pathogenesis of depression is better understood as the result of an accumulation of a number of different risk factors (Akiskal, 1985; Aneshensel and Stone, 1982; Brown and Harris, 1978). This point has been largely overlooked in the construction of animal models of depression, which in general have assumed a single causal factor. This may be counterproductive, since few of the identified aetiological factors appear sufficiently potent to precipitate depression in an otherwise risk-free individual.

Although attempts to assess the theoretical rationale of animal models are limited by this lack of theoretical structure at the clinical level, a number of generalizations are possible. The major group of animal models of depression are based on responses to stressors of various kinds, and are usually justified by reference to the role of stressful life events in the aetiology of depression (Brown and Harris, 1978, 1988; Lloyd, 1980). However, if very severe acute stress is used (e.g. Weiss *et al.*, 1982), the relevance of these procedures to depression, rather than, for example, to posttraumatic stress disorder (PTSD) is questionable. Furthermore, the adverse consequences of life events endure for a prolonged period of six to twelve months, in part by exacerbating ongoing life difficulties (Brown, 1989; Brown and Harris, 1988). Thus, life events should not be viewed as acute stressors (indeed, in the case of bereavement, a diagnosis of depression is explicitly excluded during the period of acute loss); from this perspective, it may be more appropriate to use chronic stress regimes, rather than acute stressors, to model the aetiological role of life events. Other factors have been identified that confer a long-lasting vulnerability to depression, in particular, an inadequate level of social support, which to a large extent arises from inadequate socialization (Aneshensel and Stone, 1982; Brown, 1989; Brown and Harris, 1978, 1988; see Willner, 1989b). From these starting points, a number of animal models of depression have been developed that are based on the adverse effects of social isolation. However, with the exception of some of the primate studies (see below), these models have largely ignored both the complexity of childhood social deprivation phenomena and the mediation of their effects through later social relationships.

Although assessment of the theoretical rationale of animal models of depression is limited by the paucity of theory, construct validity can also be evaluated at the level of constructs: that is, whether the behavioural phenomena are correctly described. This approach is best exemplified by the extensive experimental analysis of whether 'learned helplessness' is an appropriate term to describe the impairments of escape learning that follow exposure to inescapable electric shock (Seligman, 1975). The term 'learned helplessness' implies that the animals perform poorly because they have learned that their responses are ineffective in controlling their environment (Seligman, 1975). However, inescapable shock has a variety of other, simpler effects that could also explain many of the behavioural impairments, such as decreased locomotor activity (Anisman *et al.*, 1979; Glazer and Weiss, 1976) and analgesia (Lewis *et al.*, 1980). In addition, while inescapable shock results in 'cognitive' impairment (Jackson *et al.*, 1978) such behavioural effects arise from impairment at the level of attentional processing rather than 'helplessness' (Minor *et al.*, 1984). Thus, there are many reasons to question whether the learned helplessness procedure does in fact produce 'helplessness'.

Anhedonia is another construct that has been subjected to this kind of experimental attention. In this case, the question is whether the animals are failing to perform rewarded behaviour because they are insensitive to rewards or for some other reason. This question has been most extensively investigated in relation to the effects of chronic sequential exposure to a variety of mild stressors (chronic mild stress; CMS). CMS has been shown to cause an antidepressant-reversible decrease in the consumption of dilute sucrose solutions, which is hypothesized to reflect a decrease in the reward value of the sucrose (Willner, 1997; Willner *et al.*, 1987). Initial studies showed that, in contrast to the effect on dilute solutions of sucrose or saccharin, CMS did not decrease the intake of plain water, food pellets or concentrated sucrose solutions; thus the effects are not simply nonspecific changes in consummatory behaviour (Muscat and Willner, 1992). Some studies have suggested that decreases in sucrose intake reflect loss of body weight (e.g. Matthews *et al.*, 1995), but there is ample evidence (reviewed in Willner, 1997) to reject this suggestion, including the fact that the effects are seen in the absence of body weight changes. Furthermore, the effects of CMS are not confined to consummatory behaviours. CMS also attenuated or abolished place preferences established using a variety of natural or drug rewards. However, drug-induced place aversions were unaffected by CMS. Thus, the effect of CMS on place conditioning cannot be explained by nonspecific motivational impairments or a failure of associative learning (reviewed in Willner, 1997). Finally, CMS has also been shown to cause an increase in the threshold for brain-stimulation reward (Moreau *et al.*, 1992). Together, these results support the position that CMS causes a generalized decrease in sensitivity to rewards, and that under appropriate experimental conditions, decreases in sucrose intake provide a simple means of detecting this anhedonia.

These examples of successful experimental analyses of construct validity contrast sharply with a third example, the 'behavioural despair' test (Porsolt *et al.*, 1979). This term was introduced to describe the immobility seen in rats or mice forced to swim in a confined space, based on the assumption that when the animals adopted an immobile posture, they had 'despaired of' escaping. Unfortunately, this interpretation is not susceptible to experimental analysis. Consequently, although the test continues to be very widely used, the name 'behavioural despair' has been largely abandoned, and replaced instead by the theoretically neutral term 'the forced swim test'.

Heterogeneity of Depression

It has frequently been remarked (e.g. Fibiger, 1991) that the approach adopted in successive editions of the DSM, which identifies psychiatric disorders by the presence of a sufficient number of symptoms from a longer list, results in very different clinical presentations receiving the same diagnosis. However, discussion of the heterogeneity of depression is several decades older than the DSM system. It has been recognized since the 1920s that there are at least two forms of depression, but only one of them has enjoyed general agreement as to its nature (Willner, 1985). This is the syndrome that has been known, variously, as autonomous depression, endogenous depression, endogenomorphic depression and, more recently, melancholia. Because a consensus exists as to its existence and symptom profile, melancholia has emerged as the major subtype of depression, and its definition in DSM-IV is somewhat more restrictive than that of the parent syndrome, major depressive disorder.

Little attention has been paid to the question of subtypes of depression in the development of animal models, which have tended to focus either on an undifferentiated depressive state or on melancholia. Melancholia is defined, in DSM-IV (major depressive episode with melancholic features), by the presence of anhedonia,

which is also the only core symptom of major depression that is amenable to modelling in animals. Anhedonia may be readily modelled as decreased sensitivity to rewards, although, as noted above, this construct is considerably more complex than a simple decrease in the performance of a rewarded behaviour. In relation to the other symptoms that can be modelled, the diagnosis of melancholia specifies the direction of changes in appetite and sleep (decreased), although, as in the diagnosis of major depressive disorder, psychomotor changes could be in either direction (retardation or agitation). In many empirical studies, psychomotor retardation has emerged as the symptom most characteristic of melancholia, while psychomotor agitation tends to be more closely associated with psychotic features such as delusions of guilt (Nelson and Charney, 1981). Nevertheless, there is considerable overlap between these two groups of symptoms, and agitated melancholias are not uncommon. Unlike non-melancholic depressions, melancholia is characterized by a decrease in the latency to enter the first period of rapid eye movement (REM) sleep (Kupfer and Thase, 1983) and an increased activity of the hypothalamic–pituitary–adrenal (HPA) system, usually detected by the dexamethasone suppression test (DST) (Carroll, 1982). In a valid simulation of melancholia, these biological markers would be expected to coexist alongside decreased sensitivity to rewards. However, the specificity of both of these markers for melancholia is less than originally claimed.

Unlike melancholia, no consensus exists that non-melancholic depression is a homogeneous entity, and this issue has been extensively debated. Within the 'non-melancholic' spectrum, two syndromes have been described that are of interest in the present context. One of these is not recognized in DSM-IV, but has features which suggest that it might represent a coherent biological entity. This is a form of depression in which central 5HT activity is decreased, as indicated by low concentrations of the 5HT metabolite 5-hydroxyindole acetic acid (5-HIAA) in cerebrospinal fluid. These depressions are characterized by high levels of anxiety and agitation, and Van Praag (1994) has suggested that these symptoms are primary, with depressed mood as a secondary response to a failure to cope with the consequences. It has been suggested that some animal models of depression, which are characterized by high levels of locomotor activity and/or aggression, may represent models of this subtype. There is as yet little further evidence on which to judge the validity of these claims.

A second 'non-melancholic' subtype of depression is delusional depression (previously known as psychotic depression). Delusional depression (DSM-IV major depressive episode with psychotic features) is difficult to translate into behavioural terms, being differentiated from non-delusional depression only by a greater association with psychomotor agitation (Nelson and Charney, 1981). Delusional depressions are pharmacologically distinct in that they respond to ECT or combinations of TCAs with neuroleptics, but not to TCAs alone (Nelson, 1987). The development of an animal model that responds to ECS but not to TCAs has not yet been achieved.

Bipolar disorder is another well-defined diagnostic category for which there are no animal models. There are a number of animal models of mania (see Lyon, 1991), but the alternation of depressive-like and manic-like behaviours in an animal model has not yet been systematically addressed. Indeed, the episodic nature of unipolar depression (e.g. brief recurrent depression) also remains to be explored in animal models.

THE DIATHESIS/STRESS CONCEPT

It is widely assumed that individuals within the population vary in their risk of contracting psychiatric disorders, and that such disorders are typically precipitated by external or internal (e.g. hormonal) events. The term diathesis refers to a predisposition to contract the disorder, while the term stress refers simply to the precipitant (note that the term 'stress' is used here in a different sense from the usual). A predisposition to become depressed (a depressive diathesis) may arise in a variety of ways, which may involve different levels of explanation. For example, a number of genetic diatheses have been identified, particularly in relation to bipolar disorder (see Souery *et al.*, Chapter XVIII-10). Some early life experiences are also known to increase the risk for depression, particularly parental deprivation (Robertson and Bowlby, 1952). The mechanisms by which these experiences increase the risk of depression (and other psychiatric disorders) are largely unknown, but probably involve psychological and psychosocial constructs. For example, parental deprivation may decrease the ability to form close relationships, and so dilute the quality of social support available in later life. Early life experiences also determine characteristic styles of processing information in relation to the self. For example, the negative thinking that characterizes the depressed person is thought to reflect the activation of a negative 'cognitive schema', learned through adverse childhood experiences such as rejection, criticism, or living with a depressed parent (Beck, 1967).

It follows from the diathesis/stress concept that a person who has a weak depressive diathesis would only succumb to an intense stress, whereas a person with a strong depressive diathesis may succumb to minor or trivial stresses. As these minor events might be relatively common, a depressive diathesis might result in a chronic presentation that looks very similar to an acute major depressive episode. Indeed, there is a depressive (gloomy, pessimistic, introverted) personality type, and where the symptoms are intense and disabling, this is classified as a depressive personality disorder. (This syndrome, present in DSM-III-R, was removed from DSM-IV as a clinical diagnosis, but is retained for research purposes.) A depressive personality disorder can shade into dysthymia, a more intense and more variable presentation. The diagnostic criteria for dysthymia include a number of somatic symptoms that are absent in the criteria for depressive personality disorder, but DSM-IV comments: 'It remains controversial whether the distinction between depressive personality disorder and dysthymic disorder is useful. The research criteria given for this proposed disorder differ from the diagnostic criteria for dysthymic disorder by their emphasis on cognitive, interpersonal, and intrapsychic personality traits.' This emphasis means that the symptoms of depressive personality disorder fall outside the scope of animal models. Dysthymia, however, can be modelled in animals. Dysthymia may be present from early childhood, or may have a more definable later onset. In either case, the distinction between dysthymia and a major depressive episode rests largely on the fact that dysthymia is less intense, and of chronic duration. In other respects, major depression and dysthymia are very similar in their presentation (for example, DSM-IV specifies that there are even common abnormalities in sleep electroencephalogram (EEG) recordings). Indeed, the only significant differences are in some of the subjective symptoms that are not amenable to animal modelling, such as excessive guilt and suicidal ideation, which are less prominent in dysthymia.

Thus, depressive diatheses may present as 'silent' until activated by stress (e.g. a genetic risk factor that does not directly elicit depressive symptoms, or a tendency towards depressive thinking that is well controlled), as a sub-syndromal depressive condition, or as a chronic condition that is difficult to distinguish from a major depressive episode. Consequently, animal models of a depressive diathesis could appear either as essentially 'normal' animals that are more sensitive to a precipitant used to induce 'depressive' behaviour, or could present 'depressive' features in the absence of any specific experimental manipulation.

In surveying changes over time in the landscape of animal models of depression, the major recent development has been a growth in the number of models that are better thought of as models of a

predisposition to depression rather than as a depressive response to a precipitating event. We have therefore adopted this distinction as the organizing principle of this chapter. In the remainder of the chapter, we discuss first those models of depression that are based on exposure to adversity, and later, models that create or uncover a depressive diathesis. For the sake of completeness, we also mention briefly some of the pharmacological screening tests that were among the earliest animal models of depression to be developed.

MODELS OF DEPRESSION AS A RESPONSE TO ADVERSITY (i.e. STRESS)

A variety of adverse events have been used to produce animal models of depression, based on the observation that in general, the behavioural effects of stress are reversible by antidepressant drugs. However, the extent to which these effects meet the criteria for predictive validity, and the quality of the resulting models in terms of face and construct validity, varies greatly. For ease of presentation, we have grouped the models reviewed according to the nature of the stressor: acute experimenter-applied stressors; chronic experimenter-applied stressors; and models based on social stress and social isolation.

Acute Stress Models

Learned Helplessness Models

As noted earlier, the learned helplessness paradigm is based on the observation that animals exposed to uncontrollable stress (usually electric shocks) are subsequently impaired in learning to escape shock, an effect that is not seen in animals exposed to comparable, or indeed, identical, patterns of controllable shock. The protective effect of control appears to result from lower levels of fear (Jackson and Minor, 1988), which raises important questions about the relationship between depression and anxiety. Seligman (1975) proposed that exposure to uncontrollable stress provides the basis, in animals as in people, for learning that stress is uncontrollable (helplessness), and that this learning has a number of debilitating consequences, including depression. However, this interpretation has been the subject of considerable controversy, in both the human (Abramson *et al.*, 1978, 1989) and the animal literature (Minor *et al.*, 1984, 1988; Weiss *et al.*, 1982) and is probably incorrect (see above, and Willner, 1986, 1990). Nevertheless, the learning difficulties that follow exposure to uncontrollable shock are reversed with reasonable selectively by multiple acute, subchronic (3–5 days), treatment with TCAs or atypical antidepressants (Sherman *et al.*, 1982). SSRIs also appear to be effective, but only within a limited dose range (Martin *et al.*, 1989), as well as a number of other potential antidepressants such as 5-HT_{1A} receptor agonists (Giral *et al.*, 1988), β-adrenoceptor agonists (Martin *et al.*, 1986a), although some of these effects have been disputed (Christensen and Geoffroy, 1991), and extracts of St John's wort (*Hypericum perforatum*) (Gambarana *et al.*, 1999a; Kumar *et al.*, 2001). Interestingly, chronic, but not acute, treatment with lithium prevents the development of learned helplessness in rodents (Teixeira *et al.*, 1995). There are a few false positives, including the 5-HT_2 receptor antagonist, methysergide (Brown *et al.*, 1982), *p*-chlorophenylalanine (*p*CPA) (Edwards *et al.*, 1986) and piracetam (Cavoy *et al.*, 1988), while neuroleptics, stimulants, sedatives and anxiolytics are generally ineffective (Porsolt *et al.*, 1991; Sherman *et al.*, 1982).

The learned helplessness paradigm shows many symptomatic parallels to major depression — much so that it has been suggested that rodents subjected to uncontrollable shock could meet DSM diagnostic criteria (Weiss *et al.*, 1982)! However, the learned helplessness paradigm is implemented in a variety of different ways in different laboratories, and the version of the paradigm giving rise to the broadest range of symptoms (Weiss *et al.*, 1982) uses extremely high shock levels (4–6 ma) which are of doubtful relevance to depression. Furthermore, the effects of this regime largely dissipate within 2–3 days, and the effects of antidepressant pretreatment have not been studied using this procedure. The effects of antidepressants have typically been studied using shocks of a considerably lower intensity (1.0–1.5 ma) which cause a far less pervasive pattern of behavioural impairment.

Paradoxically, the effects of milder shock intensities are of longer duration, making it possible to interpose multiple acute (subchronic) drug treatment (typically for 3–5 days) between the initial uncontrollable shock session and the learning test. Furthermore, the duration of learned helplessness may be prolonged indefinitely by repeatedly exposing the subjects to the environment in which inescapable shock had occurred (Maier, 2001), which strengthens the face validity of this model of depression. However, a further problem is that different components of the learned helplessness syndrome may be related to different aspects of the induction procedure. For example, a long-term (7 weeks) suppression of home-cage locomotor activity has been reported following a single shock session (Desan *et al.*, 1988), but this effect is unrelated to shock controllability, and so clearly distinct from other learned helplessness phenomena (Woodmansee *et al.*, 1991). It is clear that the learned helplessness paradigm should more correctly be considered as a mixture of paradigms, and care should be taken in generalizing conclusions between them.

Among the consequences of low-intensity uncontrollable (but not controllable) shock is a poor performance of rewarded behaviour, which, as noted above, may be of particular relevance to melancholia. One manifestation of this effect is a long-lasting decrease in responding for brain-stimulation reward (intracranial self-stimulation; ICSS), in mice, which is specific to certain electrode placements, and therefore suggests a subsensitivity within part of the brain mechanism of reward, rather than, for example, a motor impairment (Zacharko *et al.*, 1983, 1987). Normal sensitivity to ICSS was restored by chronic, but not acute, treatment with TCAs (Zacharko and Anisman, 1991; Zacharko *et al.*, 1983). Interestingly, a long-lasting anhedonia is seen only if the animals are tested in the immediate aftermath of stress; otherwise the effect dissipates rapidly (Zacharko *et al.*, 1983). A related observation is that mild stressors, which are without effect in normal animals, may reinstate behavioural deficits resulting from an initial exposure to severe stress (Anisman and Zacharko, 1982). These studies suggest that it may be possible to develop conditioning models to explain how the risk of depression is elevated for several months in the aftermath of a stressful life event.

Another important observation is that uncontrollable electric shock has variable behavioural effects (most of which are antidepressant-reversible) in different inbred mouse strains. To take an extreme example, in the C57BL/67 strain, uncontrollable shock severely impaired subsequent learning to escape shock, but had no effect on responding for brain-stimulation reward, while the DBA/2J strain showed exactly the opposite pattern of deficits (Shanks and Anisman, 1988; Zacharko *et al.*, 1987). These studies may provide a starting point from which to investigate the physiological mechanisms underlying individual differences in responses to stress.

Vollmayr and Henn (2001) have recently described a procedure in which mild shocks induce learned helplessness in only some of the subjects, which may mimic the variable human predisposition for depressive illness. This procedure has been used as the basis for a selective breeding programme, which has produced a 'congenital learned helplessness' (cLH) and a 'congenital non-learned helplessness' strain. An impressive recent neuroimaging study of cLH rats reported that metabolism was decreased in dorsal frontal, medial orbital and anterior cingulated cortex, but increased in the subgenual region of the cingulated cortex (Shumake *et al.*, 2000); exactly these changes have been described in depressed patients (Drevets

and Raichle, 1998; Mayberg *et al.*, 1999). However, there are also anomalies: in particular, cLH rats show a decreased adrenocortical response to stress (King *et al.*, 2001). There is also uncertainty as to just what is being selected in the cLH breeding programme. cLH animals have been shown to exhibit stress-induced analgesia (King *et al.*, 2001), raising the possibility that an increase in pain threshold might provide a very simple explanation of their escape learning impairments. And given the separation of shock-induced escape deficits and anhedonia in inbred mouse strains (Shanks and Anisman, 1988; Zacharko *et al.*, 1987), there is no reason to assume that the cLH strain, which was bred by selecting on the basis of shock-induced escape deficits, would also exhibit shock-induced anhedonia.

There is a relatively extensive literature dealing with the neurochemical bases of stress-induced motor inactivation (see Maier, 1984; Willner, 1985). Briefly, the debilitating effect of uncontrollable stress on later performance may be reversed by agonists at μ-opioid, dopamine (DA), α_1- and β-adrenergic receptors or by anticholinergic drugs; conversely, helplessness may be simulated pharmacologically by drugs that reduce DA and/or noradrenalin (NA) function, or by drugs that increase cholinergic function (Anisman and Zacharko, 1982; Besson *et al.*, 1996, 1998, 1999). Any influence of DA neurotransmission on learned helplessness appears to be mediated by the D1 dopamine receptor (Gambarana *et al.*, 1995a, b). Furthermore, reduced catecholamine neurotransmission, either by inhibition of catecholamine synthesis with α-methyl-ρ-tyrosine (De Montis *et al.*, 1993) or by treatment with α_1- and β-adrenoceptor antagonists (as well as the opiate receptor antagonist naloxone) has been shown to block the therapeutic effect of TCAs (Martin *et al.*, 1986b). Similarly, blockade of NMDA-receptor-mediated neurotransmission also blocks TCA-induced reversal of learned helplessness (Meloni *et al.*, 1993). Gamma-aminobutyric acid (GABA) neurotransmission also seems to be involved in learned helplessness behaviour. Thus a long-term increase in $GABA_B$ neurotransmission is associated with exacerbation of learned helplessness, while the action of antidepressant drugs in this model is associated with a long-term reduction in $GABA_B$ neurotransmission (Nakagawa *et al.*, 1996a, b). In contrast, studies of the role of 5-HT in learned helplessness are inconsistent (see Willner, 1990). Some 5HT receptor agonists may reverse learned helplessness, while 5-HT lesions did not prevent the action of TCAs in this model (Soubrie *et al.*, 1986). The induction of behavioural depression/learned helplessness may be regulated, at least in part, by serotonergic input into the hippocampal CA3 subfield. Papolos *et al.* (1996) have shown that intracerebroventricular administration of an antisense oligonucleotide to the $5\text{-}HT_{2A}$ receptor reduced receptor density in the CA3 area and induced learned helplessness behaviour.

Forced Swim Test

In the forced swim test, rats or mice are forced to swim in a confined space. While mice are subjected to a single swim test following a single drug administration, rats are generally subjected to two tests, usually spaced 24 h apart, to generate increased immobility scores. The onset of immobility exhibited by rats in the second test is delayed by pretreatment with a wide variety of antidepressants, usually administered in a multiple acute (subacute) treatment schedule consisting of three injections over the time period between the two swim sessions. The fact that the forced swim test responds to acute drug treatment has been a frequent source of criticism. Small antidepressant effects may be present after a single high dose. Larger effects are observed after multiple acute treatments at lower doses, but these changes may simply reflect an elevation of brain drug concentrations.

Effective treatments include TCAs, MAOIs, most atypical antidepressants, the selective NA reuptake inhibitor, reboxetine (Connor *et al.*, 1999), $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$ and $5\text{-}HT_{2C}$ receptor agonists (Cryan and Lucki, 2000; O'Neill and Conway, 2001), extracts of St John's wort (*Hypericum perforatum*) (Butterweek *et al.*, 1997), ECS and REM sleep deprivation (Borsini and Meli, 1988; Porsolt, 1981; Porsolt *et al.*, 1991). The test is not usually sensitive to SSRIs (Borsini, 1995; Borsini and Meli, 1988; Connor *et al.*, 2000), although activity of these compounds has sometimes been reported, and this appears to reflect a subtle technical change, an increase in the water depth (e.g. Cryan and Lucki, 2000; van der Heyden *et al.*, 1991). There have also been a variety of other negative reports; nevertheless, some 90% of clinically active antidepressants are active in the forced swim test, with the proportion rising in studies using chronic drug treatment (Borsini and Meli, 1988; Porsolt *et al.*, 1991). It has been suggested that both serotonergic and noradrenergic systems may be involved in mediating antidepressant-induced reductions in immobility; serotonergic-mediated effects result from increased swimming time while noradrenergic-mediated effects reflect increased climbing behaviour (Detke *et al.*, 1995).

Reduced immobility scores have also been observed following treatment with neurosteroids (Reddy *et al.*, 1998), neuropeptide Y (Stogner and Holmes, 2000) and NMDA receptor antagonists (Panconi *et al.*, 1993; van der Bos *et al.*, 1992). The effect of NMDA receptor antagonists is to reduce calmodulin-mediated activation of nitric oxide (NO) synthase. Likewise, NO synthase inhibitors have been reported to be as effective in reducing immobility as imipramine (Harkin *et al.*, 1999a). In rat studies, selective antagonists for subtypes of the cholecystokinin (CCK) receptor have both been shown to reduce immobility score. However, these positive effects are dependent on time of drug administration. Thus, the CCK_A antagonist devazepide is only effective when given before the conditioning pre-test (Hernando *et al.*, 1996) while the CCK_B antagonist L-365,260 is effective when given immediately prior to the re-test (Hernando *et al.*, 1994). These observations suggest a role for CCK in behavioural adaptations to acute stress.

On the negative side, while the test successfully discriminates antidepressants from neuroleptics and anxiolytics (Porsolt *et al.*, 1977), false positives have been reported for stimulants, convulsants, anticholinergics, antihistamines, pentobarbital, opiates, a variety of brain peptides (see above) and a number of other drugs (Borsini and Meli, 1988; Porsolt *et al.*, 1991). Some false positive responses have been reported to disappear if chronic drug treatment is used, or the duration of the test is prolonged (Kawashima *et al.*, 1986; Kitada *et al.*, 1981), but the generality of these effects has not been established.

While the predictive validity of the forced swim test may be better than has sometimes been assumed, its face and construct validity are minimal. The only symptomatic resemblance to depression is an inability or reluctance to maintain effort. Interestingly, positive responses to antidepressant drug treatment (i.e. reduced immobility) are only observed if subjects are forced to swim in water at least 10 °C below core body temperature (P.J. Mitchell, personal observations) and consequently develop severe hypothermia. If mice are forced to swim in water maintained at body temperature then immobility still occurs, but the response to antidepressant treatment is abolished. These observations clearly implicate the importance of severe cold stress and the consequent induction of hypothermia in this model, which further weakens the parallel with depression.

The theoretical rationale of this test derives entirely from its supposed relationship to learned helplessness. However, the relationship between the two models is unclear. Prior inescapable, but not escapable, shock has been found to increase immobility in the forced swim test, (Nomura *et al.*, 1982; Weiss *et al.*, 1981), but in view of the consistent finding of decreased motor activity following inescapable shock (see Anisman and Zacharko, 1982), it would be surprising were this not the case. The reciprocal finding has not been demonstrated: forced swimming did not impair subsequent escape performance in a shock avoidance task in which

performance deficits are typically seen following inescapable shock (O'Neill and Valentino, 1982).

Nevertheless, the two tests do seem to share similar physiological substrates. Immobility in the swim test is also reversed by stimulating 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors (Cryan and Lucki, 2000; O'Neill and Conway, 2001; van der Heyden *et al.*, 1991), DA or α_1-adrenergic receptors or by anticholinergics, and potentiated by treatments that decrease the activation of DA (e.g. amisulpride: Papp and Wieronska, 2000) or NA systems or increase cholinergic transmission. Similarly, the therapeutic actions of antidepressants in this model are blocked by DA receptor antagonists and by treatments that reduce NA function, including neurotoxic destruction of the ascending NA pathways and α_1-adrenoceptor antagonists. As in the learned helplessness test, impairment of 5HT transmission neither increases immobility nor blocks its reversal by antidepressants (see Borsini and Meli, 1988; Willner, 1990).

Tail-Suspension Test

A number of variants of the forced swim test have been proposed. One of these, the tail-suspension test, has been claimed to be ethically superior to the forced swim test, as stress levels appear to be reduced (Porsolt *et al.*, 1986; Steru *et al.*, 1985). In this model, mice suspended by the tail show a temporal pattern of struggling followed by immobility, similar to that seen in the forced swimming test. Antidepressants, at strikingly low doses, have been shown to increase the duration of mobility and also, in an automated version of the test, to increase the power of the movements (though the latter effect is rather less convincing). Effective agents include TCAs, MAOIs and atypical antidepressants; the latter include mianserin, the selective NA reuptake inhibitor, reboxetine (Wong *et al.*, 2000), extracts of St John's wort (*Hypericum perforatum*) (Butterweek *et al.*, 1997), the NMDA receptor antagonist MK-801 (Panconi *et al.*, 1993) and, significantly, some (but by no means all) 5-HT uptake inhibitors (David *et al.*, 2001; Perrault *et al.*, 1992; but see also Fujishiro *et al.*, 2001; Teste *et al.*, 1993), which are usually ineffective in the forced swim test (see above). Immobility was also reduced by stimulant drugs, but was potentiated by neuroleptics or anxiolytics (Porsolt *et al.*, 1986; Steru *et al.*, 1985).

While it is tempting to see the tail-suspension test as a more sensitive version of the forced swim test, there are subtle differences between them, which are not at present understood but involve significant mediation by a noradrenergic-receptor-mediated mechanism(s) (Ferrari *et al.*, 1991). In particular, $5HT_{1A}$ receptor agonists are active in the forced swim test, but are ineffective in the tail-suspension test (Porsolt *et al.*, 1991), whereas the mixed 5-$HT_{1A/1B}$ receptor agonist RU 24969 was active in the latter. Like the forced swim test, the face and construct validity of the tail-suspension test are minimal.

Restraint Stress

Rats subjected to 2 h restraint stress exhibit hypolocomotion when examined in an open field 24 h later. Chronic treatment with desmethylimipramine (desipramine, DMI) or sertraline prior to restraint stress reverses the hypolocomotion (Kennett *et al.*, 1987). There is little basis on which to evaluate the validity of this model, which is included here simply to illustrate the generality of the reversibility of stress effects by antidepressants. Note, however, that, unlike the models described earlier, which respond to acute antidepressant treatment, this model requires chronic treatment.

Chronic Stress Models

Chronic Severe Stress

Repeated presentation of the same stressor usually leads to adaptation. However, adaptation can be prevented by presenting a variety of stressors in an unpredictable sequence. Katz and colleagues showed that three weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle and a variety of other stressors caused a decrease in the activating effect of acute stress in an open field test. However, the activating effect of acute stress was maintained in animals receiving daily antidepressant treatment during the chronic stress period; the effects of administering antidepressants after exposure to stress have not been studied in this model. A variety of antidepressant drugs, as well as ECS, were found to prevent the effect of chronic stress, but the MAOI tranylcypromine was ineffective. Various non-antidepressants failed to prevent the effect of stress. In addition to causing changes in open field activity, chronic stress also increased plasma corticosteroid levels. This effect showed the same spectrum of pharmacological sensitivity, with the exception that an anticholinergic was also effective (Katz *et al.*, 1981; see Willner, 1990 for review). Similar effects have also been reported in mice; corticosteroid levels and the response to an acute stress were normalized by TCAs, but not by the SSRI fluoxetine (Soblosky and Thurmond, 1986).

A further effect observed in rats after chronic stress was a failure to increase fluid consumption when saccharine was added to the drinking water, suggesting that chronic stress might cause anhedonia; this deficit was partially restored by imipramine (Katz, 1982). The chronic stress model has been used very little since the original series of publications, in part because the levels of severity employed raise serious ethical problems. However, a variant of the model has been devised which employs very mild stressors, and this model is described next.

Chronic Mild Stress (CMS)

The CMS model was developed in an attempt to achieve the same endpoints as the chronic stress model, but in a more ethically acceptable manner. The procedure involves relatively continuous exposure of rats or mice to a variety of mild stressors, such as periods of food and water deprivation, small temperature reductions, changes of cage mates, and other similarly innocuous manipulations. Over a period of weeks of chronic exposure to the mild stress regime, rats gradually reduced their consumption of a preferred dilute sucrose solution, and in untreated animals this deficit persisted for several weeks following the cessation of stress (Willner *et al.*, 1987, 1991). As discussed earlier (see the section on Construct Validity, above), a variety of studies have been performed to confirm that these effects reflect a generalized insensitivity to reward; in particular, chronic mild stress also impairs responsiveness to reward as assessed by different methods, including suppression of place preference conditioning (Papp *et al.*, 1991, 1992, 1993) and increased threshold for ICSS (Moreau *et al.*, 1992). Studies of the effects of the individual elements of the chronic mild stress regime revealed that one element, social housing (in animals usually housed individually), was particularly potent, but no individual element (including social housing) was either necessary to induce anhedonia or sufficient to maintain anhedonia for a prolonged period (Muscat and Willner, 1992).

Antidepressant treatment has no effect on sucrose consumption or ICSS threshold in non-stressed animals, but following the reduction of sucrose intake by stress, normal behaviour was gradually restored by chronic treatment (2–5 weeks) with a wide variety of antidepressants, including TCAs, SSRIs, a specific NA reuptake inhibitor (maprotiline), MAO-A inhibitors, atypical antidepressants such as mianserin, buspirone, and amisulpride, and ECS. Also effective were some agents of uncertain antidepressant status, such as antihistaminic and anticholinergic drugs. Ineffective drugs include the anxiolytic chlordiazepoxide, various neuroleptics, amphetamine and morphine (reviewed by Willner, 1997; Willner

and Papp, 1997). Fluoxetine, maprotiline and mianserin (but not chlordiazepoxide) were also found to restore the rewarding properties of food, as assessed in the place conditioning paradigm (Cheeta *et al.*, 1994; Muscat *et al.*, 1992). In contrast to the extensive array of drugs correctly classified in CMS experiments, very few false positives or false negatives have been reported.

The reversal of an established behavioural deficit during the continued presence of the stressor is an important feature of this model: if, as seems likely, chronic stress does play a role in the aetiology of melancholia (e.g. Aneshensel and Stone, 1982; Monroe *et al.*, 1985; Rodgers, 1991), its continued presence during antidepressant therapy would usually be the norm. Also important is the extended time course of antidepressant action, which makes it feasible to detect rapid onset of action. Many novel agents have been identified as potential antidepressants using the CMS procedure, and these include some that appear to act more rapidly than TCAs or SSRIs, including the D2/D3 receptor antagonist amisulpride (Papp and Wieronska, 2000), the glycine antagonist 1-aminocyclopropanecarboxylic acid (ACPC) (Papp and Moryl, 1996), the $5HT_{1A}$ receptor agonist BIMT-17 (D'Aquila *et al.*, 1997), and the SNRI venlafaxine (M. Papp, personal communication). Potentiation of antidepressant action by lithium and pindolol has also been reported (Sluzewska and Szczawinska, 1996a, b). In at least three instances (the D2/D3 receptor agonist pramipexole, the corticosterone synthesis inhibitor ketoconazole, and the catechol-*O*-methyl transferase (COMT) inhibitor tolcapone), the antidepressant effect of these compounds was demonstrated in the clinic only after these actions had been predicted in the CMS model (see Willner, 1997).

In addition to decreasing responsiveness to rewards, CMS also causes the appearance of many other symptoms of major depressive disorder. Behavioural changes in animals exposed to CMS include decreases in sexual, aggressive and investigative behaviours, and decreases in locomotor activity. These are seen during the dark phase of the light–dark cycle, which is the rat's active period; EEG measures of active waking are also decreased during the dark phase. In contrast, CMS did not cause the appearance of an 'anxious' profile in two animal models of anxiety, the elevated plus-maze and the social interaction test, suggesting that the behavioural changes are specific for depression. Animals exposed to CMS show an advanced phase shift of diurnal rhythms, diurnal variation, with symptoms worst at the start of the dark (active) phase, and a variety of sleep disorders characteristic of depression, including decreased REM sleep latency, an increased number of REM sleep episodes, and more fragmented sleep patterns. They also gain weight more slowly, leading to a relative loss of body weight, and show signs of increased activity in the HPA axis, including adrenal hypertrophy and corticosterone hypersecretion. Abnormalities have also been detected in the immune system, including an increase in serum, decreases in thymus weight, natural killer cell activity and reactivity to T-cell mitogens, and an increase in acute phase proteins that was reversed by chronic antidepressant treatment. Taken together with the generalized decrease in responsiveness to rewards, these parallels to the symptoms of depression, and in particular, to melancholia, are both extensive and comprehensive (reviewed by Willner, 1997). Indeed, it is arguable that the only symptoms of depression that have not been demonstrated in animals exposed to CMS are those uniquely human symptoms that are only accessible to verbal enquiry. Even without these symptoms, a rat exposed to CMS could, in principle, legitimately attract a DSM-IV diagnosis of either major depressive disorder or major depressive disorder with melancholic features.

Studies of the neural basis of the CMS-induced anhedonia have focused primarily on the mesolimbic DA system. The behavioural changes in animals subjected to CMS are accompanied by a decrease in D2/D3-receptor binding and D2-mRNA expression in the nucleus accumbens, and a pronounced functional subsensitivity to the rewarding and locomotor stimulant effects of the D2/D3 receptor agonist quinpirole, administered systemically or within the nucleus accumbens. All of these effects are also reversed by chronic antidepressant treatment (Dziedzicka-Wasylewska *et al.*, 1997; Willner and Papp, 1997). In other studies, animals successfully treated with antidepressants were treated acutely with D2/D3 receptor antagonists, at low doses that were without effect in non-stressed animals or in untreated stressed animals. This treatment reversed the effects of a wide variety of antidepressants on rewarded behaviour (Willner and Papp, 1997). These data argue strongly that an increase in D2/D3 receptor responsiveness may be responsible for the therapeutic action of antidepressants in this model (Willner and Papp, 1997). A similar reversal of SSRI action by the D2 antagonist sulpiride has been observed in a clinical study, as predicted from the CSM data (see Willner, 1997).

A recent CMS study suggests a neural mechanism that could mediate the negative information processing bias characteristic of depression (Beck, 1967). In this study, DA release in nucleus accumbens and prefrontal cortex was monitored by microdialysis following exposure to a palatable reward and a stressor (tail pinch). CMS markedly inhibited DA release in response to rewards, but potentiated responses to the stressor; and both of these effects of CMS were reversed by chronic treatment with the TCA, DMI (Di Chiara *et al.*, 1999).

While the CMS model has a great many positive features, and is probably the most valid animal model of depression currently available (see below), a major drawback is that the model has proven extremely difficult to implement reliably, and while many laboratories have succeeded in doing so, many others have not. The reasons for this lack of reliability have been extensively debated (see Willner, 1997), but as yet are unresolved.

Stress Induction and Mild Stress Maintenance

Tagliamonte and colleagues have described a model that represents a cross between learned helplessness and CMS. In this procedure, an altered state is first induced by acute exposure to a session of moderately intense inescapable tail shock, and then maintained, apparently indefinitely, by exposure to milder stressors (brief restraint, a small number of shocks, or exposure to the inescapable shock apparatus), presented at two-day intervals. This treatment resulted in an impairment of shock escape learning, as in learned helplessness, and a failure to learn a simple maze task reinforced by a highly palatable food (vanilla sugar), as in CMS. Both of these deficits were prevented by chronic pretreatment with TCAs or fluoxetine (De Montis *et al.*, 1995; Ghiglieri *et al.*, 1997). The stress procedure also decreased basal levels of extracellular DA in the nucleus accumbens shell, and this change also was prevented by chronic TCA treatment (Gambarana *et al.*, 1999b).

We noted earlier (see the section on Construct Validity, above) that the effect of severe life events to precipitate depression was best characterized as an acute severe stress followed by a chronic increase in mild stress. Stress induction followed by mild stress maintenance exactly parallels these processes, and is the only animal model of depression to do so. This procedure could potentially provide a more reliable and robust alternative to the CMS procedure, but few data are as yet available. In particular, while antidepressants have been shown to prevent the development of behavioural abnormalities, they have not as yet been shown to reverse an established deficit. Also, the portability of the procedure to other laboratories has not yet been demonstrated.

Withdrawal from Chronic Psychomotor Stimulants (Amphetamine and Cocaine)

A number of studies have reported that responding for ICSS was reduced in the days following withdrawal from chronic

amphetamine treatment. In these studies, amphetamine was administered to rats for between 4 and 10 days, typically using several administrations each day, at increasing doses (Barrett and White, 1980; Kokkinidis and Zacharko, 1980; Leith and Barrett, 1976; Simpson and Annau, 1977). The threshold for ICSS was elevated following amphetamine withdrawal, confirming that the rate reduction reflects a subsensitivity to brain-stimulation reward rather than a depression of motor activity (Cassens *et al.*, 1981; Leith and Barrett, 1980). After 14 days of amphetamine treatment, the decreased sensitivity to ICSS did not recover during 18 days of further testing (Leith and Barrett, 1980) reflecting subsensitivity of reward systems rather than simple depression of motor activity. In a single pharmacological study, this effect was alleviated by two days of imipramine or amitriptyline treatment, and with continued treatment, normal responding was restored (Kokkinidis *et al.*, 1980).

In a variant of this procedure, animals self-administer cocaine, rather than being administered amphetamine (Koob, 1989). In these experiments, the threshold for brain-stimulation reward, administered through electrodes in the posterior lateral hypothalamus, was obtained using a discrete trial procedure (Kornetsky and Esposito, 1981), which is sensitive to changes in reward value, but not to changes in motor performance (Markou *et al.*, 1989). Following 24 h of cocaine self-administration, ICSS thresholds were elevated for several hours (Koob, 1989), indicating that cocaine withdrawal induced a state of anhedonia. Acute administration of the DA receptor agonist bromocriptine restored ICSS thresholds to normal (Markou and Koob, 1989). Only one conventional antidepressant has been tested in this procedure: repeated administration of the TCA DMI was reported to shorten the duration of post-cocaine anhedonia (Markou *et al.*, 1992).

It has frequently been assumed that stimulant drug treatment is a form of stress, since in many respects stimulant treatment and stress appear to be interchangeable (Antelman *et al.*, 1980; Post, 1975). Indeed, withdrawal from chronic cocaine treatment in rats is associated with intense anxiety-related behaviour and extrahypothalamic-limbic corticotropin-releasing hormone (CRH) hypersecretion (Sarnyai *et al.*, 1995). The similarities between stress and stimulant withdrawal are thought to arise from the fact that, like stimulant drugs, stressors activate the mesolimbic and mesocortical DA projections (Blanc *et al.*, 1980). Independent of a relationship to stress, there is an obvious parallel between the effects of stimulant withdrawal and the depressions that frequently follow the cessation of chronic stimulant use (Watson *et al.*, 1972), although in the animal model, the time course is rather more compressed.

As the stimulant withdrawal models are based exclusively upon changes in ICSS behaviour, their construct validity depends largely on the assumption that brain-stimulation reward activates natural reward pathways (Hoebel, 1976). Although early studies suggested that ICSS had unusual properties compared to natural rewards (for example, rapid extinction), it was later recognized that these properties derive from differences in the experimental procedures typically employed (such as the delay of reinforcement); when such extraneous factors are equated, ICSS appears very comparable to a high-incentive natural reward presented under conditions of low drive (see Gibson *et al.*, 1965). These parallels, together with the observation that responding for ICSS performance is influenced by many of the factors that control responding for natural rewards, have justified the assumption that the ICSS electrode stimulates directly the neural substrates that are activated indirectly by natural rewards (Hoebel, 1976). A degree of caution is required, since although people implanted with ICSS electrodes report a variety of pleasurable sensations, they also report other reasons for stimulating, such as curiosity (Atrens, 1984; Valenstein, 1973). Nevertheless, the commonality of anatomical substrate between ICSS and other types of reward supports the use of this procedure as an animal model of hedonic behaviour (see also Koob, 1989; Wise, 1989). However, the relationship of drug-induced depressions to major depressive disorder is uncertain, and the validity of this model is therefore questionable.

Social Dominance Models

Parallels have often been noted between depressive and submissive behaviours (Gardner, 1982; Price, 1972), and a number of laboratories have attempted to model depression by using animals of low social rank. Subordinates, and those who have lost status as a result of defeat in social conflict situations, are at greater risk for psychopathology. Biological similarities between defeated animals and human depression have frequently been noted (see Gilbert and Allan, 1998; Henry, 1982; Toates, 1995), and there is considerable evidence that depressed individuals see themselves as inferior and behave submissively (reviewed by Gilbert and Allan, 1998). Indeed, social skills training, of which assertiveness training represents a major component, is used clinically as a psychotherapy for depression, which has been found to be as effective as TCA treatment (e.g. Hersen *et al.*, 1984). Like the chronic mild stress procedure, animal models based on social dominance employ realistic inducing conditions, which are of particular ecological relevance.

Social Defeat

Social defeat is a potent stressor, and repeated defeat is a form of chronic stress, which is associated with a decrease in aggressive behaviour (Albonetti and Farabollini, 1994). A single social defeat has been reported to produce a gradual, but long-lasting, increase in immobility in the forced swim test, which was prevented by chronic treatment with clomipramine (Koolhaas *et al.*, 1990). A similar model has been developed in submissive C57BL/6J mice: a single defeat by a dominant male mouse of the same strain was reported to cause a gradual increase over weeks in passive behaviour in response to a mild stressor, which could be antagonized by clomipramine; defeated animals also had higher immobility times in the forced swim test (Korte *et al.*, 1991). In a chronic version of a similar procedure, mice were housed in social contact, but were physically separated except for one daily 3-minute encounter. Again, increased immobility in the forced swim test was observed in repeatedly defeated animals, and this effect was prevented by chronic treatment with imipramine (Kudryatseva *et al.*, 1991), though in this study, imipramine did not normalize social behaviour in the defeated animals (Kudryatseva *et al.*, 1991).

In a modified rat model, defeat of dominant pair-housed rats by rats of a different, more aggressive strain resulted in the loss of dominant status relative to their previously submissive partners, which was restored by chronic imipramine treatment (Willner *et al.*, 1995). This loss of status was accompanied by the abolition of morphine-induce place conditioning, most likely reflecting a decrease in hedonic tone (Coventry *et al.*, 1997). This suggests that it may be appropriate to view submission models, potentially, as models of melancholia. However, more evidence is needed for any firm conclusion.

In a modified murine social defeat paradigm, subordinate mice subjected to repeated social defeat showed reduced growth compared to dominant subjects, together with citalopram-sensitive anxiogenic-like behaviour (Keeney and Hogg, 1999; Keeney *et al.*, 2001). The defeated mice exhibited a maintained increase in both core temperature and circulating corticosterone levels indicative of chronic stress, although social defeat had no effect on either ethanol consumption or immobility time measured in the forced swim test. Similarly, a single social defeat of Lewis rats resulted in hypophagia and weight loss, together with increased measures of anxiety,

and these effects were reduced following acute fluoxetine treatment (Berton *et al.*, 1999).

Overall, these various studies suggest that social defeat may in principle provide a valid and ecologically sound model of depression. However, a variety of procedures have been used, and at present, the data are not entirely consistent.

Social Hierarchy

Rats housed in closed groups develop a social hierarchy and the relative social position of each group member may be identified by assessing each individual's level of success during agonistic encounters with other group members. If rats are housed in triads then a social hierarchy consisting of a dominant, subdominant and subordinate develops. Chronic administration (2-week) of either clomipramine or mianserin to the subdominant animal results in an increase in that subject's rank position at the expense of the level of dominance enjoyed by the dominant group member (Mitchell and Redfern, 1992a). The increase in the social position of the antidepressant-treated subdominant rat is likely to be related to increased assertiveness expressed during social encounters. An attractive feature of this model is that daily assessment of social structure allows the time course of antidepressant-induced elevation of social position to be determined.

However, not all features of subordinate animals are necessarily of relevance to depression, and the relationship between social dominance and social competition is potentially problematic. In group-housed rats competing for limited access to a high-incentive reward, the performance of subordinate animals was improved by acute or chronic anxiolytic treatment (Gentsch *et al.*, 1990; Joly and Sanger, 1991), suggesting that the social competition test is relevant to anxiety rather than depression. Consistent with this view is the observation that chronic treatment with *m*-chlorophenylpiperazine (*m*CPP), a major metabolite of the antidepressant trazodone, which also possesses antidepressant activity, failed to increase the performance subdominant rats in a social competition test (Moledina *et al.*, 2000) at a dose previously shown to increase the aggressive behaviour of resident rats in a resident–intruder test (see below) (Mitchell and Redfern, 2000). However, the benzodiazepine anxiolytic diazepam, which improves performance in a social competition test, did not increase aggressive behaviour in dyadic encounters, after either acute or chronic administration (Mitchell and Redfern, 1992b). Further studies will be necessary to confirm this ineffectiveness of anxiolytics in social dominance tests.

The Resident–Intruder Test

Antidepressant treatment has consistently been shown to have profound effects on rat, but not necessarily mouse, social and agonistic behaviour. While acute treatment with pharmacologically disparate antidepressant drugs (including TCAs, MAOIs, SSRIs, SNRIs, 5-HT_{1A} receptor agonists and partial agonists, and 5-HT_{2C} receptor agonists) commonly reduces the aggressive behaviour of resident rats when confronted with an unknown conspecific intruder, chronic antidepressant treatment (including repeated ECS) increases such aggressive behaviour (Cobain *et al.*, 1994a, b; Mitchell and Fletcher, 1993, 1994; Mitchell and Forster, 1992; Mitchell and Hogg, 2001a, b; Mitchell and Redfern, 1992b, 1997a, b, 2000; Willner *et al.*, 1981). These observations are consistent with the view that aggression is the only type of rodent social behaviour consistently increased following chronic treatment with antidepressants (File and Tucker, 1986).

The fact that chronic antidepressant treatment increases aggressive behaviour appears at first sight to be incompatible with the use of SSRIs in the clinical treatment of impulsive aggression (Coccaro and Kavoussi, 1997; Evenden, 1999a; Fava and Rosenbaum, 1993). However, this is a clinical, rather than an experimental, paradox, since clinically, antidepressants both increase aggression in submissive depressed individuals (manifest as a reversal of intropunitive aggression and/or impaired sociability: see Dixon *et al.*, 1989; Kaplan *et al.*, 1961; Priest *et al.*, 1980) and decrease pathological aggression (e.g. Hollander, 1999; Vartiainen *et al.*, 1995). A resolution of this paradox may be that antidepressant treatment increases assertiveness, since this would increase low levels of social dominance while at the same time decreasing high levels of physical aggression. Hence, the ability of chronic antidepressant treatment to increase aggression in rats may reflect the increased assertiveness and associated externalization of emotions expressed during recovery from depressive illness. Such increased assertive/aggressive behaviour is consistent with the effects of such treatment in the social hierarchy model (see above). However, face validity of the resident–intruder model is reduced by the fact that the test involves 'normal' unmanipulated animals; non-depressed people do not respond to antidepressant treatment. Interestingly, and in contrast to the rat studies, the aggressive behaviour of male mice in resident–intruder studies is particularly sensitive to anxiolytic, rather than antidepressant, drug activity (e.g. Lumley *et al.*, 2000).

By programming daily dyadic encounters, the resident–intruder paradigm can be used to compare the rate of onset of antidepressant-induced elevation in aggression between antidepressant treatments and to assess the utility of potential adjuvant treatment to accelerate antidepressant-related changes in rodent behaviour. Indeed, the first published studies to demonstrate the ability of a selective 5-HT_{1A} receptor antagonist (WAY-100635) to accelerate time-dependent antidepressant-induced behavioural changes used the resident–intruder test (Mitchell and Redfern, 1997b).

Social Separation

The presumed aetiological role in depression of loss events, and particularly loss of a loved one, has led to the development of a number of animal models of depression based on separation phenomena.

Although the evolutionary proximity of primates has led some authors to consider primate separation models to be of particular importance (e.g. Everitt and Keverne, 1979), they have produced remarkably little of value. Precisely because of their evolutionary proximity, the use of primates sacrifices many of the advantages of animal models, such as the easing of ethical constraints, and the possibility of testing adequately sized groups of subjects.

Neonatal Isolation

The most familiar of these models involve non-human primates, either infants isolated from their parents, or juveniles isolated from their peer group. The separation response consists of an initial stage of 'protest', characterized by agitation, sleeplessness and distress calls, followed by 'despair', characterized by a decrease in activity, appetite, play and social interaction, and the assumption of a hunched posture and 'sad' facial expression (see Henn and McKinney, 1987; Suomi, 1976). These symptoms are strikingly similar to those of 'anaclitic depression' in institutionalized children (Robertson and Bowlby, 1952). However, while parental loss in childhood, and loss events such as bereavement in adults, are implicated in the aetiology of depression, they also increase the risk of a variety of other psychiatric and non-psychiatric disorders (Brown *et al.*, 1973; Schmale, 1973). The nature of the separation response is sensitive to the environment in which the experiments are carried out (e.g. Reite *et al.*, 1981; Suomi, 1976), and the incidence of 'depressive' behaviours may in some experiments be as low as 15% (Lewis *et al.*, 1976).

The few pharmacological studies using these models have not been impressive. Very few published studies have attempted to use antidepressant treatments to modify primate separation behaviour, and because of the expense of using primates, the size of experimental groups in most studies has usually been too small to provide reliable data. Chronic treatment with DMI (Hrdina *et al.*, 1979), imipramine (Suomi *et al.*, 1978), oxaprotiline (McKinney and Kraemer, 1989) and ECS (Lewis and McKinney, 1976) have been reported to reverse some, but not all, of the effects of separation in monkeys. Trifluoperazine, amphetamine and diazepam did not affect responses to social isolation in chimpanzees (Menzel *et al.*, 1963, Turner *et al.*, 1969), but some therapeutic effects of chlorpromazine were seen in rhesus monkeys (McKinney *et al.*, 1973). 'Depressive' — like behaviours in singly-housed rhesus monkeys are associated with decreased concentrations of NA in the cerebrospinal fluid, with relatively little effect on DA or 5HT (Kraemer *et al.*, 1989). Following a return to social housing, NA levels normalize, but the animals remain hypersensitive to pharmacological challenges of the NA system (Kraemer *et al.*, 1984a, b).

In fact, separation phenomena of 'protest' followed by 'despair' are present to some extent in many other species, including cats, dogs, rodents and precocial birds (Katz, 1981; McKinney and Bunney, 1969), and several of these phenomena have also been used as the basis for the development of animal models of depression. One of these, the reactivation of distress calling in one-week-old chicks, appears to perform relatively well as an antidepressant screening test (Lehr, 1989). The vocalizations of guinea-pig pups separated from their mothers also respond to antidepressants, and this test was used successfully to detect antidepressant-like activity of the Substance P neurokinin 1 (NK1) receptor antagonists (Rupniak *et al.*, 2000).

Adult Isolation

Chronic (4–6 weeks) isolation of adult rats has been found to cause a disruption of cooperative social behaviour (Berger and Schuster, 1982) reminiscent of the poor social performance of depressed people (Lewinsohn, 1974). In a single study of the effects of antidepressant treatment, the impairment of social cooperation in isolated rats was reversed by chronic treatment with either imipramine or fluoxetine, and the effect of imipramine in this model was abolished by the 5-HT antagonist metergoline (Willner *et al.*, 1989).

MODELS OF PREDISPOSITION TO DEPRESSION (i.e. DIATHESIS)

Depressive diatheses have been modelled genetically, genomically, developmentally and by brain lesioning. These areas are reviewed in turn.

Genetic Models

Selective Breeding for Muscarinic Hypersensitivity (FSL)

The Flinders Sensitive Line (FSL) rat is the result of selective breeding for sensitivity to the hypothermic effect of cholinergic agonists and is based on the hypothesis that central cholinergic systems are important in depression since increased cholinergic sensitivity has been reported in depressed patients. Although bred for cholinergic hypersensitivity, FSL animals also show a number of other pharmacological abnormalities, including serotonergic hypersensitivity and dopaminergic hyposensitivity (Overstreet and Janowsky, 1991). Relative to the control Flinders Resistant Line, FSL animals have a number of characteristics reminiscent of depression, including cholinergic supersensitivity, increased REM sleep and reduced locomotor activity, which is further pronounced following electric footshock (Overstreet, 1993). FSL animals also show greater immobility in the forced swim test (Overstreet and Janowsky, 1991). This behaviour was normalized by chronic treatment with a high dose of imipramine, and partly reversed by DMI or sertraline, but was not restored by chronic amphetamine or scopolamine (Overstreet, 1991; Overstreet *et al.*, 1995; Schiller *et al.*, 1992). FSL rats also exhibit a greater vulnerability to the suppressive effect of chronic mild stress on responsiveness to sweet reward (Pucilowski *et al.*, 1991, 1993) but behave normally in the elevated plus-maze, a putative animal model of anxiety (Overstreet, 1991).

While these observations are consistent with a depressive diathesis and greater behavioural responsiveness to stress, other data are not. FSL rats have markedly elevated levels of 5-HT, NA and DA in specific brain areas, which are normalized during chronic treatment with DMI (Zangen *et al.*, 1997, 1999), and hypothalamic levels of CRH and circulating levels of adrenocorticotrophic hormone (ACTH) are lower in FSL rats (Owens *et al.*, 1991), indicating reduced HPA axis activity, in contrast to the increased HPA axis activity observed in depression. Similarly, acute and chronic treatments with nicotine, a non-antidepressant, both exhibit an antidepressant-like effect on the behaviour of FSL rats in the forced swim test (Tizabi *et al.*, 1999), which was blocked by prior treatment with the nicotinic receptor antagonist mecamylamine (Tizabi *et al.*, 2000). It should be noted that the major pharmacological evidence supporting the FSL strain as a model of depression derives from studies using the forced swim test, which is itself of questionable validity.

The Roman Low-Avoidance (RLA) Strain

The Roman low-avoidance (RLA) rat strain has been selectively bred for poor avoidance in the shuttle box paradigm, while the Roman high-avoidance (RHA) counterparts have been selectively bred for their good performance in that task. Compared to RHA rats, the behaviour of RLA rats expressed in the open-field, elevated plus-maze and hole-board tests are consistent with high levels of emotionality (Escorihuela *et al.*, 1999) and reflect differences in response to or the ability to cope with stress. In contrast, RLA and RHA rats react similarly to social defeat (Meerlo *et al.*, 1997). This latter finding suggests that the RLA strain is likely to prove of limited relevance to depression.

The Fawn-Hooded Strain

The Fawn-Hooded (FH) rat strain exhibits hypercortisolaemia and blunted response to dexamethasone-induced suppression of cortisol secretion (Owens and Nemeroff, 1991) consistent with the hyperactivity of the HPA axis which is observed in depressive illness. The elevated levels of plasma cortisol are reduced by chronic treatment with the TCAs imipramine and clomipramine, and with the MAO-A inhibitor clorgyline (Aulakh *et al.*, 1993). FH rats also exhibit reduced growth rate consistent with an abnormality of the hypothalamic-somatotrophic (HSM) axis (Gomez *et al.*, 1999). FH rats exhibit increased immobility in the forced swim test in some studies (but not all; see Hall *et al.*, 1998a; Lahmame *et al.*, 1996) and increased ethanol intake and preference (Overstreet *et al.*, 1992). Furthermore, ethanol consumption in FH rats is increased by social isolation (Hall *et al.*, 1998b). Recent studies also indicate higher levels of anxiety in FH rats (Hall *et al.*, 2000; Kantor *et al.*, 2001) together with impaired social behaviour (Kantor *et al.*, 2000). These observations indicate that the FH rat may be a suitable model

of comorbid depressive illness, alcoholism and anxiety, but clearly, much more work would be needed to substantiate this conclusion.

Like FSL rats, FH rats exhibit cholinergic supersensitivity (Overstreet *et al.*, 1992). In contrast, FH rats exhibit functional hyposensitivity to a variety of 5-HT and NA receptor agonists (Aulakh *et al.*, 1994, 1996) reflecting altered serotonergic and noradrenergic function in the FH strain.

Genomic Models

HPA Transgenic

In depressive illness, the secretion of CRH from the paraventricular nucleus of the hypothalamus is under the control of a glucocorticoid-receptor-mediated inhibitory feedback system. Conditions of extreme, prolonged stress cause reduced sensitivity of the glucocorticoid receptor, thereby reducing the effectiveness of the feedback loop, resulting in hypercortisolaemia. A transgenic mouse strain expressing glucocorticoid-receptor antisense has been described which exhibits dysfunction of the HPA axis in a manner similar to that seen in depressive illness (Montkowski *et al.*, 1995; Pepin *et al.*, 1992a). Chronic treatment with antidepressants reduces plasma cortisol and ACTH levels (Montkowski *et al.*, 1995; Pepin *et al.*, 1992b), by increasing glucocorticoid-receptor mRNA expression (Barden, 1996), with a subsequent elevation of glucocorticoid-receptor activity resulting in increased sensitivity of the HPA axis to glucocorticoid negative feedback (Barden, 1999). However, while behavioural and neuroendocrine responses to stress are modified in transgenic mice (Farisse *et al.*, 1999; Linthorst *et al.*, 2000), the direction of these changes is inconsistent with a model of depressive illness: relative to wild-type controls, transgenic mice exhibit reduced immobility in the forced swim test and less anxiety-related behaviour on the elevated plus-maze but increased aggressive behaviour (Beaulieu *et al.*, 1993; Montkowski *et al.*, 1995). All of these changes are in the opposite direction to those expected.

5-HT Transporter Knockout

The serotonin reuptake transporter (5-HTT) is pivotal in the inactivation and control of serotonergic neurotransmission, and blockade of the serotonin transporter is the principal target of the TCA, SSRI and SNRI drugs. Investigations of subjects which lack the serotonergic transporter, while not a model of depression, may provide an insight into the adaptive mechanisms associated with a permanent lack of serotonin reuptake. Recent studies suggest that 5-HTT knockout mice show regional differences from wild-type mice in terms of 5-HT_{1A} receptor protein and mRNA expression (reduced in dorsal raphe, hypothalamus, amygdala and septum, but increased in hippocampus: Fabre *et al.*, 2000; Lanfumey *et al.*, 2000; Li *et al.*, 2000), which are associated with reduced functional sensitivity to the hypothermic effects of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) (Li *et al.*, 1999). Similarly, 5-HT_{2A} receptor density is also reduced in 5-HTT knockout mice (Rioux *et al.*, 1999). Interestingly, the desensitization of 5-HT_{1A} autoreceptors in 5-HTT knockout mice is further enhanced by exposure to stressful conditions (Lanfumey *et al.*, 2000).

CRH Receptor Subtype Knockouts

CRH is a critical coordinator of the HPA axis and subtypes of CRH receptors, CRH-R1 and CRH-R2, are found throughout the central nervous system and peripheral tissue. CRH has higher affinity for the CRH-R1 receptor, while urocortin (a CRH-related peptide) exhibits 40-fold selectivity for the CRH-R2 receptor. CRH-R1 knockout mice exhibit anxiolytic-like behaviour, which may be due to impaired spatial recognition memory (Contarino *et al.*, 1999), together with characteristic responses to stress (Timpl *et al.*, 1998) indicative of disrupted HPA axis (Smith *et al.*, 1998). In contrast, CRH-R2 knockout mice exhibit increased anxiety-like behaviour and are hypersensitive to stress (Bale *et al.*, 2000).

Tachykinin Receptor Knockout

The NK1 receptor is expressed in brain areas associated with the control and management of depressive illness, anxiety, stress and sensitivity to the rewarding properties of food and drugs of abuse. Indeed, antagonists at the NK1 receptor have been suggested as potential antidepressant drugs with a novel mode of action (Froger *et al.*, 2001; Rupniak *et al.*, 2000, 2001). The NK1 receptor knockout mouse is not an animal model of depression. Rather, these mice show behavioural changes similar to those elicited by antidepressants in normal mice, including a decrease in neonatal vocalization following maternal separation, decreased aggressive behaviour in the resident–intruder test, and decreased immobility in the forced swim and tail-suspension tests (Rupniak *et al.*, 2001). NK1 receptor knockout mice also exhibit a loss of the rewarding properties of morphine, together with reduced physical response to opiate withdrawal, but their response to cocaine is unchanged (Murtra *et al.*, 2000), suggesting that this may reflect a specific interaction with opioid systems, rather than a general effect on brain reward mechanisms.

Developmental Models

Neonatal Antidepressant Treatment

Neonatal treatment of rat pups with the TCA clomipramine has been reported to cause a spectrum of symptoms reminiscent of depression, including decreases in sexual and aggressive behaviour, a shortening of REM sleep latency, and subsensitivity to ICSS. Treated animals were also hyperactive in some tests (Vogel *et al.*, 1990, 1996). Animals were typically tested when mature, at ages that varied between tests. Most of the behavioural and sleep abnormalities were present on first testing at approximately 3 months; however, the ICSS abnormalities were absent at 4–5 months, but present at 6–8 months. ICSS was not tested in older animals, but some other abnormalities appeared to normalize at around 11 months (Vogel *et al.*, 1990). These effects are probably not specific to clomipramine, since neonatal treatment with DMI, zimeldine or the SSRI Lu 10-134-C has also been reported to increase immobility in the forced swim test in adult rats (Hansen *et al.*, 1997; Hilakivi and Hilakivi, 1987; Velazquez-Moctezuma and Diaz Ruiz, 1992), while neonatal treatment with the SSRI citalopram similarly reduces adult aggressive behaviour (Manhaes de Castro *et al.*, 2001). Zimeldine, like clomipramine, but not DMI, also disrupts adult rat sleep patterns by shortening the duration of REM sleep bouts (Frank and Heller, 1997). In contrast, neonatal treatment with scopolamine, a cholinergic receptor antagonist, suppressed REM sleep, as observed with neonatal clomipramine treatment, but unlike clomipramine, facilitated adult male sexual behaviour. These results suggest that the neonatal clomipramine-induced reduction in male sexual behaviour is not due to early REM sleep deprivation (Velazquez-Moctezuma *et al.*, 1993).

As yet, information concerning the pharmacological responsiveness of this model is minimal: some effects of imipramine and REM sleep deprivation have been reported, but the numbers of subjects tested were too low to allow reliable conclusions to be drawn (Vogel *et al.*, 1990). While this model appears to have good face validity, in terms of the range of symptoms displayed and the insidious onset of anhedonia, the mechanisms by which neonatal antidepressant treatment has adverse effects in mature animals are unknown. In particular, it is unclear what, if any, is the relationship between

neonatal antidepressant treatment and the aetiology of depression in humans. The extent to which the symptoms can be reversed by antidepressants is uncertain. Nevertheless, these studies raise the disconcerting possibility that a breast-fed infant could develop a susceptibility to melancholia by ingesting TCAs prescribed to the nursing mother for the relief of post-partum depression.

Prenatal/Neonatal Stress

Prenatally stressed (PS) rats (where the dam is subjected to repeated footshock during the early stages of pregnancy) exhibit elevated activity of the HPA axis and defensive behaviour before weaning, and the heightened defensive behaviour, as well as exaggerated behavioural, physiological and neuroendocrine responses to stressful stimuli, persists into adulthood (Takahashi *et al.*, 1992; Ward *et al.*, 2000; White and Birkle, 2001). Current behavioural data suggest similarities between the behavioural profiles of PS rats and the anxiogenic changes in behaviour induced by yohimbine and idazoxan (White and Birkle, 2001); these data indicate high levels of anxiety in PS rats. However, female, but not male, PS mice exhibit an antidepressant-reversible increase in immobility time in the forced swim test, indicating that PS might induce a gender-dependent increase in the risk of depression-like behaviour (Alonso *et al.*, 1999; 2000).

Neonatal stress in non-human primates has been shown to induce hypersecretion of CRH (Coplan *et al.*, 1996) and abnormal social behaviour in adulthood, indicative of enhanced response to stressors (Clarke and Schneider, 1993). Similarly, neonatal stress in rat pups, achieved by maternal separation, also increases CRH levels in adulthood (Ladd *et al.*, 1996).

Waiting Behaviour

Two procedures have been described in which antidepressants increase the ability of rats to withhold responses. They are not based on developmental — or indeed, any other — manipulations, but they do involve constitutional predispositions, and are reported at this point for convenience.

In one paradigm (differential reinforcement of low rate-72 s; DRL-72) premature responding delays the delivery of reward, and the ability of drugs to improve performance in this model has been claimed to be an efficient antidepressant screening test (Seiden *et al.*, 1985), although this has been disputed (Pollard and Howard, 1986). In the second paradigm, a larger reward may be earned by waiting; again, the improvement of performance in this model appears to be a specific property of a wide range of antidepressants (Bizot *et al.*, 1988a). Drugs which possess 5-HT_{1A} receptor agonist activity are antidepressant-like in both of these waiting paradigms (Archer, 1991; Bizot *et al.*, 1988b; Kostowski *et al.*, 1992; Richards *et al.*, 1994), and in the DRL-72 test, antidepressant effects were blocked by a 5-HT receptor antagonist (Marek *et al.*, 1989). The relevance of these behaviours to depression is unclear. In a different schedule designed to examine accurate estimation of time intervals, acute treatment with antidepressant drugs (imipramine, clomipramine and zimeldine) failed to modify timing accuracy (Bayley *et al.*, 1998) suggesting that improvement in performance on DRL schedules, including SSRIs (Sokolowski and Seiden, 1999), is most likely due to reduced rate of lever-pressing rather than improved timing.

Clinical evidence strongly suggests that similar disorders of 5-HT function cut across diagnostic boundaries, and are expressed as 'pathologically impulsive behaviour' rather than as any particular disorder (Soubrie, 1986; Willner, 1989b). Indeed, mice lacking the 5-HT_{1B} receptor (5-HT_{1B} knockout mice) exhibit more impulsive aggressive behaviour, drink more ethanol and acquire cocaine self-administration faster than wild-type mice (Brunner and Hen, 1997; Scearce-Levie *et al.*, 1999), which reflects (in part at least) reduced 5-HT function (Evenden, 1999b), and may provide an animal model of addiction and motor impulsivity. In vervet monkeys (*Cercopithecus aethiops sabaeus*) there appears to be a clear inverse relationship between serotonin turnover and social impulsivity (Fairbanks *et al.*, 2001). The inability to withhold responses in the two rodent 'waiting' models may constitute instances of impulsive behaviour, and as such, the effect of antidepressants in these paradigms may not relate specifically to depression.

The constitutional predisposition referred to earlier is that antidepressant effects in the DRL vary considerably between rats of different strains, or rats of the same strain obtained from different suppliers (Balcells-Olivero *et al.*, 1998).

Lesion Model

Olfactory Bulbectomy

The other major animal model of depression is based on the destruction of the olfactory bulb in rats, which disrupts the limbic-hypothalamic axis. Bulbectomized (OB) animals display a variety of behavioural changes, including irritability, hyperactivity, and an impairment of passive avoidance learning (Cairncross *et al.*, 1979). Recent observations suggest that OB rats exhibit increased reactivity together with a reduced rate of habituation to novel stimuli (Mar *et al.*, 2000) together with changes in the immune system and NA, DA, 5-HT, GABA, cholinergic and glutaminergic neurotransmitter systems (Kelly *et al.*, 1997). They also show an elevation of circulating corticosteroid levels (as do stressed animals), which appears to be an increased corticosteroid response to stress rather than an increase in basal levels (Broekkamp *et al.*, 1986). These changes are reversed by antidepressants, including compounds with 5-HT_{1A} receptor agonist activity (Borsini *et al.*, 1997; McGrath and Norman, 1999), SNRIs (McGrath and Norman, 1998) and selective NA reuptake inhibitors (Harkin *et al.*, 1999b) in addition to the TCAs. The most specific antidepressant effect is the reversal of the passive avoidance learning deficit (Cairncross *et al.*, 1979; Van Riezen and Leonard, 1990), although attenuation of hyperactivity in the open field is nowadays the most commonly used behavioural predictor of antidepressant activity (Kelly *et al.*, 1997). However, while all TCA and atypical antidepressants appear to be effective in this test, MAOIs are not (Jesberger and Richardson, 1986). While repeated administration of TCAs is necessary to normalize behaviour in this model, a limited number of studies suggest that some SSRIs act after acute treatment (Joly and Sanger, 1986; Lloyd *et al.*, 1982). Recent studies suggest that the OB rat model of depression is insensitive to the potential rapid-onset antidepressant action induced by concomitant treatment with 5-HT_{1A} receptor antagonists (Cryan *et al.*, 1998, 1999). The implication that the model involves primarily serotonergic mechanisms is supported by the observation that the effects of subchronic treatment with imipramine and mianserin were reversed by acute administration of the 5-HT_2 receptor antagonist metergoline (Broekkamp *et al.*, 1980).

Although pharmacologically specific for antidepressants, the face validity of the olfactory bulbectomy model appears slight. Unlike the stress models, the bulbectomized rat resembles an agitated hyposerotonergic, rather than a retarded, depression (Lumia *et al.*, 1992), but beyond hyperactivity, it is difficult to discern any further points of behavioural resemblance. Indeed, increased irritability and aggression together with exaggerated reactivity to auditory and tactile stimulation may reflect additional septal damage. Nevertheless, bulbectomized rats do resemble depressed humans on a surprisingly wide range of peripheral neurochemical and immunological markers, such as abnormalities of platelet 5HT transport and neutrophil phagocytosis (Leonard, 1991).

PHARMACOLOGICAL SCREENING TESTS

Finally, we mention briefly some older tests, now little used, based on interactions between antidepressants and a range of other pharmacological agents. Generally speaking, the predictive validity of these tests is poor, and their face and construct validity as simulations of depression is minimal or zero.

Reserpine and Tetrabenazine Reversal

The rationale behind the reserpine model of depression is from clinical observations of patients treated with reserpine for hypertension who subsequently developed signs of depressive illness. Later observations indicated that the symptoms induced by reserpine in laboratory animals (including ptosis, hypothermia and sedation) were due to depletion of central monoamines. It is generally accepted that an ability to reverse the reserpine syndrome is due to reduced inactivation of central monoamines, either by inhibiting monoamine reuptake or by inhibiting the enzyme monoamine oxidase. Indeed, the classical TCAs in use today were developed on the basis of their positive effects in this model. False negatives include iprindole, mianserin, rolipram and SSRIs, while false positives include stimulants, anticholinergics and analgesics. This model possesses little or no face or construct validity to depression. Even so, it is still used in drug development although its use is largely limited to confirmation of pharmacological activity, in relation to monoamine reuptake inhibition (Rogoz *et al.*, 1999a, b; Wong *et al.*, 2000) and MAOI activity (e.g. Caille *et al.*, 1996; Iwata *et al.*, 1997; Kato *et al.*, 1998).

Tetrabenazine is chemically related to reserpine and likewise depletes central, although not peripheral, stores of NA and 5-HT. It therefore similarly induces sedation and hypothermia. Consequently the validity profile of this model is no better than that of reserpine. It is still used in drug development but, again, simply to confirm pharmacological activity (e.g. Darias *et al.*, 1999; Ferris *et al.*, 1995).

5-HTP Potentiation

5-Hydroxytryptophan (5-HTP) is the immediate precursor of 5-HT, and the consequences of 5-HTP loading in animals are behaviours associated with 5-HT excess (hypothermia, sedation, head-twitch response), which may be potentiated by drugs which attenuate 5-HT inactivation. As with other pharmacological screening tests this model has no validity as a model of depression, and is largely employed in mechanistic studies to confirm pharmacological activity (Ferris *et al.*, 1995; Porsolt, *et al.*, 2000).

Yohimbine Potentiation

Yohimbine preferentially inhibits presynaptic α_2-adrenoceptors, so increasing the release of NA and causing behavioural excitation and increased heart rate and blood pressure. A combination of increased NA release together with reduced NA inactivation following antidepressant treatment results in a lethal cocktail and it is this phenomena that is employed in this test. Almost all antidepressants potentiate the lethal effects of yohimbine, but how such effects are related to depressive illness is unclear, and the test has no validity as a model. As with other pharmacological screening models, the use of yohimbine potentiation is primarily limited to mechanistic studies (e.g. Eguchi *et al.*, 1997).

Apomorphine and Clonidine Antagonism

On acute treatment, some antidepressants, including TCAs, NA reuptake inhibitors, the SNRI venlafaxine (Redrobe *et al.*, 1998) and β-adrenoceptor agonist drugs, antagonize the hypothermia induced by a single high dose of apomorphine (Puech *et al.*, 1981). On chronic treatment, TCAs, MAOIs and some atypical drugs antagonize the hypolocomotion and/or hypothermia induced by a single dose of clonidine (e.g. Kostowski and Malatynska, 1983; Kostowski and Obersztyn, 1988; von Voigtlander *et al.*, 1978). False positives in both cases include the psychostimulants amphetamine, *p*-chloro-methyl-amphetamine (PCA) and L-dihydroxyphenylalanine (L-DOPA), while MAOIs, SSRIs and atypical drugs are generally inactive (Maj *et al.*, 1986; Pawlowski and Mazela, 1986).

Potentiation of Apomorphine- and Clonidine-Induced Aggression

Acute treatment of rats with apomorphine or of mice with clonidine increases aggressive behaviour. Prior chronic, but not acute, treatment with antidepressants usually potentiates apomorphine- or clonidine-induced aggression (Maj, 1984; Maj *et al.*, 1979, 1980, 1981, 1982). False negatives in these tests include the SSRIs (e.g. Matto *et al.*, 1998a), while false positives include examples of 5-HT antagonists and neuroleptics (Maj, 1984; Maj *et al.*, 1982). The aggressive behaviour induced by daily administration of apomorphine was attenuated by acute treatment with trazodone at non-sedative doses, which had little or no effects on forced swim behaviour or on levels of anxiety, while chronic treatment with trazodone slowed the development of apomorphine-induced aggression (Rudissaar *et al.*, 2001). Other studies (e.g. Matto *et al.*, 1998b) suggest that 5-HT reuptake inhibition has no major effect on apomorphine-induced aggressive behaviour. Available data for clonidine-induced aggression are rather equivocal (e.g. Rogoz *et al.*, 1999a, b) although this behaviour was potentiated by venlafaxine (Maj and Rogoz, 1999). The predictive validity of these tests is limited, and their face and construct validity, non-existent.

EVALUATION

Table XVIII-1.1 presents a summary of the models surveyed, showing our evaluation of each model against the accepted validation criteria. (The pharmacological screening tests are excluded, as they have little or no validity on any of the criteria.) The first three columns score each model on a scale of 0 to 3, using the following scoring system:

0	no positive evidence, or no excess of positive evidence over negative
+	small amount of positive evidence or small excess of positive evidence over negative
++	moderate amount of positive evidence or large excess of positive evidence over negative
+++	large amount of positive evidence, with little or no negative evidence

(A score of 3 does not, however, imply, that there is no room for improvement.) The fourth column presents an overall validity estimate arrived at by summing across columns 1–3.

It should be borne in mind that these judgements to some extent reflect the authors' subjective impressions, and they should not be taken too seriously.

With this caveat, we draw the following conclusions:

1. Easily ahead of the pack, the CMS model scores a maximum 9, against a highest score of 5 for any of the other models. We reiterate, however, that this strength is to some extent offset by the limited reliability of the procedure.

Table XVIII-1.1 Evaluation of animal models of depression

Animal model	Validity				Evaluation		Sensitivity to chronic drug treatment
	PV	FV	CV	Sum	Depression	Melancholia	
Stress models							
Acute stress models							
Learned helplessness	++	++	+	5	++	++	+
Forced swim	++	0	0	2	+	0	0
Tail suspension	++	0	0	2	+	0	0
Restraint stress	+	+	0	2	+	0	+
Chronic stress models							
Chronic severe stress	+	++	+	4	++	+	++
Chronic mild stress	+++	+++	+++	9	+++	+++	+++
Induction and maintenance	+	++	++	5	++	++	0
Psychostimulant withdrawal	+	+	+	3	+	+	+
Social dominance models							
Social defeat	+	+	+	3	+	+	+
Social hierarchy	+	++	++	5	++	0	+++
Resident–intruder	+++	+	+	5	++	0	+++
Social isolation models							
Neonatal isolation	+	++	+	4	++	0	+
Adult isolation	+	+	+	3	+	0	+
Diathesis models							
Genetic models							
Muscarinic hypersensitivity (FSL)	+	++	0	3	+	+	0
Roman Low-Avoidance	0	0	0	0	0	0	0
Fawn-Hooded	+	+	0	2	+	0	+
Genomic models							
HPA transgenic	+	0	+	2	+	0	+
5-HT transporter knockout	0	0	0	0	0	0	0
CRH receptor subtypes knockouts	0	0	0	0	0	0	0
Tachykinin receptor knockout	0	0	0	0	0	0	0
Developmental models							
Neonatal antidepressant treatment	0	++	+	3	+	0	0
Prenatal/neonatal stress	0	+	0	1	0	0	0
Waiting behaviour	++	0	0	2	+	0	0
Lesion model							
Olfactory bulbectomy	++	+	+	4	++	0	++

PV, predictive validity; FV, face validity; CV, construct validity; sum = PV + FV + CV.
See text for an explanation of the scoring system.

2. High overall scores (4–5) are achieved by models from all of the groups classified as 'stress' models, but by only one of the 'diathesis' models, olfactory bulbectomy.
3. This means that the genetic, genomic and developmental models all require further development. In the case of genomic models in particular, it would not be surprising to see this achieved rapidly.
4. Good predictive validity can be achieved in the absence of face and construct validity. Thus, scores of 2/3 for predictive validity are awarded to the forced swim, tail-suspension and waiting behaviour models, which score 0 elsewhere. (Note, however, that the high score for the forced swim test refers to the modification of this procedure introduced by Lucki and colleagues [e.g. Cryan and Lucki, 2000; Detke *et al.*, 1995], which responds to SSRIs. We would only award 1/3 to the traditional version of this test.)

The next two columns present estimates of the overall validity of each model, on a scale of 0–3, in relation to major depressive disorder, and to melancholia. We must emphasize that a score of +++ means only that a model performs better than those scored ++, which in turn perform better than those scored +; there is no implication that a model receiving a score of 3 cannot be improved. We also note that in some cases (e.g. social defeat) the low score reflects a paucity of positive evidence, rather than the presence of negative evidence. In making the overall estimates, we have weighted face and construct validity a little more highly than predictive validity. In estimating validity with respect to melancholia we have weighted the available information in relation to the specific feature of melancholia. Again, the CMS model stands out as the only model receiving a +++ rating in both categories. Seven further models perform relatively well as simulations of depression (score ++): learned helplessness, chronic severe stress (note, however, that ethical considerations would usually rule this model out of use), the induction and maintenance model, social hierarchy, resident–intruder test, neonatal isolation, and olfactory bulbectomy. Two of these models, learned helplessness and induction and maintenance, also perform well as models of melancholia. It should also be noted that the simple metric used to compute the overall ratings does not take account of discrepancies between the different components of the assessment. In particular, the ++ rating for the resident–intruder model derives largely from the excellent predictive validity of this model, face and construct validity being very limited.

The final column evaluates each model against the criterion that is central to current efforts in drug development, sensitivity

to chronic drug treatment, by which we mean that the model responds to chronic, but not acute, treatment with conventional antidepressants. The importance of this feature is that only when a model displays a gradual onset of action is it possible to detect a more rapid onset. The estimates in the table are conservative, in the sense that some of the models (e.g. induction and maintenance) have the capability, potentially, to demonstrate a gradual onset of action, since they have an extended time course, but this has not yet been investigated. The three models for which the clearest evidence for gradual onset of action has been obtained are CMS, social hierarchy and the resident–intruder test.

SIMULATIONS OF DEPRESSIVE ILLNESS VERSUS DRUG SCREENING TESTS

While the focus of this chapter has been on the development of simulations of depression, to be used as research tools, many of the same behavioural models are also used for drug development purposes. The universal use of cheap, quick drug screening tests by the pharmaceutical industry since the beginning of the 1970s resulted in a large array of antidepressant drugs being discovered, all of which have pharmacological and therapeutic profiles qualitatively similar to that of the archetypal TCA, imipramine, or MAOI, isoniazid. Unfortunately, the success of such acute treatment screening tests (e.g. the pharmacological procedures reviewed, or the forced swim test) not only restricted the development of animal models with improved face and construct validity, but has also largely been responsible for delaying the development of animal models in which antidepressants are active only following their chronic administration. The majority of models used routinely as antidepressant screening tests, and which respond acutely to drug treatment, are of no value in the drive to identify rapid-onset antidepressant drugs and are of limited or minimal validity as simulations of depression (cf. Willner, 1984). Generally, the use of drug screening models has simply resulted in the identification of further 'me-too' compounds (compounds of novel chemical structure whose acute pharmacological profile is similar to that of compounds already available to the clinician), provides no information on speed of onset of desirable drug activity, has been of very limited use in furthering our knowledge of the mechanisms associated with the psychopathology of depressive illness or adaptive changes associated with the recovery process from depression, and has largely failed to identify novel mechanisms and targets for future drug discovery.

In order to ascertain whether views within the pharmaceutical industry are changing, one of us (P.W.) conducted a small survey, which was carried out in the summer of 1999. A total of 28 questionnaires were sent out to companies with a known interest in CNS pharmacology, asking about the use of 12 named behavioural tests in antidepressant development, and the respondent's level of confidence in the outcome of each test. Assurances were given about anonymity, confidentiality and the absence of any commercial interest on the part of the investigator. Fourteen replies (50%) were received, of which twelve provided usable data. Six tests were reported to be in routine use by six or more (i.e. >50%) of the respondents: forced swim test in rats and mice, tail-suspension test, CMS; DRL-72s, and social separation. The first five of these tests are evaluated here (Table XVIII-1.1) as providing reasonably good predictive information, while varying considerably in their validity as simulations of depression. (The sixth test, social separation, performs less well, but it was realized subsequently that different companies were using very different tests under this general heading, none of which alone met the 50% criterion.)

Confidence in a positive outcome that a novel agent detected by the model would be an effective antidepressant in clinical trials was rated on a scale of 0 (not at all confident) to 6 (totally confident). All of the models achieved a mean rating of 3, with the exception of CMS, which was rated 5. It would appear that drug industry scientists are well aware of the literature summarized in Table XVIII-1.1.

It has traditionally been a major consideration in the design of antidepressant screening tests that they respond to acute or subacute drug administration. As a direct consequence, they are incapable, by virtue of their design, of responding to the major current challenge of discovering new antidepressants that have a shorter onset of action. By contrast, it is implicit in their protracted time course that chronic realistic animal models do have the capacity to detect a rapidly acting novel antidepressant. In fact, most pharmaceutical companies have now abandoned the high-volume, random screening approach in favour of the development of a small number of compounds specifically designed to meet predetermined pharmacological criteria. In such a programme, the place of behavioural screening methods shifts from the discovery phase to the development phase, and the logistical disadvantages of using complex, chronic models are small, relative to the costs of testing an ineffective drug in the clinic.

WHERE NEXT?

We conclude with some comments on likely future developments in this area. The greatest immediate contribution of animal models of depression is likely to be in relation to the elucidation of the mechanisms of action of antidepressant drugs. Thirty years of clinical experience has given rise to two widely accepted axioms: that only rarely do antidepressants cause discernible clinical improvement within the first two weeks of treatment, and that they are devoid of mood-elevating effects in normal human subjects. The clinical requirement for chronic treatment regimes has led to a considerable literature describing the effects of chronic antidepressant administration in normal animals, and numerous changes in pre- and/or postsynaptic receptor function have been reported, in a variety of systems. These descriptive studies are an essential first step towards establishing mechanisms of antidepressant action. However, the inability to determine which of the many effects of antidepressants are responsible for their therapeutic actions constitutes a fundamental limitation of this approach, which has not been widely recognized. The development of chronic animal models, in which an abnormal state is induced and maintained for a prolonged period during which 'therapy' can be administered, provides a powerful methodology for investigating these problems.

Although they have as yet made little impact, it is to be expected that genomic models will play a significant role in these developments. However, it will be important not to lose sight of the fact that genetic and genomic models are mostly of relevance to depressive diatheses. Studies in which valid and realistic models of depressive diatheses are combined with valid and realistic stress models are awaited with interest.

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XVIII-2

Monoaminergic Transmitter Systems

Alexander Neumeister and Dennis S. Charney

INTRODUCTION

There is considerable evidence available in the literature supporting the idea that brain monoamine systems play a key role in the pathogenesis of affective disorders, in particular depression. These hypotheses have primarily taken the form of proposing abnormal regulation in serotonin (5-HT) (Coppen, 1967) and the catecholamines norepinephrine (NE) (Bunney and Davis, 1965; Schatzberg and Schildkraut, 1995) and dopamine (DA) (Kapur and Mann, 1992)in depression. Early studies have focused largely on levels of monoamines and their receptors and have stimulated several theories about the pathophysiology of depression, including the monoamine deficiency and receptor sensitivity hypotheses. However, we have to acknowledge today that these hypotheses have not provided us with an ultimate explanation about the role of monoamines in depression. Nor can the pathophysiology of depression be explained simply by dysregulation of 5-HT and/or NE or DA transmission. Recent advances in molecular and cellular neurobiology have offered new insights into mechanisms possibly involved in the pathophysiology of depression, and also into the mechanisms of action of antidepressant treatment modalities (for reviews see Duman *et al.*, 1997; Manji *et al.*, 2001; Sulser, 1989). These studies have shown that chronic antidepressant treatment regulates intracellular signal transduction pathways and the expression of specific target genes.

The purpose of this chapter is to provide a concise review of clinical studies on the role of 5-HT and NE transmission in depression. However, several caveats need to be considered. First, depression is not a homogeneous disorder, and classifying a given patient with depression is a clinical decision and remains a subjective interpretation of a syndrome, even though the decision should be based on established diagnostic criteria such as DSM-IV criteria (American Psychiatric Association, 1994). However, identifying homogeneous groups of patients with depression has proven to be a virtually impossible task. This might explain some of the variability of biological findings in depression and the lack of consistent replication of many intriguing findings. Second, almost all patients being studied in clinical studies have been exposed to different pharmacological and non-pharmacological treatments before entering studies. This may confound the results of clinical and preclinical studies. Finally, there is no methodological homogeneity in processing experimental samples and assays. It is remarkable that despite these methodological problems a number of neurochemical findings have been replicated in the past in patients with depression and have provided researchers with insight into the underlying biology of this devastating illness.

SEROTONIN

Serotoninergic neurons are located in the brainstem where they can project to virtually every part of the central nervous system, often modulating neuronal responses to other neurotransmitters. As a result of this widespread projection pattern, 5-HT is known to be involved in the regulation of a wide variety of functions, including mood, anxiety, aggression, sleep, arousal, appetite and sexual function. However, it has to be acknowledged that the precise details of the mechanisms involved are not fully understood. Interest into the potential role of 5-HT in the pathophysiology of psychiatric disorders was spurred by the observation that hallucinogens such as lysergic acid diethylamide and psilocybin inhibit the peripheral actions of 5-HT. This led to the hypothesis that brain serotonergic function might be altered in patients with psychiatric disorders (Gaddum and Hameed, 1954; Wooley and Shaw, 1954). Further evidence for the importance of serotonergic mechanisms in depression was inferred by the observation that imipramine improved mood and boosted psychomotor activity (Kuhn, 1958). This initial observation of the antidepressant properties of imipramine led to more intensive testing in clinical trials of this compound and later the other tricyclic antidepressants and monoamine oxidase inhibitors for the treatment of depression. The results from these clinical trials, indicating that the action of antidepressant drugs involves enhancement of brain serotonergic activity, and further evidence for dysfunction at multiple levels in the serotonergic system of depressed patients culminated in the 'serotonin hypothesis' of depression (Maes and Meltzer, 1995). Whether this serotonergic dysfunction is the primary cause of depression or is a necessary risk factor remains unclear and is the subject of intensive research.

Seasonality of Serotonergic Function

One factor that has to be considered when evaluating the potential role of 5-HT in depression is the seasonal variation in central and peripheral 5-HT function in humans. There is considerable evidence in the literature suggesting a seasonal variation in several phenomena, such as mood, feeding behaviour and suicide, and that these phenomena may be related to changes in central and peripheral 5-HT function (Maes *et al.*, 1995). In healthy subjects and non-psychiatric patients several studies have described seasonal variations in central and peripheral 5-HT function. Studies of humans distinguish whether measures are static (e.g. biochemical levels in body fluids or blood elements) or dynamic (e.g. neuroendocrine responses to pharmacologic challenges).

Several lines of evidence based on static measures support the hypothesis of seasonal fluctuations of 5-HT function in humans: (1) hypothalamic 5-HT concentrations in human post-mortem brain specimens are decreased in winter after values peak in autumn (Carlsson *et al.*, 1980), (2) levels of plasma tryptophan, the precursor of 5-HT, show a bimodal seasonal pattern (Maes *et al.*, 1995), (3) platelet 5-HT uptake and ^{3}[H]-imipramine binding show a seasonal pattern, albeit with some differences in seasonal peaks and troughs (Arora and Meltzer, 1988; DeMet *et al.*, 1989; Tang

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and Morris, 1985; Whitaker *et al.*, 1984), (4) levels of 5-HT and its metabolites in cerebrospinal fluid show seasonal fluctuations, varying with latitude and population studied (Asberg *et al.*, 1981; Brewerton *et al.*, 1988), (5) serum melatonin concentrations demonstrate summer and winter peaks in healthy males (Arendt *et al.*, 1977), and (6) recently Neumeister *et al.* (2000) reported *in vivo* a significant reduced availability of hypothalamic 5-HT transporter sites in winter compared with summer in healthy female subjects (Figure XVIII-2.1).

There are few reports in the literature about seasonal variations in 5-HT function using dynamic measures. Joseph-Vanderpool *et al.* (1993) report a seasonal variation in behavioural responses to the administration of *meta*-chlorophenylpiperazine (mCPP) in patients with SAD, with higher 'activation/euphoria' scores in SAD patients during winter compared with summer or after successful light therapy. More recently, Cappiello *et al.* (1996) demonstrated a seasonal variation in neuroendocrine (prolactin) responses to intravenous tryptophan administration in unipolar, non-melancholic depressed patients. Interestingly, seasonality was more pronounced in female than in male patients. No such seasonal variability was found in bipolar, melancholic or psychotic patients or in healthy controls.

Altogether, substantial evidence is published in the literature arguing for a seasonal variation of central and peripheral 5-HT function in patients suffering from depression and also in healthy controls. Thus, we can hypothesize that seasonality of central and peripheral 5-HT function is physiological. It is not clear whether seasonal 5-HT fluctuations may represent a predisposing factor for depression with and without a seasonal pattern. It has to be said that the variability in the specific seasonal peaks and nadirs reported by different research groups reflects the use of different study designs, methodologies, sample sizes and measures of 5-HT function. Consequently, further studies are needed to clarify the role of seasonal variations in central and peripheral 5-HT function in the regulation of human behaviour and in the pathogenesis of mood disorders.

Tryptophan-Depletion Studies

The situation regarding how to evaluate the potential role of 5-HT in depression has been hampered by the fact that, until recently, it has not been possible to measure brain 5-HT directly, which means that researchers had to rely on indirect evidence for the involvement of this transmitter in the pathogenesis of depression and its role in antidepressant treatment modalities. Over the past few years, neurotransmitter depletion paradigms have provided another means of examining the potential role of 5-HT systems in the pathophysiology of depression and their role in pharmacological and non-pharmacological treatment modalities for depression.

The aim of tryptophan depletion is to lower brain 5-HT by depleting its precursor tryptophan. Most of the tryptophan in plasma is protein-bound, with only about 5% being left free and available to be transported into the brain across the blood–brain barrier by an active protein shuttle for which five other large amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine) also compete. Once in the brain, the initial step in the biosynthesis of 5-HT is the conversion of L-tryptophan to 5-hydroxytryptophan, a reaction catalysed by the rate-limiting enzyme tryptophan hydroxylase (Fernstrom, 1983). The Michaelis constant for tryptophan hydroxylase is higher than tryptophan concentration in the brain, suggesting that under physiological conditions the activity of this enzyme is limited by the availability of the substrate (Friedman *et al.*, 1972). Animal studies have shown that the synthesis and content of 5-HT in rat brain vary in parallel with brain tryptophan concentrations (Fernstrom and Wurtman, 1971). Moreover, it has been shown that increase in brain tryptophan concentration raises 5-HT release *in vitro* (Auerbach and Lipton, 1985; Schaechter and Wurtman, 1989) and *in vivo* (Carboni *et al.*, 1989; Sharp *et al.*, 1992), although some studies disagree with these findings (Elks *et al.*, 1979; Marsden *et al.*, 1979). In summary, the concentration of brain 5-HT depends upon the availability of its precursor tryptophan.

Preclinical data show that the acute administration of a tryptophan-free amino acid mixture of essential amino acids produces a rapid and substantial decrease in plasma tryptophan levels, associated with a decrease in brain tryptophan, brain 5-HT and 5-HIAA levels in rats (Gessa *et al.*, 1974). Studies in humans show profound decreases of plasma tryptophan levels (Bell *et al.*, 2001; Moore *et al.*, 2000; Neumeister *et al.*, 1997b) and cerebrospinal fluid levels of 5-HIAA (Carpenter *et al.*, 1998; Moreno *et al.*, 2000b; Williams *et al.*, 1999) after oral administration of an amino acid mixture without tryptophan (Table XVIII-2.1).

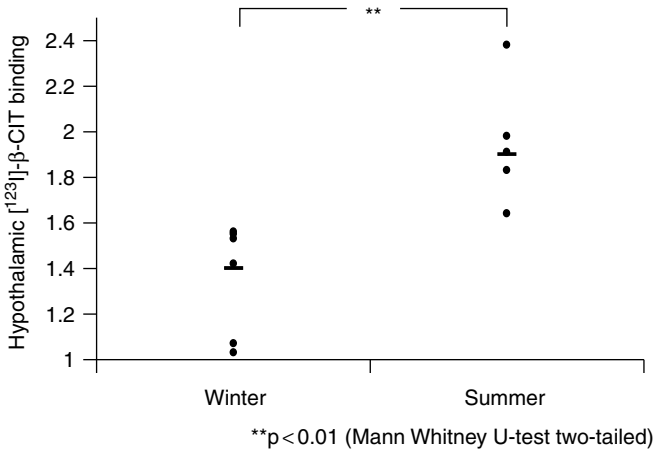

Figure XVIII-2.1 Hypothalamic serotonin transporter availability is significantly reduced in winter compared with summer in healthy female controls, as studied *in vivo* using [^{123}I]-β-CIT single photon emission computed tomography (SPECT) (Neumeister *et al.*, 2000)

Table XVIII-2.1 Amino acids used for tryptophan depletion versus sham depletion

Makes one beverage	
L-Alanine	5.5 g
Glycine	3.2 g
L-Histidine	3.2 g
L-Isoleucine	8.0 g
L-Leucine	13.5 g
L-Lysine	11.0 g
L-Phenylalanine	5.7 g
L-Proline	12.2 g
L-Serine	6.9 g
L-Threonine	6.9 g
L-Tyrosine	6.9 g
L-Valine	8.9 g
L-Methionine	12.0 g
L-Arginine	19.6 g
L-Cysteine	10.8 g
During sham depletion the beverage contains additional amino acid:	
L-Tryptophan	2.3 g

Three mechanisms appear to be responsible for the transient decrease in brain 5-HT activity during tryptophan depletion: (1) the amino acid mixture (Table XVIII-2.1) that is given to the subjects during tryptophan depletion stimulates protein synthesis in the liver, which uses up plasma tryptophan, (2) the amino acid mixture contains large amounts of the other neutral amino acids, which compete with tryptophan for the transport across the blood–brain barrier and thus restrict uptake of tryptophan into the brain and (3) a recent study showed an increase of plasma neopterin levels induced by tryptophan depletion (Stastny *et al.*, submitted). It has been shown that increased neopterin levels may facilitate catabolism of free plasma tryptophan, possibly via an interferon γ-induced activation of the tryptophan-cleaving cellular enzyme indoleamine 2,3-dioxygenase (Mellor and Munn, 1999). These mechanisms lead to the rapid and substantial, albeit transient, reduced synaptic availability of 5-HT in the brain.

The value of a depletion paradigm depends on whether the method is reliable, reversible and specific. All three issues have been addressed in several studies and have shown that the tryptophan-depletion method fulfils all three requirements for a meaningful research tool (Delgado *et al.*, 1990; Ellenbogen *et al.*, 1996; Moja *et al.*, 1989; Smith *et al.*, 1987). In particular, studies in monkeys (Young *et al.*, 1989) and humans (Moreno *et al.*, 2000b; Neumeister *et al.*, 1998c) showed that tryptophan depletion did not change the metabolism of other neurotransmitters, whereas levels of tryptophan and 5-HIAA in plasma and cerebrospinal fluid, respectively, were lowered. Thus, if the effects of tryptophan depletion can be attributed to changes in a transmitter system in the brain, it is probably 5-HT systems that are affected.

Tryptophan-Depletion Studies in Healthy Subjects

Studies of tryptophan depletion in healthy subjects have shown conflicting results. Healthy male subjects with their baseline ratings of depression in the upper normal range exhibit a transient worsening of their mood during tryptophan depletion, although never amounting to clinical depression (Smith *et al.*, 1987; Young *et al.*, 1985). In contrast, healthy male subjects who were euthymic at baseline and who were rigorously screened for any psychiatric or somatic illness remained unaffected by tryptophan depletion (Abbott *et al.*, 1992). Healthy male controls with a multigenerational family history for major affective disorders reported a greater reduction in mood induced by tryptophan depletion than healthy controls without a positive family history (Benkelfat *et al.*, 1994). Tryptophan-depletion studies in female subjects with no personal history of depression showed an increased risk to develop depressive symptoms during tryptophan depletion. In some tryptophan-depletion studies in healthy female controls family histories of affective disorders were assessed and were positive (Klaassen *et al.*, 1999), negative (Ellenbogen *et al.*, 1996; Klaassen *et al.*, 1999), or unknown (Weltzin *et al.*, 1995). These studies showed consistently an increased risk for an exacerbation of depressive symptoms during tryptophan depletion with more pronounced effects when the family history for affective disorders is positive. This is supported by a recent study in unaffected relatives of patients with bipolar affective disorders, who experience a transient lowering of their mood after tryptophan depletion (Quintin *et al.*, 2001). However, another study reported no mood-lowering effects of tryptophan depletion in healthy females with a negative family history of depression (Delgado *et al.*, 1989), and several studies report no mood-lowering effects of tryptophan depletion in healthy female subjects (Salomon *et al.*, 1997; Voderholzer *et al.*, 1998).

Other studies have focused on the memory and cognitive effects of tryptophan depletion, and have shown that tryptophan depletion impairs long-term memory formation and interferes with the process of memory consolidation (Riedel *et al.*, 1999; Schmitt *et al.*, 2000). It is noteworthy that tryptophan depletion did not affect other measures of frontal functioning. Sleep disturbances represent another key symptom of depression, so studies have been conducted to determine whether tryptophan depletion is capable of inducing sleep abnormalities, including changes in sleep continuity or architecture. Studies reported reduced REM latency after tryptophan depletion (Bhatti *et al.*, 1998; Moja *et al.*, 1979), but not unequivocally (Voderholzer *et al.*, 1998). The combination of tryptophan depletion plus administration of the 5-HT1A receptor agonist ipsapirone produced a significant suppression of REM sleep whereas tryptophan depletion alone did not significantly alter any REM sleep measure (Moore *et al.*, 2001). This differs markedly from the consistent tryptophan-depletion-induced REM-disinhibiting effect seen in medicated depressed patients. Another area of interest is whether reduced 5-HT activity during tryptophan depletion results in increased aggression since reduced brain serotonergic activity is believed to be associated with aggressive behaviour. It was shown that tryptophan depletion induces a rise in ratings of aggression in subjects with high-trait aggression but has little effect in those with low-trait aggression (Bjork *et al.*, 2000). Another study found an association between decreased serotonergic transmission and increased aggression in women who have been studied during the late luteal phase of their menstrual cycle (Bond *et al.*, 2001). Significantly, acute ethanol consumption may be associated with a decrease in tryptophan availability, and may induce aggressive behaviour in susceptible individuals (Badawy *et al.*, 1995).

Behavioural responses to tryptophan depletion in healthy subjects show a high variability. There are subgroups of subjects who appear to be at a greater risk to develop depressive symptoms during tryptophan depletion. Possible explanations include a positive family history of depression, gender and possibly high, albeit not reaching the levels of clinical depression, baseline ratings of depression. Studies that have included men and women have reported a gender difference, with a tendency for tryptophan depletion to produce more prominent mood-lowering effects in women, despite similar effects of tryptophan depletion on plasma tryptophan levels. This suggests that women may be more susceptible to the effects of tryptophan depletion than men. This is of particular interest considering the results of a positron emission tomography study of humans showing gender differences in 5-HT metabolism, with tryptophan depletion producing greater biochemical effects in women than in men (Nishizawa *et al.*, 1997). However, it has to be acknowledged that uptake of alpha-methyl L-tryptophan is not clearly established as a reliable indicator of 5-HT synthesis.

An intriguing finding is the association between the allelic distribution of the serotonin transporter gene promoter polymorphism (5-HTTLPR) and the behavioural responses to tryptophan depletion in a group of healthy women with and without family history of depression (Neumeister *et al.*, in press). The study showed subjects with the short allele of the 5-HTTLPR at increased risk to develop depressive symptoms during tryptophan depletion relative to subjects who are homozygous for the long allele. Future epidemiological studies will have to confirm the relevance of this finding, and will answer the clinically and scientifically relevant question as to whether the short allele of the 5-HTTLPR polymorphisms is associated with an increased risk for developing depression, as the authors had hypothesized. Brain imaging studies may be helpful in studying the underlying neural processes.

Tryptophan-Depletion Studies in Depression

To test the hypothesis that decreased 5-HT function is associated with depression, several studies were performed including untreated, symptomatic depressed patients prior to initiation of an antidepressant treatment (Delgado *et al.*, 1994; Neumeister *et al.*,

1997c; Price *et al.*, 1997, 1998). It was hypothesized that tryptophan depletion would lead to an exacerbation of the depressive syndrome. However, the results of these studies were somewhat unexpected. Consistently, it was shown that tryptophan depletion did not exacerbate depressive symptoms in these subjects. Remarkably, in two studies (Delgado *et al.*, 1994; Price *et al.*, 1998) some patients showed an improvement of their condition on the day after tryptophan depletion. The failure to aggravate depression by depleting brain 5-HT can be explained by the hypothesis that brain 5-HT function is already maximally dysfunctional in depressed patients and thus further lowering of 5-HT activity has no further effects on depressive symptoms. Alternatively, it can be hypothesized that disturbed 5-HT function does not explain the biological basis of depression, and that there is no direct relationship between severity of depressive symptoms and brain 5-HT function. A possible explanation for the improvement in symptoms the day after tryptophan depletion is an upregulation of postsynaptic 5-HT receptors because of the decreased release of 5-HT at the synapse during tryptophan depletion. Typically, 5-HT levels are restored the day after tryptophan depletion and the net effect is an enhancement of brain 5-HT function, resulting in an improvement of the patient's condition.

A recent study (Berman *et al.*, in press) evaluated whether combined tryptophan depletion and catecholamine depletion compared with tryptophan depletion and sham depletion would aggravate the depressive syndrome in unmedicated, symptomatic depressed patients. The authors report a progressive decrease in Hamilton depression scores in both groups with no difference between the combined monoamine-depletion group and the tryptophan-depletion group. This finding that simultaneous disruptions of 5-HT function and catecholamine function do not exacerbate depressive symptoms in untreated symptomatic depressed subjects supports the hypothesis that monoamines have an indirect role in regulating mood in actively depressed patients.

Intensive research using tryptophan depletion has been done during the past few years to study the role of 5-HT in the mechanism of action of antidepressant drugs, and non-pharmacological treatments for depression, such as light therapy and sleep deprivation. The hypothesis of these studies was that antidepressant treatments lead to an enhancement of brain 5-HT function and that tryptophan depletion will disrupt the antidepressant effects. This has now been tested in multiple studies, and researchers have found that tryptophan depletion reverses the antidepressant effects of antidepressant medications, in particular of agents with a predominantly serotonergic mode of action (Aberg-Wistedt *et al.*, 1998; Bremner *et al.*, 1997; Delgado *et al.*, 1990, 1999; Spillmann *et al.*, 2001). It should be noted, however, that tryptophan depletion causes clinically relevant symptoms only in about 50–60% of the patients, and one study reported no mood effects of tryptophan depletion at all (Moore *et al.*, 1998). However, the majority of studies clearly demonstrated that the depressive symptoms evoked by tryptophan depletion were often similar to those the patients had experienced during their depressive episode. As noted above, the behavioural responses to tryptophan depletion were substantially more prominent in subjects who had been successfully treated with selective 5-HT reuptake inhibitors (SSRIs) relative to the responses in those subjects who had responded to a treatment with noradrenergic antidepressants (Delgado *et al.*, 1999; Miller *et al.*, 1996a). This finding, and the finding that catecholamine depletion predominantly induces a depressive relapse in subjects treated with noradrenergic antidepressants, suggests that enhanced serotonergic or noradrenergic transmission is necessary to maintain the antidepressant responses to SSRIs or noradrenergic agents, respectively (Figure XVIII-2.2). Other variables that may affect the reoccurrence of depressive symptoms during tryptophan depletion are the length of the remitted state of the patient, with a greater likelihood of depressive symptom exacerbation when the duration of remission

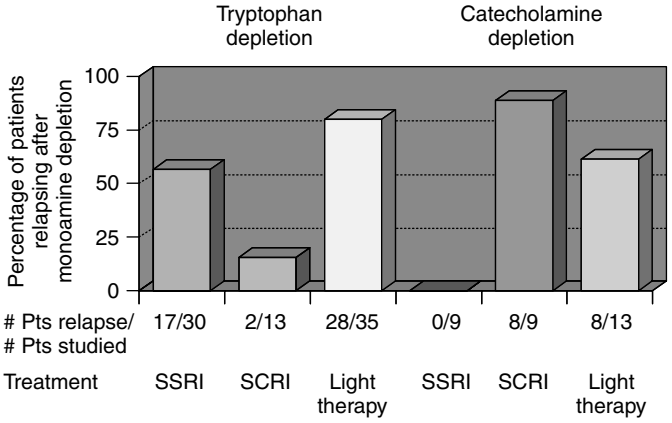

Figure XVIII-2.2 Tryptophan depletion induces a depressive relapse predominantly in patients remitted on selective serotonin reuptake inhibitors (SSRIs), but not in patients remitted on selective catecholamine reuptake inhibitors (SCRIs). In contrast, catecholamine depletion induces an exacerbation of depressive symptoms in patients remitted on SCRIs but not in patients remitted on SSRIs. Patients remitted on light therapy show a depressive relapse in both, tryptophan depletion and catecholamine depletion

is short, the number of previous depressive episodes, and the pattern of response to the antidepressant treatment. Patients who responded to treatment with placebo seem to be less vulnerable to the effects of tryptophan depletion than 'true' drug responders. This suggests that antidepressants induce biological changes in the neuron that make the subjects less vulnerable to acute changes in brain 5-HT function.

Non-pharmacological, albeit biologically based, treatments for depression include light therapy and sleep deprivation. Light therapy is the treatment of first choice for patients suffering from seasonal depressions during autumn and winter, and has been shown to be effective in non-seasonal depression when given in conjunction with other antidepressant treatment modalities (Neumeister *et al.*, 1999b). Tryptophan depletion (Neumeister *et al.*, 1997a, 1998c) and catecholamine depletion (Neumeister *et al.*, 1998c) reversed the antidepressant effects of light therapy, suggesting that both transmitter systems are involved in the mechanism of action of light therapy. Several lines of evidence suggest that sleep deprivation exerts its antidepressant effects by enhancing serotonergic transmission. To test this hypothesis, patients who responded to a single night of total sleep deprivation underwent tryptophan depletion or sham depletion (Neumeister *et al.*, 1998b). It was expected that tryptophan depletion would reverse the antidepressant effects of sleep deprivation. Unexpectedly, tryptophan depletion did not reverse the antidepressant effects of sleep deprivation, but rather prevented the otherwise naturally occurring depressive relapse after the recovery night. The authors concluded that it seems to be unlikely that serotonin alone mediates the antidepressant effects of sleep deprivation; however, serotonergic mechanisms may play an important role.

The effects of tryptophan depletion in formerly depressed, fully remitted patients, off medication is of particular interest to understand the role of 5-HT in the pathogenesis of the disorder. It might be expected that these subjects are specifically vulnerable to the depressiogenic effects of tryptophan depletion. The majority of the studies (Moreno *et al.*, 1999; Neumeister *et al.*, 1998a; Smith *et al.*, 1997) reported a depressive relapse induced by tryptophan depletion whereas subjects remained well during sham depletion. However, two studies found no exacerbation of depression during tryptophan depletion (Lam *et al.*, 2000; Leyton *et al.*, 1997). These discrepancies may be explained by the differing

length of remission among the different studies, and by differing study populations. Furthermore, a recent study in remitted depressed patients shows, similar to the effects of antidepressant treatment, that tryptophan depletion is associated with a decrease in 5-HT_2 receptor binding (Yatham *et al.*, 2001). This might have explained the failure of tryptophan depletion to exacerbate depressive symptoms in this study. The depressive relapse induced by tryptophan depletion in remitted patients off therapy suggests that at least a subgroup of patients with depression remain vulnerable to changes in 5-HT function. Interestingly, two studies showed that the majority of subjects who relapsed during tryptophan depletion experienced further depressive episodes in the near future (Moreno *et al.*, 2000a; Neumeister *et al.*, 1999a). Thus, tryptophan depletion may be capable of predicting the future course of depression.

Altogether, tryptophan-depletion studies have been shown to provide a way of examining the role of 5-HT in the pathophysiology of depression and the mechanism of action of antidepressants. The behavioural responses to tryptophan depletion provide insight into the underlying biology of depression, and suggest that serotonergic mechanisms may play a key role in the disorder. Many questions remain unanswered but tryptophan depletion may be a research tool to study gene–environment interactions in the future, and therefore may lead to further understanding of the processes involved in the pathophysiology of depression.

Serotonin 1A Receptor Function in Depression

Most 5-HT receptors belong to the family of G protein-coupled receptors (GPCRs), a large group of proteins that transduce signals through coupling to guanine nucleotide-binding regulatory proteins. Serotonin receptors are classified into seven groups according to their ligand-binding affinity profiles, molecular structures and intracellular transduction mechanisms. The human 5-HT_1 receptor subfamily includes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} receptors. Multiple lines of evidence suggest that 5-HT_{1A} receptor functions are abnormal in depression. The data supporting this hypothesis have been obtained by assessing neuroendocrine and temperature responses to 5-HT_{1A} receptor agonists in depressed subjects versus healthy controls (Lesch, 1992), measuring 5-HT_{1A} receptor density in brain tissue acquired post-mortem (Bowen *et al.*, 1989; Lopez *et al.*, 1998) and examining 5-HT_{1A} receptor functions in rats following administration of antidepressant drugs (Artigas *et al.*, 1996; Chaput *et al.*, 1991; Haddjeri *et al.*, 1998).

Human 5-HT_{1A} receptors are expressed presynaptically on 5HT cell bodies in the raphe (somatodendritic autoreceptors) and postsynaptically in other brain regions (Azmitia and Whitaker-Azmitia, 1991). In humans and monkeys, the density of 5-HT_{1A} receptors is very high in the raphe and parts of the hippocampal formation, high in hypothalamus, insula, temporal, cingulate and ventral prefrontal cortices, moderately high in occipital and parietal cortices, and very low in the cerebellum, striatum, thalamus and white matter. In the cortex, hippocampus and amygdala postsynaptic 5-HT_{1A} receptors are located on pyramidal cells and interneurons, and stimulation of these receptors generally inhibits glutamate-mediated depolarization of parent neuron (Azmitia and Whitaker-Azmitia, 1991; Sprouse and Aghajanian, 1988).

5-HT_{1A} autoreceptors constitute an important control point for serotonergic activity in the brain. Stimulation of presynaptic 5-HT_{1A} autoreceptors similarly inhibits the 5-HT neuron firing in the raphe, reducing 5HT release, and can reduce 5HT synthesis via inhibition of tryptophan hydroxylase (Briley and Moret, 1993; Chaput and de Montigny, 1988; Chaput *et al.*, 1986; Sprouse and Aghajanian, 1988). Postsynaptic 5-HT_{1A} receptors are also abundantly expressed by astrocytes and some other glia (Azmitia *et al.*, 1996). These 5-HT_{1A} receptors are expressed by the majority of astrocytes in frontal and limbic cortex, but essentially none of the astroglia in striatum, thalamus or cerebellum (Whitaker-Azmitia *et al.*, 1993). Stimulation of astrocyte-based 5-HT_{1A} sites causes astrocytes to acquire a more mature morphology and to release the trophic factor S-100B, which promotes growth and arborization of serotonergic axons (Whitaker-Azmitia and Azmitia, 1989; Whitaker-Azmitia *et al.*, 1990). S-100B is primarily released by astroglia in the developing brain, when it plays a role in the development of the serotonergic system. S-100B also plays a role in maintaining the cytoskeleton in adult animals by promoting tubulin polymerization and inhibiting PKC-mediated breakdown of microtubules (Azmitia, 1999). In addition, stimulation of neuron-based 5-HT_{1A} receptors inhibits PKA-mediated disassociation of the proteins comprising the tubulin polymers of the cytoskeleton. Administration of 5-HT_{1A} receptor antagonists, antibodies to S-100B or agents that deplete 5-HT all produce similar losses of dendrites, spines and/or synapses in adult and developing animals, effects which are blocked by administration of 5-HT_{1A} receptor agonists or SSRIs. The role of postsynaptic 5-HT_{1A} receptor function in maintenance of the cytoskeleton has led to the hypothesis that a reduction of 5-HT_{1A} receptor function may comprise a risk factor for the neuropathological abnormalities identified in limbic and paralimbic cortical areas in mood disorders (reduced cortex volume, reduced synaptic proteins, increased neuronal density, reduced glial counts) (Drevets, 2000; McEwen, 1999).

5-HT_{1A} Receptor Imaging in Depression

Positron emission tomography (PET) studies obtained *in vivo* show evidence of reduced pre- and postsynaptic 5-HT_{1A} receptor binding in depression. Drevets and colleagues (Drevets *et al.*, 1999) demonstrated that the mean 5-HT_{1A} receptor binding potential (BP) was reduced in the mesiotemporal cortex and raphe in unmedicated depressives relative to controls using PET and [*carbonyl*-^{11}C]WAY-100635. A similar reduction was evident in the parietal cortex, striate cortex and left orbital cortex/ventrolateral prefrontal cortex. These data were consistent with those of Sargent *et al.* (2000), who found decreased 5-HT_{1A} receptor BP in unmedicated depressives relative to healthy controls in the raphe, mesiotemporal cortex, insula, anterior cingulate, temporal polar cortex, ventrolateral prefrontal cortex and orbital cortex. A subgroup of the subjects were scanned both pre- and post-paroxetine treatment, and 5-HT_{1A} receptor BP did not significantly change in any area.

These data were compatible with 5-HT_{1A} receptor agonist challenge results showing that unmedicated depressed subjects have blunted hypothermic and adrenocorticotropin (ACTH) and cortisol release in response to ipsapirone or buspirone, relative to healthy controls (Lesch, 1992). The degree of blunting correlated with depression severity (Rausch *et al.*, 1990). Since 5-HT_{1A}-receptor-stimulation-induced hypothermia and ACTH/cortisol release are thought to distinguish pre- and postsynaptic 5-HT_{1A} receptor stimulation, respectively, in humans and rats, these findings were compatible with the PET data implicating both pre- and postsynaptic 5-HT_{1A} receptors in depression. Abnormally decreased 5-HT_{1A} receptor BP in depression may reflect either downregulation of receptor density or a reduction in the number of brain cells expressing 5-HT_{1A} receptors. The likelihood that abnormal 5-HT_{1A} receptor binding in primary mood disorders is accounted for by differences in nonspecific binding is reduced by the high selectivity of [*carbonyl*-^{11}C]WAY-100635 for 5-HT_{1A} receptors. Furthermore, reduced [^{11}C]WAY-100635 binding in depression is not expected to reflect a compensatory response to abnormal 5-HT release, or an effect of endogenous 5-HT concentrations on radiotracer binding. The 5-HT_{1A} receptor density and mRNA expression appear insensitive to reducing 5-HT transmission by lesioning the raphe or administering PCPA, or to pharmacologically

induced increases in 5-HT concentrations. Moreover, in baboons administration of agents which increase 5-HT release, including fenfluramine, citalopram, and amphetamine, does not alter [^{11}C]WAY-100635 binding (Parsey *et al.*, 1998). The insensitivity to endogenous 5-HT is partly related to the ~50-fold greater 5-HT$_{1A}$ receptor affinity of WAY-100635 relative to endogenous 5-HT (based upon K_D).

Altogether, reductions in 5-HT$_{1A}$ receptor binding in depression may reflect alterations in the cellular elements that express these receptors, or in the factors that downregulate genetic expression of these receptors. Preclinical and clinical data suggest that 5-HT$_{1A}$ receptors play a key role in the regulation of brain 5-HT activity and thus may be a target for novel antidepressant and antianxiety drugs with a more directed mechanism of action and possibly with a more favourable side effect profile.

NORADRENALIN

There is much data available supporting the hypothesis of a noradrenergic deficiency in depression (Delgado, 2000; Heninger *et al.*, 1996). The original catecholamine deficiency hypothesis of depression proposed that depression is a result of decreased catecholaminergic activity, whereas mania is associated with a relative excess (Schildkraut, 1965). In early reports urinary levels of MHPG were found to be significantly lower in patients with unipolar depression relative to healthy controls. However, these findings have not been consistently replicated. The original concept that depression is associated with decreased levels of catecholamines (for overview see Schatzberg and Schildkraut, 1995) as assessed with measurements of norepinephrine and its metabolites in peripheral samples has been invaluable in developing concepts of the biological mechanisms underlying depressive disorders and has served as a rich source from which researchers have generated testable experimental hypotheses. A recent study showing pronounced and sustained central hypernoradrenergic function in depression with melancholic features (Wong *et al.*, 2000) supports these early observations. New techniques such as brain imaging or depletion paradigms have provided new insight into the role of catecholaminergic mechanisms in the pathophysiology of mood disorders. Such studies have confirmed initial hypotheses that have suggested a pivotal role for noradrenergic systems in the pathophysiology of depression.

Catecholamine Depletion Studies

Of various methods employed for modifying the functions of sympathetic nervous systems, a unique and successful one has been the inhibition of catecholamine biosynthesis by α-methyl-para-tyrosine (AMPT) (Engelman *et al.*, 1968a; Sjoerdsma *et al.*, 1965). AMPT decreases norepinephrine and dopamine levels via inhibition of tyrosine hydroxylase, a rate-limiting enzyme in the synthesis of both transmitters (Widerlov and Lewander, 1978). AMPT is adequately absorbed from the gastrointestinal tract and the degree of inhibition achieved in man approximates the values, which could be predicted from the plasma levels of the drug (Engelman *et al.*, 1968b). In clinical investigations, AMPT (in dosages ranging from 1 g per day to 4 g per day) leads to profound decreases in urinary excretion of catecholamine metabolites and cerebrospinal fluid levels of the dopamine metabolite homovanillic acid (HVA) with no change in the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Brodie *et al.*, 1971; Engelman *et al.*, 1968a; Sjoerdsma *et al.*, 1965). A maximal reduction of catecholamine metabolites during AMPT treatment occurs after 2–3 days of treatment (Bunney *et al.*, 1971; Engelman *et al.*, 1968b). It has been shown that about 20% of the urinary MHPG is derived from the central nervous system pool (Potter *et al.*, 1984).

Catecholamine Depletion Studies in Healthy Subjects

In order to evaluate the role of catecholamines in regulation of mood, anxiety and alertness, and its potential role in the pathogenesis of psychiatric disorders, it is also important to study the effects of catecholamine depletion in healthy volunteers. Treatment with AMPT has been shown to induce pronounced increases in sleepiness and mild increases in negative mood and anxiety, when administered to healthy male controls (McCann *et al.*, 1995). Significantly, replacement of catecholamine stores with L-DOPA reversed the effects of catecholamine depletion, and was associated with a more rapid recovery from AMPT's effects than when subjects were treated with AMPT alone. Another study of the same group comparing the psychological effects of AMPT alone versus AMPT plus 40.5 hours of total sleep deprivation (McCann *et al.*, 1993) suggests that catecholamines may be involved in mood changes during sleep deprivation. Combined treatment with AMPT and sleep deprivation led to significant increases in negative mood, whereas no treatment alone produced consistent mood changes. With the use of a different methodology to deplete catecholamines, by administering an amino acid mixture deficient of phenylalanine and tyrosine, Leyton and colleagues (Leyton *et al.*, 2000b) induced lowered mood and energy and increased irritability scores in a group of healthy women. Interestingly, the behavioural changes induced by catecholamine depletion were similar to those found during tryptophan depletion in the same group of subjects. In both conditions, the behavioural effects were more pronounced following exposure to aversive psychological events. Another study suggests that acutely decreased catecholamine transmission may disrupt mechanisms mediating alcohol self-administration (Leyton *et al.*, 2000a). Altogether, these studies support the role of catecholamines in a variety of human behaviours and also suggest that disturbed catecholamine transmission may predispose humans to various psychiatric disorders, including depression and different anxiety disorders.

Catecholamine Depletion Studies in Depression

The role of norepinephrine and dopamine in the pathogenesis of depression and in the mechanisms of action of antidepressant drugs have been subjects of intensive research during the past decades. A comprehensive overview of catecholaminergic function in depression is beyond the scope of this chapter. The purpose of the present chapter is to show the biochemical and behavioural effects of catecholamine depletion in depressed patients in different states of their illness, and their implications in our understanding of the pathogenesis of depression and its treatment. In the initial studies performed by Bunney and colleagues at the National Institute of Health (Bunney *et al.*, 1971) the authors demonstrated the antimanic effects of AMPT in a small group of manic patients, whereas three of four unipolar psychotic depressed patients showed an increase of depression on AMPT. However, small sample sizes and the lack of a control group or a placebo condition limit the interpretation of these initial findings. More recent studies addressing these methodological shortcomings showed that drug-free, untreated, symptomatic depressed patients had significant increased visual analogue ratings of 'tired' and decreased ratings of 'energetic'. However, there was no alteration in mood reported by the authors (Miller *et al.*, 1996b). This finding suggests that a simple norepinephrine or dopamine deficiency hypothesis is insufficient to explain the neurobiological basis of depression. Rather, the role of noradrenergic systems needs to be considered in relationship

to many other neurobiological factors that may be involved in the pathogenesis of depression. An alternative explanation could be that during a depressive state the catecholamine systems are already maximally dysfunctional and thus further manipulations do not worsen the condition of the patient.

Studies of the effects of AMPT on patients remitted on antidepressants or remitted depressed patients off medications provided further insight into the pathogenesis of depression and mechanisms of action of different antidepressants. Depressed patients in remission and on noradrenergic antidepressants (desipramine and mazindol) experienced a depressive relapse during catecholamine depletion, whereas those patients whose remissions were maintained with the selective 5-HT reuptake inhibitors (SSRIs) fluoxetine and sertraline remained well during catecholamine depletion (Miller *et al.*, 1996a). The administration of AMPT induced core symptoms of depression, such as depressed mood, loss of interest, anhedonia, cognitive disturbances, and sense of worthlessness and failure. Another study reported the reoccurrence of depressive symptoms induced by catecholamine depletion in a group of patients with seasonal affective disorder/winter type remitted on light therapy (Neumeister *et al.*, 1998c), supporting recent findings of reduced dopaminergic function in untreated patients with winter depression (Neumeister *et al.*, 2001). These findings disagree with a small study of three depressed patients with a favourable treatment response to imipramine (Shopsin *et al.*, 1975). However, beside the sample size of that study being small and there being no control situation, it has to be considered that imipramine is a potent 5-HT reuptake inhibitor as well as a norepinephrine reuptake inhibitor. The findings that catecholamine depletion disrupts the antidepressant effects of catecholaminergic, but not serotonergic, antidepressants, whereas tryptophan depletion reverses the antidepressant effects of serotonergic antidepressants, but not catecholaminergic antidepressants (Delgado *et al.*, 1999), argue against a single monoamine-related mechanism of action of antidepressants. However, such studies suggest that enhanced catecholamine function is important to maintain response to noradrenergic antidepressants, and enhanced serotonergic function is important to maintain response to serotonergic antidepressants.

State-related changes in catecholamine function have been described in depressed subjects, whereas enduring abnormalities have been less reliably identified. Recent catecholamine depletion studies in fully remitted, medication-free, formerly depressed subjects showed a depressive relapse in these subjects during catecholamine depletion, but not during sham depletion (Berman *et al.*, 1999; Lam *et al.*, 2001). The authors argue that the reoccurrence of depressive symptoms during catecholamine depletion may represent a reliable marker for a history of depression, and may well be a trait marker for the disorder. Further studies are needed to clarify the importance of these findings.

α_2-Adrenergic Receptors

Neurotransmission in the noradrenergic system is mediated by a number of different neurotransmitter receptors whose function has been implicated in either the pathophysiology of depression or in the mechanism of action of antidepressants (Duman and Nestler, 1995). The α_2-adrenergic receptors (α_2-AR) have been the focus of considerable research on their role in the pathophysiology of depression. The α_2-ARs are a heterogeneous group of receptors that bind to the naturally occurring ligands epinephrine and norepinephrine (O'Dowd *et al.*, 1989; Venter *et al.*, 1989). α_2-ARs mediate their functions through the G_i class of G proteins (Lefkowitz and Caron, 1988; Limbird, 1988). The α_2-ARs are located on nerve terminals and on the cell bodies and/or dendrites, and participate in local and neuronal feedback inhibition (Cedarbaum and Aghajanian, 1977; Hein *et al.*, 1999; Langer, 1997; Miller, 1998; Stjarne, 1989). Activation of these receptors by endogenous ligands or α_2-agonists, e.g. clonidine, decreases noradrenalin release and the firing rate of the neurons (Engberg and Eriksson, 1991; Hein *et al.*, 1999; MacDonald *et al.*, 1991) and results in bradycardia, hypotension, hypothermia, locomotor inhibition, anxiolysis, analgesia and sedation (Aantaa and Scheinin, 1993; Altman *et al.*, 1999; Hein *et al.*, 1999; Lakhlani *et al.*, 1997; Puke and Wiesenfeld-Hallin, 1993; Rohrer and Kobilka, 1998; Ruffolo *et al.*, 1993; Sallinen *et al.*, 1999). Clonidine is a partial agonist at brain α_2-ARs, but also has high affinity for non-adrenergic imidazoline-binding sites. It has been shown that the distribution of non-adrenergic [^{3}H]-clonidine binding sites is correlated but distinct from that of α_2-ARs, and that the affinity of these sites is distinct from α_2-AR sites (Piletz *et al.*, 2000). Blockade of α_2-ARs by α_2-antagonists, e.g. yohimbine, increases the firing rate and responsiveness of neurons to stimulation (Simson and Weiss, 1987; Tjurmina *et al.*, 1999) and promotes release of noradrenalin (Freedman and Aghajanian, 1984; Grossman *et al.*, 1991; Scheinin *et al.*, 1988; Starke *et al.*, 1975). It has to be acknowledged that interpretation of data from yohimbine challenge studies is complicated by mixed drug effects due to the blockade of both pre- and postsynaptic α_2-ARs in the hypothalamus and presynaptic D_2 dopaminergic autoreceptors in the pituitary (Meltzer *et al.*, 1983). In addition, yohimbine exhibits affinity to 5-HT_{1A} receptors, although yohimbine is tenfold selective for human α_{2A}-ARs versus human 5-HT_{1A} receptors (Newman-Tancredi *et al.*, 1998). In addition to these α_2-auto-AR, α_2-heteroreceptors have been identified, which are located elsewhere than on noradrenergic neurons. These heteroreceptors are involved in the regulation of release of a variety of neurotransmitters, including serotonin (Gobert *et al.*, 1997, 1998; Mongeau *et al.*, 1993; Raiteri *et al.*, 1990), dopamine (Gobert *et al.*, 1997, 1998; Trendelenburg *et al.*, 1994), and histamine (Gulat-Marnay *et al.*, 1989). Activation of α_2-ARs by α_2-agonists reduces the turnover of norepinephrine, serotonin and dopamine in the brain.

It has been demonstrated that α_2-ARs mediate a variety of physiological functions and pharmacological effects in the central nervous system, mainly by inhibiting neuronal firing and release of noradrenalin and other neurotransmitters. A wide range of functions in peripheral tissues is also mediated by α_2-ARs, including regulation of noradrenalin release from sympathetic nerves, smooth muscle contraction, platelet aggregation, insulin secretion, glomerular filtration and energy metabolism (Ruffolo *et al.*, 1993).

The α_2-ARs have been the focus of considerable research on their role in the pathogenesis of depression (Charney *et al.*, 1981; Cohen *et al.*, 1980; McKenna *et al.*, 1992; Piletz *et al.*, 1986; Smith *et al.*, 1983). An increase in the density of platelet α_2-ARs has been reported in drug-free depressives. Also, the platelet aggregation response has been reported as being enhanced in depression. The findings of increased α_2-AR density in depression, both in the central nervous system and in the periphery, have not been consistently replicated. Blunted growth hormone responses have been reported in depressive patients in different states — untreated, treated with antidepressants, and when being remitted. This argues that alterations in α_2-AR function may be a trait characteristic in at least a subgroup of depressed patients.

SUMMARY

The data obtained from studies evaluating the role of monoamines in the pathophysiology of mood disorders, in particular depression, and in antidepressant treatments provide evidence that monoamines do not have a direct effect on regulating mood, but rather have a modulator role on other neurobiological systems. However, there is substantial evidence that adequate monoaminergic activity in the brain is necessary to achieve and maintain an antidepressant response to antidepressant treatments. Future research

should try to further increase our knowledge about the interactions between serotonergic and noradrenergic transmitter systems and other neurobiological systems. This may become of clinical relevance since preclinical studies have revealed potential molecular and anatomic sites that could contribute to the development of future generations of antidepressant agents.

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XVIII-3

Evidence for GABAergic and Glutamatergic Involvement in the Pathophysiology and Treatment of Depressive Disorders

Gerard Sanacora

The majority of neurons in the brain use either γ-aminobutyric acid (GABA) or glutamate as their primary neurotransmitter. In effect these two neurotransmitters serve to regulate the excitability of almost all neurons in the brain. Therefore, it is not surprising that they are implicated in a broad range of both physiological and pathophysiological events related to brain function. Although the past three decades of research has emphasized the role of the biogenic amines and hypothalamic–pituitary–adrenal (HPA) axis in the neurobiology of mood disorders, emerging evidence now suggests that the amino acid neurotransmitter systems also contribute to the pathophysiology and pharmacological treatment of depression.

Newly developed neurochemical imaging techniques and advances in molecular pharmacology are now providing novel methods to study the amino acid neurotransmitter systems. Through the use of these modalities and novel pharmaceutical agents we are now beginning to uncover the extent to which these ubiquitous systems are related to depression and other mood disorders. In the following pages we will examine the evidence supporting the involvement of the GABAergic and glutamatergic systems in the neurobiology of mood disorders.

EVIDENCE SUPPORTING GABAERGIC CONTRIBUTIONS TO THE NEUROBIOLOGY OF DEPRESSION

Dysregulation of GABAergic neurotransmission is increasingly implicated in the neurobiology of mood disorders (Lloyd *et al.*, 1989; Petty, 1995; Sanacora *et al.*, 2000; Shiah and Yatham, 1998). Supporting evidence comes in the form of (1) animal studies showing stress-related changes in GABAergic function, and the ability of GABA modulating agents to alter animal models of depression, (2) demonstration of GABAergic abnormalities in depressed patients, and (3) GABAergic effects of antidepressant and mood stabilizing medications.

GABA and Animal Models of Stress and Depression

Multiple lines of evidence suggest stress is a major precipitating factor in the development of depressive episodes. Existing animal studies suggest that stress-related effects on the GABAergic neurotransmitter system may contribute to this relationship. Several studies have found decreased cortical $GABA_A$ receptor function following exposure to short-term stress in various rodent models (Biggio *et al.*, 1984; Concas *et al.*, 1988; Sanna *et al.*, 1992; Serra *et al.*, 1991). Additionally, stressful early life events can result in long-lasting changes in $GABA_A$ receptor function that appear related to altered expression of adult behaviours (Caldji *et al.*, 2000). Lower GABA concentrations, synthesis rates, and neurotransmitter uptake have also been reported in acute stress paradigms (Acosta *et al.*, 1993; Borsini *et al.*, 1988). Chronic stress produced similar changes in GABAergic function, including decreases in brain GABA concentrations, glutamic acid decarboxylase (GAD) activity, GABA uptake, and regional $GABA_A$ receptor binding (Acosta and Rubio, 1994; Insel, 1989; Weizman *et al.*, 1989). Interestingly, the time course of these events corresponds to changes in neuroactive steroid concentrations, which may serve as endogenous mediators of homeostatic function by maintaining GABAergic regulation during prolonged stress (Barbaccia *et al.*, 1996, 1997).

The role of GABA in the regulation of the HPA axis is now drawing increasing attention (Barbaccia *et al.*, 1996, 1997; Boudaba *et al.*, 1996; Calogero *et al.*, 1988; Owens *et al.*, 1991). Local circuits from within the hypothalamus provide a rich supply of GABAergic innervation directly on to the corticotropin-releasing hormone (CRH) containing parvocellular region of the paraventricular nucleus (PVN) (Herman and Cullinan, 1997). These stress-activated PVN-projecting GABAergic pathways appear to play a prominent role in modulating the HPA axis response to stress (Bowers *et al.*, 1998). Disruption of this system could be related to the abnormal HPA stress responses commonly observed in mood-disordered individuals.

The ability of GABA modulating agents to alter the expression of stress responses and animal models of depression further suggest a role for the inhibitory neurotransmitter system in the neurobiology of mood disorders. Initial studies by Sherman and Petty demonstrating the ability of intrahippocampal GABA injections both to prevent and reverse the induction of learned helplessness provided the initial link to the GABAergic system (Sherman and Petty, 1980). Other studies demonstrating decreased Ca^{++}-dependent GABA release from the hippocampus of helpless animals, and inhibition of imipramine's 'antidepressant-like' effects on learned helplessness behaviour by intrahippocampal administration of the GABA antagonist bicuculline (Petty and Sherman, 1981), provided additional evidence that GABA-mediated mechanisms are associated with the development of depression-like behaviours in this model.

Transient GABA reductions were reported in several brain regions following the initial session of the forced swimming test (FST) (Borsini *et al.*, 1988), another frequently used animal model of depression and a test of antidepressant activity. Similar to the actions of other antidepressant agents, GABA agonists reduce

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the immobility time during the forced swimming test (Borsini *et al.*, 1986; Poncelet *et al.*, 1987). Inhibiting GABA breakdown by administering the GABA-transaminase (GABA-T) inhibitor amino-oxyacetic acid prior to the first forced swim session elevated GABA levels, and decreased immobility time (Borsini *et al.*, 1988), suggesting that the immobility time associated with the FST is mediated in part through the GABAergic system.

Like the learned helplessness and FST models, the olfactory bulbectomized rat, another commonly used behavioural model of depression, also appears sensitive to modulation by GABA mimetics. The passive avoidance deficit (Lloyd *et al.*, 1983, 1987), muricidal behaviour (Delini-Stula and Vassout, 1978) and open field behaviour (Leonard, 1984), measures frequently used as tests of antidepressant-like activity, are all reversed following the administration of GABA agonists. In other studies $GABA_B$ binding was demonstrated to be significantly decreased in the frontal cortex of these animals (Lloyd *et al.*, 1987). GABA mimetics also produce antidepressant-like activity in other tests of antidepressant activity such as the paradoxical sleep and 5-HTP head twitch (for review see Lloyd *et al.*, 1989).

Evidence of GABAergic Abnormalities in Mood Disorder Patients

There are several forms of evidence suggesting possible widespread abnormalities of GABAergic function to mood disorders. However, the most convincing evidence linking the GABAergic system to the neurobiology of mood disorders are the studies showing decreased GABA in the plasma, cerebrospinal fluid (CSF) and cerebral cortex of depressed individuals.

CSF

Gold and colleagues first reported decreased CSF GABA levels in mood disorder patients (Gold *et al.*, 1980). They found significantly lower CSF GABA concentrations in mood disorder patients compared to a group of subjects undergoing a neurological evaluation and a group of psychotic subjects. A second CSF study (Post *et al.*, 1980) reported a non-significant trend toward lower GABA levels in 16 depressed patients, compared to 8 manic patients and 41 healthy comparison subjects. A third study (Gerner and Hare, 1981) highlighted the complexities of CSF GABA studies. It compared CSF GABA levels from groups of depressed, schizophrenic, manic and anorexia nervosa patients to the levels of healthy comparison subjects using two samples of CSF. Consistent with the initial study by Gold *et al.*, only the depressed subjects exhibited lower CSF GABA than the healthy comparison subjects when the sample was obtained from the first 12 cm^3 collected. However, when the second aliquot of CSF (15–26 cm^3, the same as in the Post *et al.*, study) was used, the trend was no longer statistically significant (Gerner *et al.*, 1984). Gerner later replicated the findings of decreased GABA concentrations in a larger study measuring CSF levels in 41 depressed patients and 38 controls. Consistent with the previous studies, lower CSF GABA levels were not observed in patients diagnosed with schizophrenia or mania. A Japanese study also reported significantly lower CSF GABA levels in depressed patients compared to a mixed group of healthy comparison subjects and hospitalized patients without neuropsychiatric illness (Kasa *et al.*, 1982). A later study by Roy and collaborators (Roy *et al.*, 1991b) also found lower CSF GABA levels in patients with a current depressive episode. However, when they controlled for age and gender there was no significant difference. Only the subgroup of patients with unipolar

Table XVIII-3.1 Review of CSF GABA studies in depression

	Comparison subjects				Depressed patients		
Reference	CSF Sample (cc)	Sample size	Gender	CSF GABA level (pmol ml^{-1}) mean ± SEM	Sample size	Gender	CSF GABA level (pmol ml^{-1}) mean ± SEM
Gold *et al.* (1980)	0–10	20 (neurologic patients)	M = 4 F = 16	218 ± 26	MDE = 17 UP = 10 BP = 5 ETOH = 1 OBS = 1	M = 1 F = 16	122 ± 10*
Post *et al.* (1980)	16–28	41 (healthy)	M = 26 F = 15	232 ± 13	MDE = 16 (sub-type not specified)	M = 6 F = 10	214 ± 13
Gerner and Hare (1981)	1–12 15–26	29 (healthy)	M = 14 F = 15	183 ± 12 200 ± 11	MDE = 24 UP = 19 BP = 5	M = 11 F = 13	134 ± 5* 172 ± 10
Zimmer *et al.* (1981)	1–6	6 (surgical patients)	Not specified	360 ± 29	DS = 6 (sub-type not specified)	M = 3 F = 3	280 ± 45
Kasa *et al.* (1982)	5–10	24 (mixed group of healthy subjects and hospital patients without neurologic and psychiatric disorders)	Not specified	138 ± 12	MDE = 13 (sub-type not specified)	M = 11 F = 2	96 ± 39*
Gerner *et al.* (1984)	0–12	38 (healthy)	M = 18 F = 20	190 ± 5	MDE = 41 Up = 30 BP = 10	M = 18 F = 23	140 ± 6*
Roy *et al.* (1991b)	1–10	20 (healthy)	M = 8 F = 12	145 ± 10	MDE = 25 UP = 18 BP = 7	M = 4 F = 21	119 ± 33

Note: *Sig. $P < 0.05$. M = male, F = female, MDE = major depressive episode, DS=depressive syndrome, UP = unipolar depression, BP = bipolar depression, OBS = organic brain syndrome, ETOH = alcohol related.

melancholic depression had significantly lower GABA levels than all other groups. Finally, a study by Zimmer and colleagues reported a non-significant decrease in CSF GABA levels with depressive mood states in a small group of poorly defined subjects (Zimmer *et al.*, 1980).

In summary, all of the CSF studies to date have found at least a trend toward decreased GABA concentrations in depressed subjects (see Table XVIII-3.1). A meta-analysis of this data (Petty *et al.*, 1993c) revealed a highly significant difference between depressed and comparison subjects. Interestingly, the finding appears to be relatively specific to depression. No significant differences were observed in schizophrenic, anorectic or manic subjects (Gerner and Hare, 1981; Gerner *et al.*, 1984; Gold *et al.*, 1980; Post *et al.*, 1980). In further support of this specificity, Roy and colleagues (Roy *et al.*, 1991a) reported CSF GABA levels to be selectively decreased in depressed alcoholic patients compared to non-depressed alcoholics. Unfortunately, lack of diagnostic specificity in these early studies and the grouping of unipolar and bipolar patients into a common class does not allow for tests of further specificity. The fact that none of the studies examined remitted patients also leaves the state versus trait dependence issue unresolved.

Plasma Levels

There are several reports of decreased plasma GABA levels in individuals with affective disorders, with the majority of studies from a single laboratory (Berrettini *et al.*, 1982a, 1986; Petty and Schlesser, 1981; Petty and Sherman, 1984; Petty *et al.*, 1990, 1992, 1993b). In general, these findings suggest a trait-dependent reduction in plasma GABA levels in patients with mood disorders. This is best noted by a markedly increased percentage of individuals having plasma GABA concentrations below 100 pmol ml^{-1}. Unlike the CSF studies, there is no clear evidence of specificity between active episodes and remitted states, or between depressed and manic states. However, the group of patients with the lowest GABA levels had more severe melancholic-like symptoms. Similar to the CSF studies, the lower GABA concentrations were somewhat specific to mood disorders. No change in plasma GABA was observed in patients with schizophrenia, generalized anxiety disorder, eating disorders or panic disorder (Petty and Sherman, 1984; Goddard *et al.*, 1996; Roy-Byrne *et al.*, 1992; Petty, 1994 for review see). However, lower plasma GABA levels were seen in alcoholism (Petty *et al.*, 1993a), Parkinson's disease (Manyam, 1982) and premenstrual dysphoric disorder (Halbreich *et al.*, 1996).

Post-Mortem and Biopsy Studies

Post-mortem studies consisting mostly of depressed suicide victims have not provided consistent evidence of GABAergic abnormalities. No significant differences were found in $GABA_B$ binding in a post-mortem sample of 16 suicide victims compared to 20 controls (Arranz *et al.*, 1992). This is consistent with earlier post-mortem studies that showed no change in $GABA_B$ binding sites in depressed suicide victims (Cross *et al.*, 1988). $GABA_A$-benzodiazepine binding was increased by 18% in the frontal cortex of 21 depressed suicide victims compared to 21 age- and sex-matched controls (Cheetham *et al.*, 1988).

Biopsy and post-mortem data on GABA concentrations also remains inconclusive, an outcome that may be due in part to the dramatic post-mortem increase in the activity of GABA's primary synthetic enzyme GAD. A negative correlation was found between GABA concentrations and severity of depression in a study measuring GABA levels from cortex removed during psychosurgery (Honig *et al.*, 1988). However, Korpi *et al.* (1988) found no significant differences in brain GABA levels of suicide victims. Similar inconsistencies are seen regarding GAD activity levels. An early study reported significantly lower rates of GABA synthesis in several brain regions from elderly depressed patients compared to control subjects (Perry *et al.*, 1977). Consistent with this, a single study found lower plasma GAD activity in depressed patients (Kaiya *et al.*, 1982). However, Cheetham *et al.* (1988) found no significant differences in GAD activity in depressed suicide victims, and a more recent study reported a significant increase in the expression of the GAD_{67} isoform of the enzyme in the prefrontal cortex of depressed patients (Toth *et al.*, 1999). No differences were found in the one study investigating the activity of GABA-T activity in depressed and non-depressed suicide victims (Sherif *et al.*, 1991), although lower platelet GABA-T activity was found in euthymic medication-free bipolar patients (Berrettini *et al.*, 1982b). Recent studies by Rajkowska and colleagues suggest that mood disorders are associated with reduced numbers of neurons in layer II in regions of the frontal cortex (Rajkowska *et al.*, 1999), and preliminary findings further demonstrate an associated reduction of calbindin-immunoreactive neurons in layer II depressed subjects (Personal Communication Dr. Rajkowska).

In vivo Brain Studies of GABA

Advances in magnetic resonance spectroscopy (MRS) have made it possible to measure GABA concentrations *in vivo*. Using this methodology, decreased GABA concentrations were demonstrated in the occipital cortex of depressed subjects (Figure XVIII-3.1) (Sanacora *et al.*, 1999a). In two other preliminary studies the occipital GABA concentrations were found to be normalized following treatment with either electroconvulsive therapy (Sanacora *et al.*, 1999b) or selective serotonin reuptake inhibitor (SSRI) medications (Sanacora *et al.*, 2002). Since these initial MRS studies were limited to the occipital cortex it is difficult to determine the pathophysiological significance of the findings. However, in light of the previous reports that consistently demonstrated decreased GABA concentrations in the plasma and CSF of depressed patients, the studies suggest that widespread alterations of GABAergic function are associated with major depression. In a single preliminary report using SPECT imaging of radiolabelled Iomazenil to examine benzodiazepine binding, no significant difference was observed between depressed subjects and a historical group of healthy control subjects despite the presence of lower occipital cortex GABA concentrations (Kugaya *et al.*, 2001).

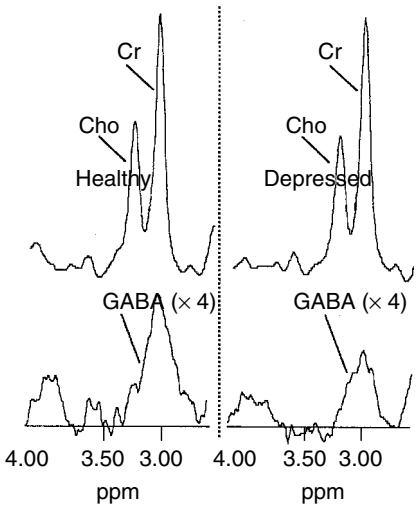

Figure XVIII-3.1 *In vivo* 1H-MRS spectra from representative healthy and depressed subjects. The relative differences in occipital cortex GABA between these two subjects is illustrated by the size of the GABA peaks on the spectra

Pharmacological Challenges

The use of pharmacological challenge studies to investigate GABAergic involvement in depression has yielded inconsistent findings. Administration of the $GABA_B$ agonist baclofen is known to increase growth hormone secretion from the anterior pituitary (Koulu *et al.*, 1979; Müller, 1987). Two studies have shown this effect to be blunted in depressed individuals, suggesting altered $GABA_B$ function in depression (Marchesi *et al.*, 1991; O'Flynn and Dinan, 1993). However, two other studies found no significant difference between healthy comparison and depressed subjects in response to a baclofen challenge (Davis *et al.*, 1997; Monteleone *et al.*, 1990).

Findings Related to Endogenous Modulators of GABA Function

Neuroactive steroids are metabolites of progesterone and deoxycorticosterone that interact with the $GABA_A$/benzodiazepine receptor complex. Decreased plasma levels of several neuroactive steroids possessing potentiating effects on the $GABA_A$ receptor were observed along with a concomitant increase in two neurosteroids that may act as functional GABA antagonists in depressed individuals (Romeo *et al.*, 1998). Treatment with antidepressant medication appears to reverse this disequilibrium. This effect of antidepressant medications on neuroactive steroid concentrations is consistent with an earlier rodent study demonstrating increased neuroactive steroids concentrations in response to fluoxetine treatment (Uzunov *et al.*, 1996), and may contribute to the mechanism of antidepressant action by altering GABAergic transmission. Furthermore, the involvement of neuroactive progesterone metabolites in the pathophysiology of depression also suggests one possible explanation for the elevated rates of depression in the post-partum period (O'Hara *et al.*, 1984), and elevated rates of depressive symptoms in the late luteal phase of the menstrual cycle (Rubinow *et al.*, 1984).

Genetic Studies

Attempts to examine the relationship between the GABAergic system and mood disorders using genetic analysis studies are yet to yield convincing evidence (Table XVIII-3.2). Puertollano and colleagues reported an association between manic-depressive illness and tetranucleotide repeat polymorphisms at the GABRB1 gene in a study of a subpopulation of Spanish females (Puertollano *et al.*, 1997). Oruc *et al.* (1996) failed to detect a significant association with GABRA1 and GABAR3 and bipolar disorder. A third study by Serretti and colleagues (Serretti *et al.*, 1998) also failed to find an association between depressive symptomatology and the GABRA1 gene in a group of unipolar and bipolar depressed patients. Petty and colleagues recently provided preliminary evidence suggesting that regulation of plasma GABA levels may be under the control of a single gene, inherited in a recessive manner (Petty *et al.*, 1999). This is consistent with an older study that showed a statistically significant correlation between plasma GABA concentrations of depressed subjects and first-degree relatives (Petty, 1994).

GABA's Role in the Treatment of Mood Disorders

The delayed clinical response to tricyclic antidepressants (TCA), SSRI and monoamine oxidase inhibitors (MAOI) suggests that other neurotransmitter systems in addition to the monoamines may be involved in the mechanism of antidepressant action. Several studies have sought to examine the possible involvement of the GABAergic system in this regard.

Receptors

Chronic administration of all three classes of antidepressants (TCA, SSRI, MAOI) and ECT have been reported to enhance $GABA_B$ receptor binding in animal studies (for review see Lloyd *et al.*, 1987). However, contrasting findings have also been reported (Cross and Horton, 1988; McManus and Greenshaw, 1991; Monteleone *et al.*, 1990; Szekely *et al.*, 1987). The upregulation of $GABA_B$ receptors appears dependent on chronic administration of antidepressant medications, as no changes were seen after acute administration. Interestingly, other neuroleptic, anxiolytic and psychostimulant medications failed to show similar effects (Lloyd *et al.*, 1987), but mood stabilizing agents such as sodium valproate, carbamazepine and lithium did enhance $GABA_B$ binding (Motohashi *et al.*, 1989).

Using the olfactory bulbectomized rat model Joly and colleagues (Joly *et al.*, 1987) reported that only desipramine 'responders' displayed a significant increase in $GABA_B$ receptor binding, thus suggesting a functional relationship between the increase in $GABA_B$ receptors and behaviour. Interpretation of the above findings is complicated by a recent study by Beck and colleagues suggesting that the potency of $GABA_B$ transmission is decreased following chronic fluoxetine treatment in selected subfields of the rat hippocampus as measured by baclofen-stimulated hyperpolarization (Beck *et al.*, 1997).

There are two reports of long-term antidepressant administration resulting in downregulation of $GABA_A$ receptors (Suranyi-Cadotte *et al.*, 1984; Suzdak and Gianutsos, 1985). However, these finding were not replicated in a later study (Kimber *et al.*, 1987), and increased numbers of benzodiazepine receptors were found in selected areas of rat brain following chronic administration of sertraline (Giardino *et al.*, 1993). Chronic administration of

Table XVIII-3.2 Summary of findings in depressed subjects

	Levels relative to controls	Comments	References
Plasma GABA	↓	Consistent findings of low plasma GABA with one exception.	Petty (1994), Petty and Schlesser (1981), Petty *et al.* (1992), Rode *et al.* (1991)
CSF GABA	↓	4 of 7 studies found significantly lower GABA concentrations, others reported trend toward lower GABA in depression.	Gerner and Hare (1981), Gerner *et al.* (1984), Gold *et al.* (1980), Kasa *et al.* (1982), Post *et al.* (1980), Roy *et al.* (1991b), Zimmer *et al.* (1981)
Brain GABA	↓	Inconsistent findings with limited post-mortem studies, *In vivo* MRS study highly significant	Honig *et al.* (1989), Korpi *et al.* (1988), Sanacora *et al.* (199b)
GABAergic neurons	↓	Single study results	Rajkowska *et al.* (1999) (Personal Communication Dr. Rajkowska)

imipramine, phenelzine and buspirone resulted in altered $GABA_A$ receptor subunit patterns in rat brains (Tanay *et al.*, 1996), but no consistent pattern of change was observed.

GABA Levels

Several older studies found elevated GABA levels and enhanced GABA release in rat brains following high-dose acute administration of TCAs and MAOIs, and daily electroconvulsive shock (Bowdler *et al.*, 1983; Korf and Venema, 1983; Patel *et al.*, 1975; Perry and Hansen, 1973; Popov and Matthies, 1969). However, other studies found GABA levels to be generally unaltered by antidepressant administration (Olsen *et al.*, 1978; Pilc and Lloyd, 1984). More recent studies consistently find the MAOI phenelzine to increase GABA levels in the rat brain (Baker *et al.*, 1991; McKenna *et al.*, 1994; McManus *et al.*, 1992). The increased GABA levels do not appear the direct result of changes in synthetic or catabolic enzyme gene expression since phenelzine produced no effect on the steady-state levels of mRNAs that encode for GABA-T, GAD_{65} or GAD_{67} (Lai *et al.*, 1998). However, the elevated brain GABA levels do appear in part related to the anxiolytic properties of the drug in animal models (Paslawski *et al.*, 1996). The true relevance of the elevated GABA levels to phenelzine's antidepressant actions remains unknown. Seemingly in contrast to the above findings, Giardino and colleagues (Giardino *et al.*, 1996) found chronic sertraline treatment to decrease GAD expression in several rat brain regions. As mentioned above, recent MRS findings suggest that GABA concentrations in the occipital cortex are elevated following treatment with antidepressant agents.

Effectiveness of Mood Stabilizers and Antidepressants with GABAergic Activity

GABA Analogues

Several studies suggest that compounds that enhance GABAergic transmission possess antidepressant properties. As noted above, GABA agonists have antidepressant-like activity in several animal models used to assess antidepressant action (Lloyd *et al.*, 1989). These findings prompted clinical investigation of GABA-enhancing drugs as antidepressants. Due to the pharmacological limitations of many of these compounds, their use in clinical trials is limited. However, progabide and fengabine (SL 79229) are two GABA-mimetic compounds that reached clinical trials as antidepressant agents. Both of these compounds appeared to be effective antidepressants in multiple controlled clinical trials, in some reports having equal efficacy to TCAs (for compilation of studies see Bartholini *et al.*, 1986). However, development of these compounds was halted due to unfavourable side-effect profiles, and no recent work has been reported as a follow-up to these initial studies.

Benzodiazepines and Anticonvulsants

The benzodiazepine class of $GABA_A$ agonists appear to be ineffective as antidepressant agents in general (Schatzberg and Cole, 1978; Tiller *et al.*, 1989). However, the efficacy of the triazolobenzodiazepine alprazolam remains contested (Petty *et al.*, 1995). Interestingly, valproic acid, a compound that appears to increase GABAergic transmission through multiple mechanisms (Löscher, 1993), has proven mood stabilizing properties (Bowden *et al.*, 1994), and possible efficacy in unipolar major depression (Davis *et al.*, 1996). Other anticonvulsants (gabapentin and topiramate) with GABAergic activity have recently attracted attention as possible mood stabilizing, and antidepressant drugs (Brown *et al.*, 1999; Post *et al.*, 1997). However, the clinical effectiveness of these treatments awaits further study.

In conclusion, the evidence supporting the hypothesis that the GABAergic system contributes to the mechanism of action of antidepressant medications remains preliminary and contestable.

GLUTAMATE

Given that glutamate is the principal excitatory neurotransmitter in the mammalian brain it is likely to contribute to a wide array of both normal and abnormal brain functions. Its involvement has been previously implicated in the pathophysiology of several neurodegenerative disorders as well as schizophrenia, obsessive compulsive disorder and alcohol dependence (Carlsson, 2001; Danbolt, 2001; Tsai and Coyle, 1998). Supporting evidence for glutamate's role in the pathophysiology of mood disorders comes from (1) animal studies showing stress-related changes in glutamatergic function, and its possible relationship to excitotoxicity, (2) demonstration of glutamatergic abnormalities in depressed patients, (3) glutamatergic effects of antidepressant and mood stabilizing medications and (4) the effectiveness of glutamate modulating agents in the treatment of depression.

Stress-Related Effects

Glutamatergic neurotransmission appears intimately involved in a stress-responsive cascade of events that result in neurotoxicity (Sapolsky, 2000). Stress has been shown to increase synaptic glutamate concentrations in the hippocampus and prefrontal cortex (Moghaddam, 1993; Moghaddam *et al.*, 1994), and to alter glutamate receptor function in the hippocampus and hypothalamus (Bartanusz *et al.*, 1995; Nowak *et al.*, 1995b). If this model is correct, stress-responsive neurotoxicity may help explain several recent findings demonstrating altered hippocampal and cortical morphology (Bremner *et al.*, 2000; Sheline *et al.*, 1996) and histology (Ongür *et al.*, 1998; Rajkowska *et al.*, 1999), associated with major depression. In support of this hypothesis, studies using glutamate transporter GLT-1 knockout mice with excessive synaptic glutamate have demonstrated pathological destruction of hippocampal neurons (Tanaka *et al.*, 1997).

Other data suggests that glutamatergic activity is closely tied to the stress-responsiveness of the paralimbic–HPA axis, and that glutamate is capable of stimulating CRF release into the portal circulation (Cratty and Birkle, 1999; Feldman and Weidenfeld, 1997; Ziegler and Herman, 2000). Consistent with glutamate's proposed role in the regulation of the stress response, *N*-methyl-D-aspartate (NMDA) receptor antagonists reduce immobility time on the FST (Maj *et al.*, 1992) and prevent many of the behavioural deficits associated with chronic stress (Ossowska *et al.*, 1997).

Evidence of Glutamatergic Abnormalities in Mood Disorder Patients

Plasma

Studies regarding plasma levels of glutamate in mood disorders remain inconclusive. An early study by Kim and colleagues (Kim *et al.*, 1982) reported elevated glutamate levels in a group of depressed patients, but could not separate the effect from the use of antidepressant medications. Altamura and colleagues (Altamura *et al.*, 1993) also initially reported elevated plasma glutamate levels in a group of mood disorder patients but in a later study found no significant differences in plasma glutamate concentrations comparing unmedicated patients with major depression to healthy controls (Altamura *et al.*, 1995). However, employing a linear discriminant analysis, the group did find a highly significant separation between major depressed subjects and normal volunteers,

using glycine, glutamate and taurine as discriminatory variables. In more recent studies, Mauri and colleagues (Mauri *et al.*, 1998) found elevated plasma and platelet levels of glutamate in depressed patients compared to controls, but Maes *et al.* (1998) found no difference in serum glutamate levels from depressed patients compared to age- and sex-matched controls. Interestingly, however, they did find significantly reduced serum glutamate levels following a 5-week period of treatment with antidepressants.

CSF

Very few CSF studies have addressed the glutamatergic system in relation to depression. Levine and colleagues used MRS technology to demonstrate significantly higher CSF glutamine concentrations in the CSF of 18 hospitalized patients with acute unmedicated severe depression compared to 22 control subjects (Levine *et al.*, 2000). In a second study, Hiraoka and colleagues (Hiraoka *et al.*, 1989) reported that mean CSF glutamine levels in functional psychosis (schizophrenia, manic-depressive illness and other psychoses) did not differ from those of neurotic patients, patients with cerebrovascular disorders or patients with metabolic neuropathy.

Brain

A single study examining neurosurgical samples from chronically depressed subjects did not find a significant difference in frontal cortex glutamate concentrations (Francis *et al.*, 1989). Another study also failed to find any significant difference in the binding of a non-competitive antagonist to the NMDA receptor among depressed suicide victims and an age-matched comparison group (Holemans *et al.*, 1993). However, Nowak and colleagues reported that the proportion of high-affinity, glycine displaceable [3H]CGP-39653 binding to glutamate receptors was reduced in age- and post-mortem interval-matched suicide victims (Nowak *et al.*, 1995a).

In vivo measures of excitatory amino acids in the brain can be made with the use of 1H-MRS. Using standard clinical field strength magnets, the visibility of these metabolites are limited by several factors that make it extremely difficult to assign unequivocal resonance peaks. This has led to the use of a combined measure termed Glx that contains glutamate, glutamine and GABA, of which the greatest proportion reflects the glutamate concentration. In one of the more unique studies, Cousins and Harper used this methodology to demonstrate temporary decreases in Glx levels coinciding with a patient's transient experience of suicidal depression following Taxol and Neupogen chemotherapy (Cousins and Harper, 1996). More recently, Auer and colleagues (Auer *et al.*, 2000) reported decreased Glx measures in the anterior cingulate cortex of severely depressed subjects. A preliminary study by Michael and colleagues also showed a similar decrease in baseline anterior cingulate Glx levels that later increased following treatment with ECT (Michael *et al.*, 2001). Interestingly, no decrease was seen in a group of bipolar depressed subjects participating in this study. Consistent with the idea that Glx measures may differ between unipolar and bipolar depressed patients, elevated levels of Glx were found in both the frontal lobe and basal ganglia of depressed bipolar children compared to a control group (Castillo *et al.*, 2000).

Glutamatergic Effects of Antidepressant and Mood Stabilizing Agents

A growing body of research suggests that the NMDA class of glutamate receptors may be involved in the mechanism of action of antidepressants (Skolnick, 1999). Downregulation of the glycine-B site of NMDA receptors appears to be a common feature of current antidepressant treatments. As shown in Figure XVIII-3.2, tricyclics, serotonin reuptake inhibitors, electroconvulsive stimuli and bupropion selectively reduced the potency of glycine to inhibit [3H]-5,7-dichlorkynurenic acid (5,7-DCKA) binding to the glycine-B site of the NMDA receptor, a change consistent with downregulation of NMDA receptor function (Paul *et al.*, 1994). In this study, the NMDA antagonist ketamine did not modify sensitivity to glycine, although it directly reduced NMDA receptor function through blockade of the calcium channel within the NMDA receptor complex. The tricyclic antidepressant desipramine, which has direct NMDA receptor antagonism at therapeutic doses (Reynolds and Miller, 1988; White *et al.*, 1990), reduces the potency of glycine to displace 5,7-DCKA binding similarly to other antidepressants and it additionally reduces the density of 5,7-DCKA binding sites (Paul *et al.*, 1994). Interestingly, pretreatment of mice with desipramine was shown to significantly reduce NMDA-induced biting and scratching behaviour (Mjellem *et al.*, 1993).

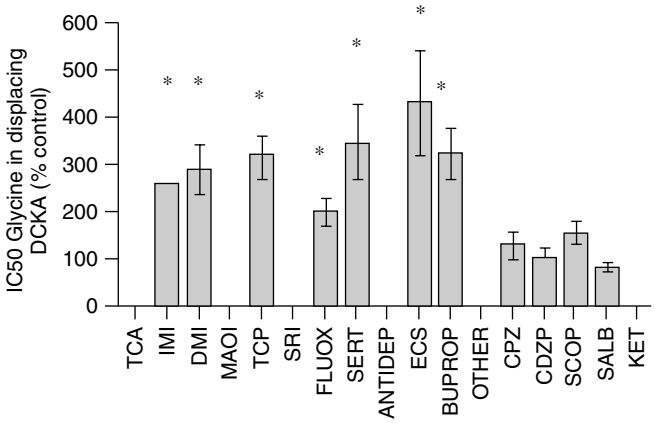

Figure XVIII-3.2 Graph demonstrating the general ability of various antidepressant treatments to reduce the potency of glycine in displacing DCKA binding on NMDA receptors. Adapted from Paul *et al.* (1994)

Several mood stabilizing compounds also appear to attenuate glutamatergic function. Repeated administration of lithium, the prototype mood stabilizer, may promote the uptake of glutamate from the synapse (Dixon and Hokin, 1998), attenuate the function of glutamate receptors (Nonaka *et al.*, 1998) and reduce the function of intracellular signalling cascades that are activated by the binding of glutamate to its receptors (Manji and Lenox, 1999). Anticonvulsant and voltage-sensitive calcium channel antagonist mood stabilizers appear to reduce glutamate release (Cunningham and Jones, 2000), and to inhibit intracellular signalling downstream from glutamate receptors (Manji *et al.*, 1999).

NMDA Antagonist as Antidepressant Agents

Animal Studies

A growing body of preclinical research suggests that NMDA glutamate receptor antagonists exhibit antidepressant-like effects in animal models. Non-competitive NMDA antagonists (amantadine and memantine), competitive NMDA receptor antagonists (CGP 37849) and glycine-B partial agonists (ACPC) have antidepressant-like effects in several tests, including the Porsolt swim test (Moryl *et al.*, 1993; Przegalinski *et al.*, 1997), the tail suspension test (Trullas and Skolnick, 1990) and the chronic mild stress paradigm (Ossowska *et al.*, 1997; Papp and Moryl, 1994).

The mechanisms through which NMDA receptor antagonism exerts this effect are not yet clear. However, several possible pathways exist. For example, NMDA antagonists are known to downregulate β-adrenergic receptors, in a manner similar to

other antidepressants (Wedzony *et al.*, 1995). NMDA receptor antagonists also enhance serotonergic function (Lejeune *et al.*, 1994; Pallotta *et al.*, 1998) by several mechanisms, including increasing the number of 5-HT1A receptors (Wedzony *et al.*, 1997). Alternatively, the mechanism may be the result of a more direct effect on cortical excitability in critical regions of brain activity.

Treatment of Humans with Glutamate Modulating Agents

In humans, the antidepressant activity of NMDA receptor antagonists has received little rigorous evaluation. Initial studies by Crane suggested that D-cycloserine (DCS), an antibiotic developed to treat tuberculosis with indirect NMDA receptor antagonist-like effects mediated through the glycine-B site, possessed antidepressant activity (Crane, 1959, 1961). He reported beneficial effects with respect to depressed mood, insomnia and reduced appetite in depressed tuberculosis patients treated with DCS in doses of 500 mg per day. Interestingly, the rapid onset of antidepressant activity was a striking feature of DCS effects in these patients.

Case reports and preliminary studies suggest that amantadine, a low-affinity non-competitive antagonist of the NMDA receptor, has clinical efficacy in treating depressive symptoms provide additional evidence of the antidepressant activity of NMDA receptor antagonists (Dietrich *et al.*, 2000, Ferszt *et al.*, 1999, Huber *et al.*, 1999). Memantine, a related non-competitive NMDA receptor antagonist, also shows signs of behavioural improvement across a number of dimensions in patients with neuropsychiatric disorders, including mood and motor activity (Ambrozi and Danielczyk, 1988; Görtelmeyer and Erbler, 1992).

Most recently, a placebo-controlled pilot study suggested that the administration of single doses of ketamine 0.5 mg kg^{-1}, intravenously, had antidepressant effects in depressed patients (Berman *et al.*, 2000). Ketamine infusion produced mild psychosis and euphoria that dissipated within 120 minutes, while the antidepressant effects of ketamine infusion emerged over the first 180 minutes and persisted over 72 hours (Figure XVIII-3.3).

Although the clinical evidence supporting the antidepressant efficacy of NMDA antagonists remains limited, if effective, NMDA antagonists may show unique onset rapidity of clinical efficacy.

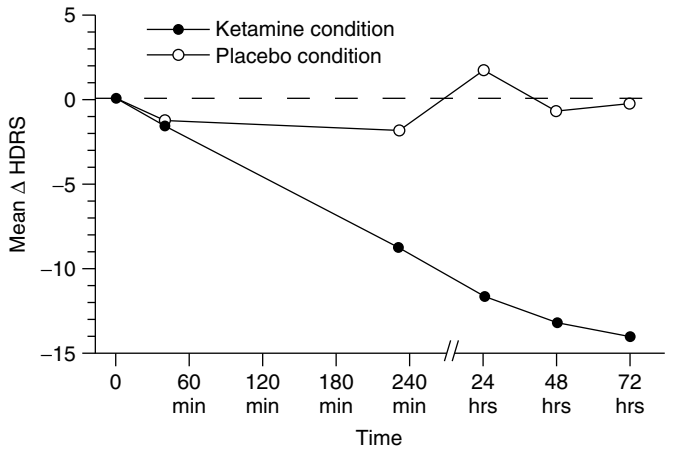

Figure XVIII-3.3 Graph illustrating the decrease in depressive symptom severity as measured by the Hamilton depression rating scale (HDRS) in seven subjects following either administration of placebo (open circle) or ketamine (closed circle) over a 72-hour period. Adapted from Berman *et al.* (2000)

CONCLUSION

Increasing evidence from several lines of research suggests that the major amino acid neurotransmitter systems contribute to the pathophysiology of mood disorders and the mechanisms of antidepressant and mood stabilizing actions. Relatively recent advances in magnetic resonance spectroscopy, along with an increased understanding of amino acid physiology and pharmacology, have now made it possible to begin our investigation into the role these systems may play in the pathophysiology and treatment of mood disorders.

The convergence of this evidence with other studies showing glial deficits and neuronal atrophy associated with stress and depression suggests that a common link may exist. Mismatches between inhibitory and excitatory drive are likely to promote atrophy and neurotoxicity, and may also be related to the reports of altered energy metabolism and cortical excitability that are commonly associated with mood disorders.

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XVIII-4

Peptidergic Transmitter Systems

Jeffrey H. Meyer

Peptides refer to short proteins consisting of chains of less than 100 amino acids. There are as many as 250 peptide neurotransmitters in the brain (see Table XVIII-4.1 for a partial list). Some, such as corticotrophin-releasing factor, have been extensively investigated with respect to mood disorders, but most have not.

Peptides may function as neurotransmitters, neuromodulators and neurohormones. Neurotransmitters are synthesized in presynaptic neurons, and are released after depolarization by presynaptic neurons in sufficient amounts to have effects upon postsynaptic neurons. Neurotransmitters have immediate effects upon postsynaptic neurons and are removed by the synaptic cleft. Neuromodulators have longer term effects upon neurons. For example, a neuromodulator may stimulate a receptor coupled to an effector that influences a second messenger, leading to a cascade that changes ongoing cellular functioning. Neurohormones are similar to neuromodulators except that neurohormones are released into blood, cross the blood–brain barrier, and then bind to receptors.

In this chapter, abnormalities of peptidergic transmitter systems in mood disorders are reviewed. Abnormal peptidergic functioning may involve neurotransmitter, neuromodulator and neurohormone effects. There will be some discussion regarding the role of specific peptidergic transmitter systems in clinical treatment.

OPIOIDS

It is well known that opioid agonist administration can influence mood, and cause analgesia in humans. Given that these behavioural states are dysregulated during depressive episodes, there has been some interest in measuring abnormalities of opioid receptors during mood disorders.

Table XVIII-4.1 A partial list of peptides in the central nervous system

Adrenocorticotrophic hormone
Androgens
Bradykinin
Calcitonin
Cholecystokinin
Corticotrophin-releasing hormone
Cortisol
Oestrogens
Glucagon
Growth hormone
Insulin
Nerve growth factor
Neuropeptide Y
Opioids
Oxytocin
Somatostatin
Substance P
Vasoactive intestinal peptide
Vasopressin

The major classes of opioid receptors are delta (δ), kappa (κ), and mu (μ). Two reports have found an increase in μ opioid receptor density in multiple brain regions of suicide victims (Gabilondo *et al.*, 1995; Gross-Isseroff *et al.*, 1990). The earlier study sampled depressed patients. In the later study, the diagnosis was not always known; however, among those in which the diagnosis was known, most had depression. These reports are consistent with preliminary, *in vivo*, imaging investigations. There is one published abstract reporting an increase in μ opioid receptor binding potential in an [^{11}C] carfentanil positron emission tomography study of depression (Zubieta *et al.*, 1995). The binding potential is proportional to receptor density and affinity (Meyer and Ichise, 2001; Mintun *et al.*, 1984).

Increased μ opioid receptor density should enhance the effects of endogenous opioids and it may seem counterintuitive that increased μ opioid receptor density is observed in depressive episodes. It is possible that these findings may be secondary to lower endogenous opioids or other abnormalities that affect the regulation of this receptor (Law and Loh, 1999).

CHOLECYSTOKININ (CCK)

Cholecystokinin is a family of short-chain peptides which bind to CCK receptors. CCK receptors are categorized into CCK_A and CCK_B receptors. CCK is of interest in mood disorders because anxiety symptoms often occur comorbidly with depressive episodes, and peptides that have CCK_B receptor agonist properties provoke panic attacks (Bradwejn and Koszycki, 1994; Rehfeld, 2000). A second reason for the interest in CCK in mood disorders is that CCK can modulate the release of dopamine in the nucleus accumbens and influence reward behaviours in animal models (Vaccarino, 1994).

Studies sampling CCK in cerebrospinal fluid tend to find no differences between depressed and healthy subjects (Gerner and Yamada, 1982; Gjerris and Rafaelsen, 1984; Gjerris *et al.*, 1984; Rafaelsen and Gjerris, 1985). One study associated state anxiety level with several CCK peptides in a large sample of depressed patients (Lofberg *et al.*, 1998). It has been reported that CCK receptors are increased in frontal cortex of young suicide victims (Harro *et al.*, 1992). The most common diagnosis of a suicide victim is a major depressive episode and major depressive disorder (Barraclough *et al.*, 1974; Robins *et al.*, 1959) so it may be that this abnormality occurs during depressive episodes. Post-mortem investigations of CCK have found no changes in entorhinal cortex concentrations in depressed subjects (Perry *et al.*, 1981) and no changes in several cortex and limbic regions in suicide victims (Kleinman *et al.*, 1985).

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These findings suggest that most CCK peptides are not globally altered during depressive episodes. They do not clarify whether CCK peptides may be altered in small, specific subregions such as the shell of the nucleus accumbens. Thus it is theoretically possible that CCK peptides may have a role within specific neurochemical brain circuits and contribute to pathological behaviour such as impaired reward.

Even if it is eventually found that CCK peptides do not contribute to the pathophysiology of depression, CCK antagonists might be considered as possible treatments to enhance reward or reduce anxiety. The main barrier to the clinical development of such compounds is that they often do not penetrate the blood–brain barrier (Wilson *et al.*, 2001).

CORTICOTROPHIN-RELEASING FACTOR

Corticotrophin-releasing factor (CRF) binds to CRF_1 and CRF_2 receptors. Hypersecretion of CRF is known to occur during depressive episodes. Evidence for this includes increased cerebrospinal fluid CRF in depressed patients (Banki *et al.*, 1987; France *et al.*, 1988; Nemeroff *et al.*, 1984) as well as several findings in suicide victims. In suicide victims, cerebrospinal fluid CRF was increased, and CRF receptor density was decreased in multiple brain regions (Arato *et al.*, 1989; Hucks *et al.*, 1997; Nemeroff *et al.*, 1988). These findings suggest that CRF is higher throughout the central nervous system during depressive episodes (Nemeroff *et al.*, 1988).

Increased CRF appears to affect other hormones during depressive episodes. The adrenocorticotrophic hormone (ACTH) response to CRF is blunted (Hartline *et al.*, 1996; Pariante *et al.*, 1995) and the decreased ACTH responsiveness to CRF can be considered a consequence of chronically excessive CRF stimulation. Since CRF stimulates ACTH and ACTH stimulates cortisol secretion, the increased cortisol secretion observed during depressive episodes is most likely attributable to increased CRF during depressive episodes.

In addition to effects upon ACTH and cortisol secretion, CRF may influence anxiety systems and have specific behavioural effects during depressive episodes. CRF has been implicated in stress response behaviours in animal models. Stressors increase the synthesis of CRF and CRF administration increases stress-related behaviours (Gray and Bingaman, 1996; Lightman *et al.*, 1993). Some of these effects may be consequent to CRF release in the hypothalamic–pituitary axis because exploratory behaviours are reduced after CRF administration to the hypothalamus (Menzaghi *et al.*, 1993).

CRF release in the amygdala may also contribute to these stress behaviours. Stress paradigms appear to activate CRF releasing neurons in the amygdala and CRF administered to the amygdala increases stress behaviours (Gray and Bingaman, 1996; Honkaniemi, 1992). CRF-containing neurons in the amygdala project to other limbic regions that contribute to the stress response (septum, hypothalamus, vagal nucleus) (Gray and Bingaman, 1996).

Glucocorticoids suppress CRF by providing negative feedback to the secretion of CRF, and this feedback is impaired during depression (Carroll, 1976; Carroll *et al.*, 1976). It has been postulated that one mechanism of antidepressants is to enhance this inhibitory feedback upon CRF secretion by increasing glucocorticoid receptor expression (Barden *et al.*, 1995; McQuade and Young, 2000). A number of antidepressants, including desipramine, imipramine, moclobemide and lithium, increase mRNA of type II glucocorticoid receptors (Barden *et al.*, 1995; McQuade and Young, 2000; Pepin *et al.*, 1989).

Another interesting direction of clinical treatment is the use of CRF antagonists to treat mood disorders. An open trial of a CRF_1 antagonist in patients with depressive episodes reported promising results with good tolerability and a reasonable response rate (Zobel *et al.*, 2000).

CORTISOL

Cortisol is a peptide that binds to both glucocorticoid and mineralocorticoid receptors. Increased cortisol levels in the serum and cerebrospinal fluid may be the most replicated finding in depressive episodes (Holsboer, 2000). It has also been demonstrated that cortisol secretion is resistant to inhibition by dexamethasone during depressive episodes (Carroll, 1976; Carroll *et al.*, 1976). Adrenal gland size is also reported to be enlarged during depressive episodes (Nemeroff *et al.*, 1992; Rubin *et al.*, 1995, 1996).

In animal models, it has been demonstrated that increased stress may result in a number of changes in cells within the hippocampus: decreased number of granule cells, decreased neurogenesis of granule cells, increased death of neurons, and atrophy of neuronal dendrites (Duman *et al.*, 2000; Fuchs and Flugge, 1998; Sapolsky, 2000). It is thought that cortisol is in part responsible for mediating these effects because stress raises cortisol levels and some of these hippocampal changes occur after high doses of glucocorticoids (Fuchs and Flugge, 1998; Sapolsky, 2000).

There are several observations of changes in depression that have been attributed to increased stress and/or increased glucocorticoids because they resemble such effects observed in the hippocampus in animal models (Duman *et al.*, 1997; Fuchs and Flugge, 1998; Sapolsky, 2000). Post-mortem investigation of both unipolar and bipolar illnesses reports decreased neuronal size, and neuronal and glial densities in some cortex layers within Brodmann's area 9 of the prefrontal cortex (Rajkowska *et al.*, 1999, 2001). Hippocampal volume as measured using structural magnetic resonance imaging (MRI) is reported to be lower in major depressive disorder (Bremner *et al.*, 2000; Sheline *et al.*, 1996, 1999).

Increased stress during depressive episodes may be causing increased cortisol. The increased cortisol during depressive episodes could then be contributing to the post-mortem and MRI changes reflecting cell loss (Bremner *et al.*, 2000; Rajkowska *et al.*, 1999, 2001; Sheline *et al.*, 1996, 1999). Increased cortisol is not specific to mood disorders and can be found in other psychiatric illnesses, most notably bulimia. Even so, the effects of high cortisol may be an important part of the pathophysiology of this illness.

VASOPRESSIN AND OXYTOCIN

Arginine vasopressin (AVP) is a neuropeptide and hormone which binds to V_1, V_2 and V_3 receptor subtypes. V_3 (also known as V_{1B}) receptors can be found in the anterior pituitary and are of particular interest in mood disorders (Thibonnier *et al.*, 1998, 2001). V_1 receptors are mostly in vasculature and V_2 receptors are mostly renal (Thibonnier *et al.*, 1998, 2001). The reason why AVP has been investigated in mood disorders is that it participates in stress responses and modulates the hypothalamic–pituitary cortisol axis. In rodent models, vasopressin secretion is increased after immobilization and novelty stress, and especially after repeated immobilization stress (Bartanusz *et al.*, 1993; Chen and Herbert, 1995; Gibbs, 1986; Ivanyi *et al.*, 1991). These findings are consistent with a report that stressful events can increase vasopressin secretion in humans (Meyerhoff, 1990).

Vasopressin can enhance the function of the hypothalamic–pituitary axis to secrete cortisol. Vasopressin facilitates CRF-stimulated ACTH production. The mechanism for enhancing the effect of CRF may involve increased production of CRF itself and/or increased synthesis of CRF_1 receptors (Aguilera *et al.*, 2001; Bartanusz *et al.*, 1993; Gibbs, 1986).

Since increased CRF secretion occurs during depressive episodes, investigators have been interested in assessing whether increased AVP secretion may contribute to increased CRF secretion observed during depressive episodes. Serum and cerebrospinal fluid levels during depressive episodes are not consistently different from those of healthy subjects (Inder *et al.*, 1997; Legros and Ansseau, 1992; Legros *et al.*, 1993; Pitts *et al.*, 1995; van Londen *et al.*, 1997); however, one study did correlate CRF levels with AVP levels during depressive episodes (Pitts *et al.*, 1995). Within specific brain structures such as the paraventricular nucleus of the hypothalamus and the suprachiasmatic nucleus, increased numbers of AVP secreting neurons are reported (Purba *et al.*, 1996; Zhou *et al.*, 2001). While the role of the latter finding is still unclear, the increased numbers of AVP secreting neurons in the hypothalamus may be important: increased arginine vasopressin release could facilitate activation of the hypothalamic–pituitary axis for cortisol production.

Oxytocin (OT) may have a similar role to AVP in major depressive disorder. The role of this peptide during stress and its influence upon ACTH secretion appear to be similar to AVP (Callahan *et al.*, 1992; Gibbs, 1986; Ivanyi *et al.*, 1991). OT secretion is increased during stressful conditions and OT can facilitate CRF stimulation of ACTH (Callahan *et al.*, 1992; Gibbs, 1986; Ivanyi *et al.*, 1991).

Since increased CRF secretion occurs during depressive episodes, investigators have been interested in assessing whether increased OT secretion also occurs during depressive episodes. Cerebrospinal fluid and serum measures of OT are not consistently abnormal during depressive episodes (Legros and Ansseau, 1992; Legros *et al.*, 1993; Pitts *et al.*, 1995; van Londen *et al.*, 1997). There is one report of increased OT cell numbers in the paraventricular nucleus of the hypothalamus during depressive episodes (Zhou *et al.*, 2001). It is possible that OT will have a role as a facilitator of CRF stimulation during depressive episodes.

NEUROPEPTIDE Y

Neuropeptide Y is a 36-amino acid neurotransmitter with five receptor subtypes. There are some reports that neuropeptide Y is reduced in the cerebrospinal fluid and plasma of patients with depressive episodes (Hashimoto *et al.*, 1996; Nilsson *et al.*, 1996; Westrin *et al.*, 1999; Widerlov *et al.*, 1988). Neuropeptide Y is also reported to be lower in the prefrontal cortex of suicide victims (Widdowson *et al.*, 1992). Neuropeptide Y mRNA was reported to be lower in the prefrontal cortex of subjects with bipolar disorder (Widdowson *et al.*, 1992) and a post hoc analysis showed increased Y_2 mRNA expression in layer IV of the prefrontal cortex in suicide victims (Caberlotto and Hurd, 2001).

An interesting argument has been made that neuropeptide Y may be inadequately inhibiting CRF secretion during depressive episodes (Antonijevic *et al.*, 2000). The role of neuropeptide Y in mood disorders is still an area of ongoing investigation.

SUBSTANCE P

Substance P is classified with the tachykinin family and binds to neurokinin (NK_1) receptors. There is only limited evidence to suggest that there are abnormalities of the function of this peptide in mood disorders: one study found an elevation of substance P and/or substance P related peptides in cerebrospinal fluid of patients with major depressive disorder, and a second found no difference in substance P levels in major depressive disorder or bipolar disorder (Berrettini *et al.*, 1985; Rimon *et al.*, 1984). One study found no difference in substance P concentrations in a number of different brain regions in suicide victims as compared to healthy subjects (Kleinman *et al.*, 1985). One study found no overall difference in NK_1 density in the anterior cingulate gyrus in patients with a history of unipolar or bipolar disorder (Burnet and Harrison, 2000). In this study a secondary analysis found a decrease in the ratio of NK_1 density between superficial and deep layers in patients with a history of unipolar disorder. Further studies are needed to replicate this post hoc finding, to determine if there are substance P or NK_1 receptor abnormalities in other brain regions, and to address whether any such changes are related to the specific state of illness, i.e. depressive episode.

Although substance P might not play a role in mood disorders, NK_1 antagonists may have useful antidepressant properties. In behavioural models in animals, NK_1 antagonists reduce stress-induced vocalizations, enhance memory performance and reduce anxiety behaviours (Hasenohrl *et al.*, 2000; Kramer *et al.*, 1998). In addition, NK_1 antagonists promote cell growth and survival, and enhance recovery from lesions (Barker, 1996). All of these properties would seem favourable for the treatment of depressive episodes.

One clinical trial of an NK_1 antagonist found a similar rate of antidepressant response as compared to paroxetine and a significantly better response rate than placebo (Kramer *et al.*, 1998). Replication of this finding will be important as the placebo response rate was very high in this study.

NEUROTROPHINS

Brain-derived neurotrophic factor (BDNF) is a peptide within the family of neurotrophins and it binds to the tyrosine kinase receptor trkB. Neurotrophins and BDNF are released in the brain after damaging process such as seizures, hypoglycaemia and ischaemia (Lindvall *et al.*, 1994). Under such conditions, BDNF and neurotrophins demonstrate neuroprotective effects, and contribute to the formation of new synapses (Lindsay *et al.*, 1994; Lindvall *et al.*, 1994).

Neurotrophins are involved in processes of neuronal plasticity that occur in response to changes in perceptual stimuli (Thoenen, 1995). It is possible that processes which interfere with neurotrophin function will interfere with neuronal plasticity. This may be very relevant to stress during depressive episodes as it has been demonstrated that elevated stress may decrease hippocampal BDNF levels in animals (Duman *et al.*, 1997).

Neurotrophins may mediate some therapeutic effects of antidepressants. Long-term antidepressant (serotonin and norepinephrine reuptake inhibitors, monoamine oxidase inhibitors) administration to animals increases the expression of BDNF and trkB (Duman *et al.*, 1997). This finding may be relevant to clinical treatment of depression: a recent post-mortem study found elevated BDNF levels in the cerebellum in antidepressant-treated patients with major depressive disorder (Bayer *et al.*, 2000).

CONCLUSIONS

The relationship between peptides and mood disorders is a new frontier that bears further investigation. Studies of peptides in mood illnesses in humans have mostly sampled unipolar depression, and the relevance of these investigations to bipolar disorder is not known. The relationship between most peptides and mood disorders has not been studied.

Some peptides, such as CRF, may play a role in behavioural symptoms of depression, whereas peptides such as cortisol and NGF may contribute to pathological changes observed. Intervening with treatments to reverse abnormalities of these neuropeptides should result in new therapeutic opportunities for mood disorders.

Other peptides (such as substance P) may have no role in the pathophysiology of the illness, yet their antagonists could influence either symptoms or pathology and have important therapeutic properties. Most current antidepressant medications share considerable commonality because they bind to monoamine receptors or influence monoamines. For treatment-refractory patients, new classes of antidepressants could be extremely important.

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XVIII-5

Neuroendocrinology of Mood Disorders

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INTRODUCTION

The major mood disorders are associated with specific, highly reproducible neuroendocrine alterations, and conversely, certain endocrine disorders (e.g. hypothyroidism and Cushing's disease) are associated with higher than expected rates of mood disturbances. Neuroendocrine abnormalities have been thought to provide a 'window on the brain', revealing clues regarding the pathophysiology of central nervous system dysfunction. This neuroendocrine strategy is based on extensive research which indicates that the secretion of peripheral endocrine hormones is largely controlled by the respective pituitary trophic hormone. The pituitary hormones are in turn regulated by hypothalamic release and release-inhibiting hormones. Mood disorders are associated with multiple alterations, specifically of the hypothalamic–pituitary–adrenal (HPA), hypothalamic–pituitary–thyroid (HPT) and growth hormone axes. In this chapter, we review the research on the alterations of these axes and also discuss the roles of melatonin, dehydroepiandrosterone, parathyroid hormone and gonadal hormones in the pathophysiology of mood disorders.

HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

Introduction

Hypercortisolism has been prevailingly linked to the hormonal stress response found in patients experiencing affective disorders, especially in depression. Stressful life events may trigger a psychiatric condition in susceptible individuals, perhaps indicating a response of the HPA axis. Current research, however, is now aimed at understanding the mechanism by which the HPA axis may be causally linked to the pathophysiology of mood disorders. A growing body of preclinical and clinical research continues to provide information on the close ties between abnormalities in the HPA axis and various regions of the brain, with their subsequent influence to alter function of crucial neurotransmitters (and their receptors), relevant to the development of affective disorders.

HPA Axis Function

Corticosteroids and Mood Effects

Cortisol and the HPA axis play a crucial role in the stress response system, acting as an important interface between the central nervous system and the peripheral endocrine response system (Holsboer, 2001). Each hormone in the HPA axis cascade, as well as the other neuropeptides, possesses important effects not only on behaviour and emotions, but have significant roles in modulating the immune, endocrine and autonomic nervous systems. Cortisol enhances the availability of glucose, the main nutrient for all cells, including those found in the brain. Hypercortisolism, as a reflection of a hyperactive HPA axis state, now is postulated to be causally linked to the pathophysiology of depression.

Patients with depression and documented hypercortisolism often present with vegetative changes, such as sleep disturbance and energy reduction, decreased attention and cognition, psychosis, suicidality, anxiety, psychomotor disturbances or decreased libido (Wolkowitz, 1994). More recent literature hypothesizes that current antidepressants stimulate corticosteroid receptor expression, causing enhanced negative feedback on the HPA axis restoring appropriate corticotropin-releasing hormone (CRH) and cortisol levels. By reducing the levels of circulating cortisol, mood and vegetative symptoms may improve.

Historically, medically ill patients who were prescribed steroids such as prednisone, dexamethasone, cortisone and other synthetic corticosteroids for various medical conditions reportedly developed behavioural and emotional changes, such as mood lability, depression, hypomania, memory and attention changes, or psychosis. Those treated with exogenous corticosteroids seem to present with hypomania, while those with an underlying condition that produces increased endogenous steroids present with depression (Plihal *et al.*, 1996). Patients with Cushing's syndrome (non-adrenocorticotropic hormone [ACTH] related) and disease (increased ACTH) develop depression at similar incidence rates, even though the disease origins are different, implying that cortisol contributes to the development of psychiatric symptoms (Murphy, 1991).

Of note, patients with Addison's disease, an immune-related disorder leading to adrenocortical insufficiency, may also develop depression which responds to cortisol and other hormone replacement therapy (Leigh and Kramer, 1984). DeBattista *et al.* (2000) reported on 22 patients with non-psychotic depression who were randomly assigned to a one-day treatment with either ovine CRH, hydrocortisone or saline. Depression rating scales given immediately prior to treatment and the day following treatment demonstrated a rapid and robust reduction in depressive symptoms. Arana *et al.* (1995) and Dinan *et al.* (1997) described brief open-label trials using high-dose dexamethasone for the treatment of depression, reporting significant symptom improvement. On the otherhand, Wolkowitz *et al.* (1996) found the contrary, albeit in a very small sample size of five patients. Wolkowitz and Reus, (1999) postulated that both anti-glucocorticoid treatment and brief high-dose steroid treatment may have similar effects on the HPA axis, such as lowering of cortisol levels through upregulation of brain corticosteroid receptors; altering levels of the other adrenal steroid hormones; and increase in ACTH levels (after dexamethasone's initial inhibitory effect on ACTH production is worn off).

The HPA Axis, Serotonin, and Hippocampal Pathophysiology

It appears that corticosteroids influence brain function through the modulation of gene expression, and direct cell membrane effects

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(McEwen *et al.*, 1979). The mechanisms by which hypercortisolism leads to affective disorders, such as depression, may be explained by effects of increased CRH, enhanced secretion of ACTH from the pituitary, increased adrenal steroid output, and impaired response to negative feedback at the levels of the hypothalamus, pituitary, adrenals and hippocampus. The hippocampus is implicated in its role of regulating the HPA axis. This stems from findings of increased expression of mineralocorticoid (type I) and glucocorticoid (type II) receptors in the hippocampus (Jacobson and Sapolsky, 1991), along with evidence of increased HPA stress reactions after hippocampal damage, and hippocampal glucocorticoid receptor blocking (Feldman and Conforti, 1980; Sapolsky *et al.*, 1984). In fact, type I receptors are predominantly found in the hippocampus, while type II receptors are distributed in many regions of the brain, including the hippocampus (Joels and Dekloet, 1992; McEwen *et al.*, 1992).

The hippocampus is densely distributed with serotonin 1A receptors, and there is early evidence that interactions between corticosteroids and the 5-HT 1A receptor (Lopez *et al.*, 1998; Meijer *et al.*, 1998) may contribute to the pathophysiology of affective disorders. In fact, 5-HT 1A receptor density reduction and downregulation appear to be mediated by the mineralocorticoid receptors (Meijer *et al.*, 1997), while adrenalectomy appeared to reverse the decrease in hippocampal 5-HT 1A receptor levels (Lopez, 1998).

With the advent of more suitable positron emission tomography (PET) imaging tracers, decreased pre- and postsynaptic binding of 5-HT 1A receptors in depressed patients have been detected. Serotonin 2A receptors demonstrated equivocal results, perhaps due to the heterogeneity of depressive disorders and varying severity of presentations. Much data also indicate a decreased density in serotonin transporters in the brains of depressed patients (Ellis and Salmond, 1994). Post-mortem studies of suicide victims examining postsynaptic 5-HT 1A receptor density in the hippocampus and frontal cortex reported inconsistent results (Lowther *et al.*, 1997; Stockmeier *et al.*, 1997). The reports of increased 5-HT 1A receptor sites among individuals who have completed suicide may suggest that these receptors are more regulated by steroid levels in the hippocampus and, less so, in other parts of the brain. Also, tracers used in PET studies of patients (antagonistic action binding to receptors in high- and low-affinity states) differ from those used in post-mortem studies (agonist action with high-affinity binding).

MRI studies of depressed patients showed decreased hippocampal size, correlating with lifetime duration of illness and treatment resistance (Shah *et al.*, 1999; Sheline *et al.*, 1999). McEwen (2000) summarized the possible causes for hippocampal atrophy, and noted evidence of its reversibility after treatment. Reduction in the volume of Ammon's horn or dentate gyrus may occur secondary to reduced dendrite branching, impaired neurogenesis, decreased neuronal survival rate or permanent neuron loss. Atrophy of other areas of the brain, such as the prefrontal cortex (Drevets *et al.*, 1997) and amygdala (Sheline *et al.*, 1998), has been linked to depressive disorders. Glial cell loss appears to contribute to atrophy of the prefrontal cortex, amygdala and possibly hippocampus, which has also been associated with depression.

For further details, Fujita *et al.* (2000) completed an excellent review of the literature on the role of the hippocampus in depression pathophysiology.

The D-fenfluramine challenge test is used in studies as an indirect indicator of central serotonergic functioning, by measuring the prolactin (PRL) response to D-fenfluramine (serotonin releasing agent) exposure. O'Keane *et al.* (1992) reported that a fenfluramine-induced prolactin response was augmented after antidepressant treatment with medications including tricyclic agents and serotonin reuptake inhibitors, as have other groups (Charney *et al.*, 1984; Kasper *et al.*, 1990; Maes *et al.*, 1991; Price *et al.*, 1989). Shapira *et al.* (1993) found that an increased prolactin response to D-fenfluramine occurred after lithium treatment for depression, which persisted even after medication cessation.

On the other hand, in a double-blinded randomized control trial comparing patients before and after treatment with two SSRIs, fluoxetine and fluvoxamine, Kavoussi *et al.* (1999) observed a lack of enhancement of the PRL response after an 8-week period of treatment. This group postulated that study patients may have been less severely depressed, and free of Axis I and II comorbidities. Indeed, certain patients had normal PRL responses to D-fenfluramine even prior to the intervention. They explained that SSRI treatment may have prevented D-fenfluramine uptake into presynaptic neurons, thus lessening the release of 5-HT from these neurons, and thereby reducing the PRL response. Lastly, the question was raised that the action of certain SSRIs, such as fluvoxamine, may preferentially downregulate 5-HT 2 receptors (Spigset and Mjorndal, 1997). Recent literature has suggested that the PRL response to D-fenfluramine may be mediated by the 5-HT2 Rec (Coccaro *et al.*, 1996).

Mannel *et al.* (1997) found the fenfluramine test not to be a useful tool for the prediction of therapeutical outcome, upon measuring PRL and cortisol responses in euthymic patients (combination of unipolar, bipolar and schizoaffective diagnoses), treated on long-term lithium or carbamazepine prophylaxis. However, they observed a trend that blunted cortisol release in response to the FEN challenge seemed to occur in those responding to lithium or carbamazepine treatment, which may reflect better mood stabilization.

Dopamine and the HPA Axis

As described in the previous section, psychotic depression appears to be associated with hypercortisolaemia and activation of the dopamine system (Schatzberg and Rothschild, 1988; Schatzberg *et al.*, 1985) while suppression of glucocorticoid (GC) release reduces dopamine activity (Piazza *et al.*, 1996). In plasma homovanillic acid (HVA) (metabolite of dopamine) studies, there is evidence of increased central dopamine activity (Aberg-Widtedt *et al.*, 1985; Devanand *et al.*, 1985; Sweeney *et al.*, 1978), while others have found the absence of effect (Wolkowitz *et al.*, 1987). Plasma HVA is derived from peripheral noradrenergic neurons and limits interpretation of these results. Measures of CSF HVA levels may be more suggestive of presynaptic dopamine activity (Pitchot *et al.*, 1992), but psychomotor activity may interfere with CSF HVA measurements (van Praag *et al.*, 1975).

Other groups have administered dopamine agonists (APOmorphine), to observe prolactin and growth hormone responses, as an indirect indicator of dopamine receptor activity in depressed patients (Duval *et al.*, 2000) and depressed, suicidal patients (Pitchot *et al.*, 2001). Results are interpreted with difficulty due to their indirect indication of central neurotransmitter activity at the hypothalamic level.

Of interest, Cyr *et al.* (2001) reported their findings in transgenic mice bearing blocked GC receptor expression, compared to control mice. They were able to show increased amphetamine-induced locomotor activity, with increased concentrations of striatal dopamine and its metabolites, dihydroxyphenylacetic acid and HVA. Transgenic mice exhibited increased D1 and D2 receptor binding. Upon exposure to amitriptyline, increased dopamine-specific transporter binding occurred (restoring dopamine uptake into synaptosomes), and upon exposure to fluoxetine, reduced striatal D1 and D2 receptor levels were observed. These results seem to indicate that increased GC levels in these transgenic mice was associated with hyperactive dopamine activity, and corrected following antidepressant exposure.

Measures of HPA Axis Function

Dexamethasone-Suppression Test (DST)

The cortisol response to challenge with the exogenous corticosteroid dexamethasone is measured to determine the intact function of the HPA axis response, by suppressing cortisol release. Patients with psychiatric disorders, particularly major depression, often fail to suppress their cortisol production, or will escape from suppression abnormally early. According to the DST protocol published by Carroll *et al.* (1981), dexamethasone 1.0 mg was administered at 11 p.m., and blood samples for serum cortisol were taken the following day, at 8 a.m., 4 p.m. and 11 p.m. The greatest sensitivity was obtained when all three samples were collected (Rush *et al.*, 1996). For convenience, outpatients are usually tested in the afternoon, thus losing sensitivity of the test results. Carroll *et al.* (1981) defined the criterion for a normal response to the DST as a cortisol level of $5.0\,\mu g\,dl^{-1}$, using the modified Murphy competitive protein finding technique (Murphy, 1968). Rubin *et al.* (1987) suggested a cortisol level cut-off of $3.5\,\mu g\,dl^{-1}$, with the more specific radioimmunoassay (RIA) technique. Rush *et al.* (1996) reported that a limit of $4.0\,\mu g\,dl^{-1}$, would achieve a 96% specificity of the DST. Rothschild *et al.* (1982) described how cortisol levels with a threshold of $15\,\mu g\,dl^{-1}$ would attain a high specificity and high predictive value for psychotic depression, and may be more clinically useful in confirming this particular diagnosis.

Interestingly, depressed patients found to be DST cortisol suppressors have higher plasma dexamethasone concentrations than DST non-suppressors (Carson *et al.*, 1988), with dexamethasone plasma levels rising with treatment (Holsboer *et al.*, 1986b). This may indicate a hypercortisolaemic induction of liver enzymes, to augment dexamethasone metabolism (Holsboer *et al.*, 1986a).

Other Measures of HPA Activity

Salivary cortisol has proven to be an accurate and valid measure of free cortisol (Kirschbaum and Hellhammer, 1994), and is closely correlated with serum and plasma cortisol levels before and after the DST. Normal values may range from 1 to $25\,nmol\,l^{-1}$. Difficulties in interpreting salivary cortisol results arise from the use of different cut-off values in different laboratories, and measurements made at different times of the day.

The 24-hour urinary-free cortisol (UFC) sample appears to closely reflect circulating unbound plasma cortisol production, and is a helpful indicator of adrenal cortical activity (Carroll *et al.*, 1976). Total creatinine is also determined, as a measure of renal function. Normal UFC excretion is $40-50\,\mu g$ in 24 hours, versus $>90\,\mu g$ in 24 hours in the (non-psychotically) depressed.

Finally, age may be an important contributing factor to increased cortisol levels, determined in the DST (Halbreich *et al.*, 1984), although others have not found this to be a significant factor (Schweitzer *et al.*, 1991). In certain studies, the population of older adults who have documented cognitive decline exhibit higher levels of 24-hour UFC, with women more than men demonstrating memory impairments (as tested by verbal free recall and spatial recognition performance, implying encoding deficits) associated with increased UFC (Seeman *et al.*, 1997).

The DST and Mood Disorders

Schatzberg *et al.* (1983) reported that using a plasma cortisol level cut-off of $15\,\mu g\,dl^{-1}$, the frequency of DST non-suppression in the group with psychotic depression (PD) was greater than in those with non-PD (71.4% versus 58.1%). Rothschild *et al.* (1982) examined patients with schizophrenia and psychotic symptoms, to compare DST responses of PD patients. None of the schizophrenic patients had DST non-suppression, while half of the PD patients showed elevated post-dexamethasone cortisol levels, $>14\,\mu g\,dl^{-1}$. A meta-analysis of 14 studies, carried out by Nelson and Davis (1997), found the DST non-suppression rate at 64% in PD patients, versus 41% in non-PD patients. In addition, there was no difference in the rate of DST non-suppression among the endogenously depressed versus those with non-endogenous depression (39% versus 36%). Patients with psychotic depression exhibit the most replicable findings of DST non-suppression, in addition to elevated post-dexamethasone cortisol levels and high levels of 24-hour UFC. Schatzberg *et al.* (1985) and Schatzberg and Rothschild (1988) hypothesized that in certain depressed patients, the hypercortisolaemic state enhanced dopamine activity, leading to psychotic symptoms.

Cassidy *et al.* (1998) reported on the cortisol responses to DST in bipolar manic patients, and although the sample size was small, mixed manic patients seemed to exhibit greater degrees of non-suppression than pure manic patients do. With disease remission, cortisol suppression became restored. Cassidy's (1998) study also summarized the literature published on the effects of mania on DST non-suppression, reporting the frequency to be from 0% to 70%. Drawing conclusions here may be difficult, due to numerous methodologic differences, such as the dexamethasone dose chosen and time of blood draw.

As for dysthymic disorder, the rate of DST non-suppression falls between that observed in patients with major depression and healthy controls. Howland and Thase (1991) reported on a meta-analysis of ten studies comparing DST results in patients with dysthymic disorder, major depression and other psychiatric diagnoses. They found that 14% of dysthymic individuals, 59% of major depressive patients and 6% of healthy controls demonstrated DST non-suppression, with no significant difference reported between the rate in dysthymics and that in controls. Interestingly, Ravindran *et al.* (1994) found the rate of non-suppression in the DST in dysthymic patients was 7%, and the non-suppressors proved to be more successful responders to antidepressant therapy (with fluoxetine).

DST and Outcome of Mood Disorders

Deshauer *et al.* (1999) observed the pattern of DST cortisol response, in a four-year follow-up study, of 19 completely remitted, unipolar depressed and bipolar disorder patients on lithium prophylaxis. Those with initial DST non-suppression demonstrated persisting HPA dysregulation (more females than males), despite symptom remission. Ribeiro *et al.* (1993) reported on a meta-analysis of long-term outcomes of depressed patients, describing those with DST non-suppression at post-treatment evaluations to have poorer outcomes.

Rothschild *et al.* (1993) found significant positive correlation between higher cortisol activity at one-year post-treatment, and impaired social and occupational functioning as measured by the Social Adjustment Scale — Self Report (SAS-SR) (Weissman *et al.*, 1978). Likely, impaired functioning was linked to cognitive deficits associated with the hypercortisolaemia. Belanoff *et al.* (2001) provide an excellent review on the effects of cortisol on cognition. Chronic hypercortisolaemia has been linked to neurotoxic effects, especially in the hippocampus, which possesses numerous glucocorticoid receptors (McEwen *et al.*, 1991). Preclinical evidence from rat and primate studies suggested evidence of hippocampal neuron loss (Sapolsky *et al.*, 1990). Cushing's syndrome patients, who have an endogenous overproduction of cortisol, demonstrated depressive symptoms, impaired cognition and decreased hippocampal volume (Starkman *et al.*, 1992). Of interest, after treatment of patients with Cushing's disease (microsurgical resection of pituitary adenomas), the hippocampal volume was increased by up to 10% (Starkman *et al.*, 1999). Further discussion on the HPA axis and hippocampal function will follow below.

DEX/CRH Test

Heuser *et al.* (1994) summarized their experience with the dexamethasone (DEX) suppression/CRH challenge test, to assess HPA axis abnormalities, by first pretreating patients with DEX 1.5 mg orally at 23 : 00 h the previous night. This is followed the next day with administering human CRH (hCRH) 100 μg intravenously, with blood samples drawn for serum cortisol and ACTH every 15 minutes from 14 : 00 to 18 : 00 h. This group reported that from a sample of 140 psychiatric patients with various diagnoses, including major depression, mania, panic disorder and schizophrenia, cortisol and ACTH release were significantly greater than healthy controls. The sensitivity of this test was 80% for major depression, exceeding that of the standard DST, which reportedly has sensitivity of 44% from a meta-analysis of available data (Arana and Forbes, 1991), depending on age, depression severity and DEX dose. Normal controls demonstrated suppression of ACTH and cortisol release.

With this test, DEX suppresses the HPA axis at the pituitary level, but does not suppress the release of CRH or vasopressin from the hypothalamus. Depressed patients may have altered glucocorticoid feedback regulation, such that pre-administered DEX was paradoxically unable to suppress ACTH and cortisol release after the hCRH bolus. In animals exposed to chronic stress conditions, vasopressinergic regulation of the pituitary–adrenal system takes over (de Goeij *et al.*, 1992). Likewise, psychiatric illness may lead to an increased hypothalamic CRH and vasopressin production, resulting in augmented ACTH and corticosteroid release, and a transient CS receptor desensitization.

Thus in a DEX-pretreated depressed patient, ACTH release in response to administered CRH would be greater than in a control patient, because of synergistic action between the CRH given and the increased available vasopressin (von Bardeleben *et al.*, 1985). Limitations include the cumbersome nature of the test, which requires intravenous administration of both DEX and human CRH, and frequent blood monitoring thereafter; and although the test is fairly sensitive for depression, the specificity remains low.

Rybakowski and Twardowska (1999) used this test to compare 16 bipolar and 24 unipolar depressed patients, with 20 healthy controls, finding that HPA dysregulation was more pronounced in patients with bipolar depression, as shown by significantly higher cortisol levels during the acute episode and in remission. Holsboer *et al.* (1995) reported that healthy probands at high risk for psychiatric disorders developed an increase in plasma cortisol response to the DEX/hCRH challenge test, at levels between depressed patients and the controls without family history.

The results of the DEX/CRH test, administered to inpatients with depression at the time of treatment initiation and at time of discharge, seemed to suggest that those individuals with increased cortisol response at discharge time exhibited a higher risk of relapse in the next six months, versus those with low cortisol responses (more appropriate response) (Zobel *et al.*, 1999), although this report did not report the average length of hospitalization for observed patients.

Of interest, Holsboer *et al.* (1995) and Krieg *et al.* (2001) (in the Munich vulnerability study) described their findings of cortisol response to the DEX/CRH challenge in apparently healthy relatives of patients being treated for acute major depression. In the Krieg study, those relatives with increased cortisol response exhibited higher scores on psychometric scales measuring 'rigidity' and 'autonomic lability', and 32% of first-degree healthy relatives had depression-like features in other neuroendocrine and polysomnographic (EEG) measures. Their prospective study continues to monitor these relatives, in order to determine whether these measures are able to predict risk for developing affective disorders in the relatives of patients already being treated.

CRH

Corticotrophin-releasing hormone is a neuropeptide with numerous sites of action in the endocrine, autonomic and immune systems, modulating various behaviours, e.g. anxiety-related behaviour, food intake, reproduction, arousal and motor function. CRH is released from the paraventricular nucleus (PVN) of the hypothalamus into portal vessels, and triggers release of ACTH from the anterior pituitary. From preclinical evidence, it is postulated that CRH behaves as a neurotransmitter, which acts at different sites in the brain to exert neuroendocrine and behavioural effects. CRH activates the HPA axis to cause increased corticosteroid release. Increased cortisol levels feed back into a circuit via three possible routes: negative feedback at the level of the PVN neurons and anterior pituitary (Swanson and Simmons, 1989), positive feedback at the level of the central nucleus of the amygdala (CeA) and the dorsal PVN (Swanson and Simmons, 1989), and negative feedback through the ventral hippocampus to the bed nucleus of the stria terminalis (BNST) (Herman *et al.*, 1992).

CRH projections from the central nucleus of the amygdala, PVN and BNST terminate in the locus ceruleus (Van Bockstaele *et al.*, 1998), and may be responsible for enhanced arousal to stressors with an emotional component, as well as affect attention. CRH interacts with serotonin at the level of the raphe nuclei (Price *et al.*, 1998), to influence behaviours and emotions (via serotonin innervations in the forebrain). CRH may also affect dopamine neurons in the substantia nigra, thereby modulating dopaminergic activity in the striatum.

For more detail, Steckler and Holsboer (1999) provide an excellent overview of preclinical data pertaining to CRH receptor subtypes and their link to emotion and other affective conditions. The activation of CRH receptors allows CRH to exert its influence on behaviour. Importantly, two receptors have been found thus far, CRH1 and CRH2. In preclinical studies, CRH2-alpha is expressed more in subcortical regions of the brain (lateral septum, ventromedial hypothalamus, dorsal raphe nucleus), and may be more responsible for behaviours such as feeding, reproduction and defence. CRH2-beta is expressed in non-neuronal tissue, such as the choroid plexus, arterioles, cardiac and skeletal muscle (Steckler and Holsboer, 1999). CRH1 receptors are found in the pituitary, cerebellum and frontal cortex, and seem to modulate the cognitive processes, e.g. attention, executive function, emotional experience, and perhaps learning and memory.

Urocortin is a neuropeptide that is 45% homologous to CRH, which binds more potently to the CRH2-alpha receptor, than CRH (Donaldson *et al.*, 1996). Binding of the different CRH receptor subtypes likely activates different functions. However, urocortin does not appear to mediate ACTH release from the anterior pituitary at baseline, after induced stress and adrenalectomy, according to preclinical studies (Turnbull *et al.*, 1999).

Ongoing basic and clinical research studies are being undertaken to further understand the effects of glucocorticoids on CRH gene expression, and the implications of CRH on fear and anxiety. Much continues to be reported on the neurobiology of stress and anxiety, including HPA dysregulation secondary to stress, and the resulting predisposition to or development of abnormal autonomic, CNS, neuroendocrine and immune system responses. Important reports and reviews include Schulkin *et al.* (1998), McEwen (2000), Thrivikraman *et al.* (2000) and Liu *et al.* (1997).

CRH, ACTH and Cortisol Responses in Mood Disorders

Symptoms of stress activation and melancholic depression have common features such as anxiety and fear, constricted affect, stereotyped thinking, including guilt preoccupations, pessimism for the future, and sense of worthlessness. There may be evidence of physiological hyperarousal, autonomic and neuroendocrine activation,

along with inhibited appetite, libido, and growth and reproduction (Gold *et al.*, 1988). Early studies by Gold *et al.* (1984), Holsboer *et al.* (1984) and Nemeroff *et al.* (1984, 1988) implicated CRH in the hypercortisolaemia found in depression: Gold and colleagues, in their study of HPA responses to ovine CRH, to glucocorticoid antagonists such as mifepristone, arginine vasopressin and to continuous infusions of CRH; Nemeroff and colleagues, in reporting elevated cerebrospinal fluid (CSF) CRH levels in depressed patients, and later describing reduced CRH receptors in the cerebral cortex of post-mortem suicide victims; and Holsboer and colleagues, in replicating findings of pituitary inhibition by hypercortisolaemia.

Norepinephrine (NE) is a neurotransmitter with numerous functions, inhibiting feeding, grooming and sleep behaviours. It activates the amygdala to enhance the coding of emotionally connected memories. NE inhibits medial prefrontal cortex functions, such as shifting mood states, developing novel versus well-rehearsed behaviours, and inhibiting the HPA axis and brainstem autonomic activity (Arnsten *et al.*, 1999; Gold and Chrousos, 1999). NE appears to stimulate the HPA axis, under chronic stress situations. Sympathetic inputs to the adrenal system are activated, thus sustaining hypercortisolaemia (Gold and Chrousos, 1999). CRH may also play a role in the learning and storage of fear responses in the amygdala. Rodent and primate research have shown that central administration of CRH activates the HPA axis, leading to sympathetic nervous system activation (Britton *et al.*, 1982; Brown *et al.*, 1982; Sirinathsinghji *et al.*, 1983; Sutton *et al.*, 1982).

Wong *et al.* (2000) postulated that melancholic depression activated a stress response via the NE-locus ceruleus and CRH systems. A study of 10 melancholic, depressed, medication-free patients and 14 healthy controls found that depressed patients exhibited elevated CSF NE (even during sleep), indicating central noradrenergic activation in depression.

The observation of elevated plasma cortisol may have resulted from possible direct NE activation of hypothalamic CRH neurons (Calogero *et al.*, 1988), NE activation of the amygdala and, subsequently, the HPA axis (Goldstein *et al.*, 1996), NE inhibition of the medial prefrontal cortex, which inhibits the HPA axis (Arnsten *et al.*, 1999), and sympathetic modulation of adrenal secretion during stress (Arnsten *et al.*, 1999). In fact, increased cortisol has been documented to directly activate brainstem noradrenergic neurons via CRH (Wong *et al.*, 2000). On the other hand, this study reported that CSF CRH levels and plasma ACTH levels were within 'normal' range. This actually represents inappropriately elevated levels of CSF CRH and plasma ACTH, in the setting of hypercortisolaemia (Kling *et al.*, 1991), thus indicating a possible dysfunction in the glucocorticoid negative feedback on hypothalamic CRH neurons, or an override of the negative feedback by CSF NE excitation.

Earlier, Gold *et al.* (1995) linked similar depressive states, those of atypical depression, seasonal affective disorder and depression accompanying Cushing's disease. Such patients usually describe symptoms of lethargy, fatigue, hypersomnia, hyperphagia and increased mood reactivity. It was postulated that with chronic hypercortisolism, these patients developed a central, hypothalamic CRH deficiency, with low ACTH levels, and a blunted response to exogenous CRH.

Vieta *et al.* (1999) compared the ACTH and free cortisol response to hCRH in 42 lithium-treated bipolar manic and hypomanic patients in remission with 21 healthy controls, and reported high baseline and peak ACTH levels in the patients. At six months follow-up, using stepwise logistic regression, the measured ACTH area under the curve, after CRH stimulation, documented a significant prediction of manic relapse with a sensitivity of 56% and specificity of 92%. After a 12-month follow-up, again enhanced ACTH response was found and the ACTH AUC predicted mania with sensitivity 75% and specificity 91%. Gold *et al.* (1984) did not find significant difference between remitted bipolar and healthy control patients (and between manic euthymic bipolar patients); however, samples sizes were small.

Heim *et al.* (2001) reported on HPA axis responses to provocation challenge tests (CRH stimulation and ACTH stimulation tests) in survivors of childhood abuse, finding that women with a history of abuse but without major depression exhibited greater ACTH responses to ovine CRH. Depressed women with and without histories of abuse both exhibited blunted ACTH responses, likely indicating CRH receptor downregulation, secondary to intrinsic chronic CRH hypersecretion from the hypothalamus. In addition, the women with abuse histories and major depression exhibited more comorbid post-traumatic stress disorder (PTSD) and recent chronic mild stress than the same cohort, without major depression. Heim *et al.* (2000) also reported on autonomic responses and ACTH and cortisol levels, after psychological laboratory stress exposure, to similar cohorts of women with a history of childhood abuse. Here, women with abuse histories, regardless of coexisting depressed mood, exhibited increased ACTH but normal cortisol responses, compared with healthy controls and depressed women without childhood abuse.

CRH Antagonists in Treatment of Depression and Anxiety Disorders

Holsboer (1999) argued that a number of clinical conditions, including anxiety, depression and alcohol withdrawal, are accompanied by an exaggerated stress response, and that certain psychiatric conditions, such as psychotic depression, seem to exhibit enhanced dopamine neurotransmission secondary to elevated cortisol. The longer-term administration of the antidepressant trimipramine appeared efficacious in treating psychotic depression, via suppression of the HPA system (Holsboer-Trachsler *et al.*, 1994). Preclinical studies to decrease CRH1 receptor function by blockade, or suppression of synthesis through receptor gene deletion, have resulted in decreased anxiety in stressed animal models. Therefore, it would seem appropriate and promising to explore the development of modalities to block the central hyperdrive mechanism of the HPA axis, inferred to be responsible for the production of anxiety and depressive symptoms (such as suppressing CRH gene activation and release, and inducing CRH receptor blockade).

In rodents, CRH1 seems to modulate anxiety, possibly through its regulation of the HPA axis, as demonstrated by the anxiolytic effects of CRH1 antagonists (Arborelius *et al.*, 2000). This study reported that in rats, chronic administration of the CRH1 antagonist CP-154,156 decreased CRH production in the paraventricular nucleus of the hypothalamus, rather than alter the synthesis of CRH1 receptors, and did not cause adrenal insufficiency.

Zobel *et al.* (2000) described the effects of administering the CRH1 receptor antagonist R121919, to 20 patients for the treatment of major depression, over a period of 30 days. Patients were randomized to two groups; one group received the lower dose of 40 mg per day, and the second group was started on 40 mg per day and titrated up to 80 mg per day. At the end of the study, depression and anxiety scores improved significantly. At the same time, CRH1 receptor blockade did not seem to impair the release of ACTH and cortisol, as shown by results of CRH challenge tests given both before and after the study.

CRH1 antagonists appear to shorten the time of onset of antidepressants, exerting antidepressant effects, and may also be beneficial in treating insomnia, which frequently accompanies anxiety and mood disorders. There are concerns, however, that in the setting of a hyperactive HPA axis (as found in depression), the CRH receptors may be downregulated or desensitized, thus resulting in the blunted ACTH response to CRH (Holsboer, 1999). Upon exposing the depressed patient to CRH1 antagonists, which would block the already desensitized CRH receptors, cognitive

impairment may be induced or worsened, because of the interaction of CRH with the cholinergic systems, at the cortical level.

There are many other possible applications for CRH1 receptor antagonists, not only for treatment of depression, but also for the treatment of substance abuse (especially drug-seeking behaviour), anxiety disorders (including PTSD) and eating disorders. Further preclinical and clinical studies to clarify the function of these receptors and their subtypes, as well as the effect of various neuropeptides on receptor activity, are needed.

Anti-Glucocorticoid Agents

Cushing's patients, managed with either surgical intervention, or anti-glucocorticoid agents such as ketoconazole, metyrapone and aminoglutethimide, remit from depression up to a rate of 70% (Nieman *et al.*, 1985; Sonino *et al.*, 1993). Wolkowitz *et al.* (1999a) reported on their double-blind trial using ketoconazole versus placebo for the treatment of depression over a four-week period, and reported significant improvements in depressive symptoms, in those patients with an underlying hypercortisolaemic state. Ghadirian *et al.* (1995) reported the use of the three anti-glucocorticoid agents mentioned above in the treatment of depression, in an open-label, two-month trial. They described 60% of the patient sample with a greater than 50% reduction in depression symptomatology, and 50% with sustained remission at eight months follow-up. Most recently, the anti-progesterone and anti-glucocorticoid agent mifepristone, also known as RU-486, has been found to rapidly reverse symptoms in patients with primary psychotic depression (see Belanoff *et al.*, 2001 for review) and psychotic depression secondary to Cushing's syndrome (Van der Lely *et al.*, 1991).

The HPA Axis and PTSD

Quite recent literature has been published on the HPA responses in adult survivors of child abuse, thus adding important information to already present literature documenting HPA axis changes in male combat veterans and Holocaust survivors, with PTSD. As in depression, patients with PTSD hypersecrete CRH, as proven by elevated levels of CRH found in the cerebrospinal fluid (Baker *et al.*, 1999). On the other hand, there is evidence suggesting that PTSD patients possess an HPA axis with an exaggerated negative feedback mechanism.

For example, male combat veterans have decreased 24-hour UFC excretion (Mason *et al.*, 1986; Yehuda *et al.*, 1990) and lower plasma cortisol levels measured in the morning and throughout the day (Boscarino, 1996; Yehuda *et al.*, 1994). Likewise, Holocaust survivors demonstrated lower 24-hour UFC (Yehuda *et al.*, 1995).

Other studies report combat veterans with higher baseline plasma cortisol levels than controls, but no differences in baseline plasma ACTH levels, in the ACTH response to stress as well as in the cortisol response to stress (Liberzon *et al.*, 1999). Surprisingly, prepubertal children with PTSD demonstrate elevated 24-hour UFC, as compared to healthy controls and children with only a primary anxiety disorder. Combat veterans also exhibited greater plasma cortisol suppression to lower doses of dexamethasone (Yehuda *et al.*, 1995).

Heim *et al.* (2000) measured the HPA axis response (afternoon plasma ACTH and cortisol levels) and autonomic response (heart rate) to a standardized psychosocial laboratory stressor, consisting of an anticipation and preparation phase, followed by 10 minutes of public speaking and mental arithmetic task performance before an audience. Women with a history of childhood abuse exhibited increased pituitary–adrenal and autonomic responses, particularly those with concurrent major depression. This increased reactivity may occur from CRH hypersecretion, as a persisting consequence of past abuse, and may predispose such individuals to developing certain affective disorders. If this is the case, it may also suggest a choice of therapy, such as the use of CRH antagonists for treatment of depression in this patient population.

Heim *et al.* (2001) elaborated on the above study, by comparing a cohort of adult survivors of childhood abuse (with and without major depression) to healthy controls, by measuring plasma ACTH and cortisol responses to the ovine CRH challenge, and plasma cortisol responses to the ACTH challenge. They discovered that abused women without depression exhibited greater than usual ACTH responses to the exogenous CRH, contrasting with both non-abused and abused women with depression, who demonstrated blunted ACTH responses to CRH. In the ACTH stimulation test, abused women without depression had lower baseline and stimulated plasma concentrations, as compared to healthy controls, and to non-abused and abused women with depression. Newport and Nemeroff (2000) provide an excellent review of the neurobiology of PTSD.

Combining current clinical and preclinical data from rat (Plotsky *et al.*, 1993), and primate studies (Coplan *et al.*, 1996), early life stress may cause alterations at the adrenal level leading to an underlying hypocortisolaemic state, as found in patients with PTSD. Second, childhood trauma may also result in the development of a hyperreactive HPA axis towards stress in adulthood, with an underlying sensitization of the anterior pituitary to CRH. This may lead to increased CRH release in times of stress, perhaps contributing to pituitary downregulation and observed hypocortisolism. At the same time, abused women with depression may have developed an override of this pituitary downregulation, explaining their blunted ACTH response to the CRH challenge. Further preclinical and clinical studies are necessary to confirm and clarify the meaning of the above findings.

DEHYDROEPIANDROSTERONE AND DHEA(S)

Dehydroepiandrosterone (DHEA) and its sulphated metabolite, DHEA(S), are adrenocortical steroids, which serve as the precursor to testosterone and oestrogen. Circulating plasma and CSF levels peak in the mid-20s, and decline with age, reaching a nadir at age 65–70 years (Azuma *et al.*, 1993), when age-related illnesses increase substantially. DHEA(S) appears to decrease in settings of medical illness as well as with chronic stress (Spratt *et al.*, 1993), in contrast to cortisol, which tends to increase or remain unchanged. In fact the plasma ratio of DHEA(S) to cortisol falls with chronic stress and increasing age (Fava *et al.*, 1989; Goodyer *et al.*, 1998; McKenna *et al.*, 1997; Reus *et al.*, 1993; Wolkowitz *et al.*, 1992).

Hypotheses for mechanism of action of DHEA, from preclinical studies, include: transformation of this compound to testosterone or oestrogen (which both have mood enhancing effects), the effect of DHEA on increasing the bioavailability of gonadal steroids (Barrett-Connor *et al.*, 1999; Morrison, 1997; Morrison *et al.*, 1998; Yaffe *et al.*, 1998a), antagonistic effects on cortisol production (Browne *et al.*, 1992; Osran *et al.*, 1993), GABA antagonistic effects (Friess *et al.*, 1995; Majewska, 1992; Spivak, 1994; Steffensen, 1995; Yoo *et al.*, 1996), GABA inverse agonist effect of DHEA(S) (Friess *et al.*, 1995; Majewska, 1992), which may then cause a secondary increase in other GABA agonist neurosteroids, potentiation of NMDA neurotransmission (Bergeron *et al.*, 1996) and of NMDA-induced hippocampal release of norepinephrine (Majewska, 1995; Monnet *et al.*, 1995) and lastly, increased serotonin and dopamine brain levels (Abadie *et al.*, 1993; Murray and Gillies, 1997; Porter and Svec, 1995). DHEA(S) has been reported to increase hippocampal cholinergic function (Rhodes *et al.*, 1996, 1997), and enhance neuronal excitation (Carette and Poulain, 1984; Diamond *et al.*, 1995; Meyer and Gruol, 1994; Spivak, 1994).

In human studies, depression has been linked to lowered levels of DHEA(S) (Legrain *et al.*, 1995; Yaffe *et al.*, 1998a), with

lower morning DHEA : cortisol ratios being reported in depressed patients (Osran *et al.*, 1993). Goodyer *et al.* (1996) found DHEA hyposecretion in morning measurements, with cortisol hypersecretion in evenings to be significantly correlated with the diagnosis and severity of depressive episodes in children and adolescents. Among post-partum women, Buckwalter *et al.* (1999), found higher DHEA levels were associated with less depression, interpersonal sensitivity and better short-term memory function; it was hypothesized that DHEA might play a role in regulating mood and cognition in pregnancy, although this remains to be tested.

More recently, Michael *et al.* (2000) reported on decreased levels of salivary DHEA in adults with major depression, with lower morning DHEA correlating with improved Hamilton Depression Scale (HAM-D) scores. This is suggestive that DHEA(S) changes reflect actual mood changes, rather than a simple medication-related effect. In this study, they recruited individuals with current major depression (mostly on antidepressants), remitted depression and still taking antidepressants, and a normal control group. Interestingly, the remitted patients demonstrated DHEA levels between the group with depression and the control group. In the data analysis, when depressed patients on treatment were separated from those patients not taking medications, there was no significant effect on DHEA levels; however, numbers were not large. Further studies on the possible interrelated effects of DHEA and cortisol are implicated, especially in light of preclinical literature reporting the actions of DHEA to antagonize damaging effects of steroids on hippocampal neurons (Kimonides *et al.*, 1999), the antidepressant effects of DHEA (Wolkowitz *et al.*, 1999b) and the ability to predict the course of adolescent depression from cortisol/DHEA measurements (Goodyer *et al.*, 1998).

Low plasma DHEA(S) has been reported with anxiety (Diamond *et al.*, 1989), perceived stress (Labbate *et al.*, 1995), cynicism and hostility (Fava *et al.*, 1987; Littman *et al.*, 1993). In contrast, higher DHEA(S) has been associated with more 'expansive personality ratings' (Hermida *et al.*, 1985) and healthier psychological profiles (Fava *et al.*, 1992). Other literature describe contrary findings, with depressed patients exhibiting increased urinary or serum DHEA(S) levels (Heuser *et al.*, 1998; Tollefson *et al.*, 1990) or unchanged levels (Fava *et al.*, 1989; Reus *et al.*, 1993; Shulman *et al.*, 1992).

Increased DHEA to cortisol ratios have also been found in panic disorder patients (Fava *et al.*, 1989). Herbert *et al.* (1996) reported that adolescents with depression and comorbid panic or phobic disorder did not exhibit low DHEA levels in morning samples.

Studies have explored the link between higher DHEA(S) levels and improved cognition and general functioning, especially among the elderly (Berkman *et al.*, 1993; Berr *et al.*, 1996; Cawood and Bancroft, 1996; Flood and Roberts, 1988; Kalmijn *et al.*, 1998; Ravaglia *et al.*, 1996, 1997; Reus *et al.*, 1993). Other studies report no effect or contrary findings, with the absence of association between DHEA(S) and cognition in women (Yaffe *et al.*, 1998b) or inverse association between DHEA(S) and declining cognition in female versus male nursing home patients (Morrison *et al.*, 1998, 2000) and female versus male Alzheimer's disease patients (Miller *et al.*, 1998).

Open-label studies showed that treatment with DHEA resulted in mood improvement, and increased interest, energy and activity levels, in healthy subjects (Morales *et al.*, 1994) and depressed patients (Wolkowitz *et al.*, 1997). Wolkowitz *et al.* (1999b) performed a double-blinded trial with 22 depressed patients, who were either already on antidepressants (with persisting depressive symptoms) or were medication-free. Patients were randomized to DHEA 90 mg per day or placebo for six weeks. Greater than 50% decreases in Hamilton Depression Rating Scale, with documented statistical significance, were reported in 5 of 11 patients treated with DHEA, compared to none of the 11 patients treated with placebo. This is a preliminary study, and larger scale trials may provide more useful information. For dysthymic patients, Bloch *et al.* (1999) treated non-medicated patients in a 12-week double-blinded placebo-controlled study, with DHEA (90 mg daily for three weeks, then increased to 450 mg daily for another three weeks), or placebo for six weeks and was able to show a robust antidepressant response with DHEA.

GROWTH HORMONE, PROLACTIN, AND OTHER ANTERIOR PITUITARY ABNORMALITIES

Alterations in the production of other anterior pituitary hormones, including growth hormone and prolactin, are associated with mood alterations. This would include conditions of over- and underproduction of such hormones.

Growth Hormone

The pulsatile release of growth hormone (GH) is regulated by the stimulatory effect of GH releasing hormone (GHRH), and the inhibitory effect of somatostatin (which itself is regulated by insulin-like growth factor-I) (Scanlon *et al.*, 1996). The secretion of GH can be increased in response to falls in blood glucose level, exercise, sleep, stress and alpha-adrenergic agonists; its release may be inhibited by beta-adrenergic agonists.

Overproduction of GH frequently occurs secondary to pituitary adenoma, rarely, hyperplasia, and results in acromegaly in adults and gigantism in children. Physical manifestations include skeletal overgrowth, especially of the hands, feet, skull and lower jaw, symptoms of headache, decreased libido, kyphosis and joint pains, and the development of hypertension, hypogonadism and diabetes mellitus (Lishman, 1998). Patients may present with psychiatric disorders, such as depression (Fava *et al.*, 1993; Ferrier, 1987). However, Abed *et al.* (1987) failed to confirm an increased rate of depression when compared with the general population.

GH deficiency causes growth failure and short stature in children, with psychiatric sequelae, including distorted body image and low self-esteem. In adults, decreased sense of well-being (Holmes and Shalet, 1995), anhedonia, fatigue and social isolation may improve from hormone replacement with recombinant GH (Degerblad *et al.*, 1990).

Neuroendocrine researchers use GH as an indirect indicator of neurotransmitter function (Porter *et al.*, 1998). Studies of children, adolescents and adults with major depression found altered responses of GH to clonidine (alpha-2 agonist)(Dolan and Calloway, 1986) and L-tryptophan (serotonin precursor) (Price *et al.*, 1991), possibly suggesting reductions in alpha-2 adrenergic and serotonin receptor functions.

Birmaher *et al.* (2000) reported a decreased GH response to GHRH in acutely depressed and recovered children and adolescents, as compared to healthy controls. Dahl *et al.* (2000) also reported low GH response to GHRH in acutely depressed children, as well as those in remission. Coplan *et al.* (2000) reported on ten-year follow-up data of now young adults, with and without major depression; those adults with lifetime depression exhibited lower levels of GH in the 100 minutes before sleep onset, in adolescence; those with a history of suicide attempts during adolescence had greater 24-hour GH release. Chronically elevated levels of corticosteroids may inhibit neurotransmitter actions, in addition to directly affecting pituitary secretion of GH, as reported by Watson *et al.* (2000).

Prolactin

Prolactin, which is secreted by the anterior pituitary gland, is under tonic inhibition from dopamine released by the hypothalamus (Lishman, 1998). Serotonin, acetylcholine, thyrotrophin-releasing hormone (TRH), oestrogen, endogenous opiates, stressors and nipple

stimulation all promote prolactin release (Rafuls *et al.*, 1987). Symptoms of hyperprolactinaemia commonly arise from prolactin-secreting adenomas, and may result in amenorrhea, galactorrhea, infertility and impotence (more in men). In addition, psychiatric manifestations, such as depression, anxiety and irritability, may occur. Mood changes appear to respond better to treatment with dopamine agonists (such as bromocriptine) to reduce prolactin levels, rather than antidepressants, which seem to be less effective (Fava, 1993). Diagnosis is made from serum screening for prolactin elevation, and confirmed with imaging studies. Transsphenoidal resection or radiotherapy to remove or shrink the adenoma are other possible treatment options.

Prolactin responses to dopamine agonists are used to indirectly measure serotonin activity, as was discussed earlier in the chapter.

HYPOPITUITARISM

This is a state which can be caused by pituitary tumours (craniopharyngioma in children), post-partum ischaemic necrosis of the anterior pituitary gland (Sheehan's syndrome) or, very rarely, secondary to a basal skull fracture (Lishman, 1998). Symptoms often exist for an extended period of time prior to diagnosis, and can include weakness, fatigue, cold sensitivity, decreased libido, amenorrhea, impotence and weight loss. Facial expressions are diminished, and the development of depression, irritability, apathy, somnolence, memory impairment and metabolic changes can occur before the onset of delirium. This presents secondary to concurrent infection, and manifests with hypoglycaemia, hypotension and hypocortisolaemia. Differential diagnosis should include hypothyroidism, Addison's disease (hyperpigmentation would be prominent), delirium, dementia and anorexia nervosa (AN) (apathy and somnolence are usually not found in cases of AN). With hormone replacement therapy, including cortisol or prednisolone, thyroxine and gonadal hormones, symptoms typically resolve.

PARATHYROID HORMONE (PTH)

The parathyroid glands are situated on the thyroid but function discretely from the thyroid axis. The synthesis and release of PTH is modulated by serum calcium levels, in which elevated calcium inhibits PTH release, while low serum calcium causes increased PTH synthesis and release. Increased serum phosphate causes an increase in PTH release (by decreasing serum calcium levels, such as in cases of renal failure). The commonest cause of hyperparathyroidism is the benign adenoma of a parathyroid gland; it is rarely associated with multiple endocrine neoplasia (MEN type I — associated with pancreatic and pituitary tumours, or MEN type II — associated with thyroid cancer and pheochromocytoma) (Lishman, 1998). Mood symptoms are a frequent presentation, with reports of up to two-thirds of patients experiencing psychiatric changes (Petersen, 1968). These can include amotivation, depression, lethargy, memory impairment, irritability and explosive outbursts (Petersen, 1968). Patients may complain of anorexia, nausea, headache, thirst and polyuria. Physical symptoms of renal calculi, bony deformities and fracture, myopathy of proximal muscles, hypotonia and corneal calcifications may develop (Lishman, 1998). Laboratory abnormalities associated with hyperparathyroidism include elevated serum calcium levels and low serum phosphate, (serum albumin values will need to be considered). Radioimmunoassay of parathyroid hormone levels will confirm diagnosis. Alkaline phosphatase is raised secondary to bony involvement; renal calculi, and calcifications in the caudate and frontal lobes may be identified on imaging. Occasionally, EEG will exhibit widespread slow activity, along with paroxysms of frontal delta waves (Lishman, 1998). Surgical resection of the adenoma improves most symptoms, but depression and other psychiatric manifestations may require treatment with psychotropic medications for complete symptom resolution.

Hypoparathyroidism can occur following neck surgery, and after thyroidectomy (occurring in up to 50% of these patients). Symptoms frequently arise from chronic tetany; signs and symptoms may include perioral, hand and feet numbness and tingling, muscle cramps, limb stiffness, carpopedal spasms, and even laryngeal stridor. Presenile cataracts, seizures, macrocytic anaemia, and prolonged QT interval are other possible manifestations (Brown, 1984). Psychiatric changes occur in up to 50% of these cases, as reported by Denko and Kaelbling (1962), and may present with delirium, difficulty concentrating, emotional lability and cognitive impairment. Other symptoms include depression, anxiety, irritability and social isolation. Psychosis, obsessions and derealization rarely occur (Denko and Kaelbling, 1962; Rafuls *et al.*, 1987). Calcium and vitamin D replacement successfully treat the above symptoms.

MELATONIN AND AFFECTIVE DISORDERS

Melatonin is an indolamine derivative of serotonin, produced in the pineal body, and secreted into the bloodstream and cerebrospinal fluid. Melatonin receptors are found in the hypothalamus, cerebellum and pineal body itself. Secretions usually peak at night and reach a nadir during the daytime. Melatonin appears to lower body temperature at night and increase sedation (Shafii and Shafii, 1990).

Nurnberger *et al.* (2000) attempted to replicate observations made in the past (Lewy *et al.*, 1980; Wetterberg *et al.*, 1981; Wetterbuerg *et al.*, 1979), that bipolar patients exhibit supersensitivity to the suppression of melatonin production by light, as this may be a marker for genetic vulnerability for affective disorders (Nurnberger *et al.*, 1988). Results from Nurnberger's more recent study observed no difference in light-induced melatonin suppression among bipolar, unipolar and healthy patients. Cortisol levels were similar in all three groups of patients. Lithium and propranolol did not produce any effect, despite past research documenting beta-adrenergic influence on melatonin production (Connolly and Lynch, 1983). Among bipolar I patients, they were able to document significantly lower melatonin levels on nights with light exposure, as well as a later peak time for melatonin on dark nights. On the other hand, both Lam *et al.* (1990) and Whalley *et al.* (1991) were not able to confirm that bipolar patients exhibited increased light sensitivity. Indeed, medication effects may account for these differences, and further study would be merited.

HYPOTHALAMIC–PITUITARY–THYROID AXIS AND MOOD DISORDERS

Thyroid dysfunction has been reported to affect up to 6% of the general population, with psychiatric patients exhibiting higher rates of abnormal thyroid metabolism, and women demonstrating a higher incidence than men (Whybrow, 1995). Joffe and Levitt (1993) have edited an excellent text on this topic, with more detailed overview on such related issues.

Historically, links between thyroid diseases and psychiatric disorders include Caleb Parry's (1825) report that 'nervous affectations' seemed to often occur with thyroid disorders. Gull (1873) reported observations of myxoedema resulting in psychosis. Later, the Clinical Society of London (1888) reported that 36% of myxoedematous patients developed 'delusions and hallucinations', and Asher (1949) coined the description 'myxoedema madness'. Robert Graves (1940) reported the case of a woman with 'symptoms ... suppose to be hysterical', who developed 'tachycardia, weakness, exophthalmos, and thyromegaly'.

Hypothyroidism

Hypothyroidism can be classified according to the level of dysfunction in the HPT axis, primary hypothyroidism referring to abnormal function of the thyroid gland (accountable for 95% of cases), secondary hypothyroidism as pituitary dysfunction, and tertiary hypothyroidism as hypothalamic dysfunction (Gharib and Abboud, 1987). Idiopathic, autoimmune, iatrogenic (psychotropic culprits, such as lithium and carbamazepine) and iodine deficiency causes explain most cases of hypothyroidism. Hashimoto's thyroiditis, an immune-related condition, is associated with elevated TSH and decreased thyroid hormone release, producing thyroid gland enlargement.

Primary hypothyroidism is subdivided into three grades. Grade I is characterized by increased levels of thyroid-stimulating hormone (TSH), decreased free thyroxine index (FTI) and an increased TSH response to thyrotrophin-releasing hormone (TRH), along with clinical findings such as weight gain, dry skin, hoarse voice, cold intolerance, fatigue, constipation, menstrual disturbances, psychomotor retardation and cognitive disturbances. Grade II hypothyroid patients have increased TSH, normal FTI, increased TSH response to TRH, but few clinical findings. Patients with Grade III hypothyroidism present with normal TSH, normal FTI, but an increased TSH response to TRH stimulation, with a clinically euthyroid presentation (Evered, 1973; Wenzel *et al.*, 1974). Traditionally, Grades II and III hypothyroidism are considered 'subclinical' forms, but ongoing reports of clinical psychiatric sequelae from these states, e.g. major depression, may imply the contrary (Haggerty and Prange, 1995; Joffe and Levitt, 1993).

The thyroid axis interacts closely with the sympathetic nervous system, with thyroid hormones potentiating central and peripheral sympathetic–adrenergic activity (Emlen *et al.*, 1972). Hypothyroidism is associated with a decrease in the number and activity of beta-adrenergic receptors, in particular. Thyroid hormones affect second-messenger systems, such as the phosphoinositol and adenyl cyclase pathways, which may influence alpha and beta adrenoreceptor activity. There appears to be a reduction in messenger RNA coding for the beta-adrenergic receptors responding to epinephrine and norepinephrine, as shown in rat studies (Gross *et al.*, 1980; Mason *et al.*, 1987; Sandrini *et al.*, 1991).

Lithium acts to inhibit the conversion of thyroxine (T4) to triiodothyronine (T3), and inhibit iodine uptake, thyroid hormone production and thyroid secretion by the thyroid gland (Burrow *et al.*, 1971; Carlson *et al.*, 1973). Patients treated with lithium may develop decreased total T4, decreased free T3, increased TSH, exaggerated TSH response to TRH challenge, anti-thyroid antibodies, and clinical evidence of hypothyroidism and/or goitre. Lithium appears to suppress thyroid function, albeit on a temporary basis. It is usually a reversible condition upon cessation of this medication (Joffe and Levitt, 1993). It would likely be prudent to screen the above laboratory tests prior to initiation of lithium, and intermittently throughout the course of treatment.

Carbamazepine has reportedly been shown to decrease thyroid function in patients treated for seizure disorders and affective illness (Ericsson *et al.*, 1985; Larkin *et al.*, 1989; Tanaka *et al.*, 1987). This occurs more often in responders than non-responders of the medication, is unrelated to the mean dose or blood level of the anti-epileptic (Roy-Byrne *et al.*, 1984), and may be a result of a reduced TSH response to TRH (Joffe *et al.*, 1984). On the other hand, valproic acid has not been demonstrated to affect thyroid function (Ericsson *et al.*, 1985; Larkin *et al.*, 1989).

Lastly, electroconvulsive therapy may cause a rise in serum TSH, during treatment, and may be correlated with seizure length (Aperia *et al.*, 1985a, b; Papakostas *et al.*, 1991). Phototherapy has not been shown to affect thyroid function tests (Bauer *et al.*, 1993; Joffe, 1991).

Hyperthyroidism

Hyperthyroidism is commonly caused by Graves' disease, an autoimmune process associated with increased circulating thyroid hormones, and with higher incidence in females than males (6 : 1 ratio). Toxic multinodular goitre, a non-malignant condition in which thyroid nodules produce excessive thyroid hormones, and overtreatment of hypothyroidism are other possible causes for hyperthyroidism. This results in physical, cognitive and affective symptoms, including increased motor activity (tremulousness, tremor, restlessness), tachycardia, weight loss, anorexia, heat intolerance, menstrual irregularities, exophthalmos, mood lability, distractibility and decreased concentration, impaired short-term memory, and, in extreme conditions, frank delusions and hallucinations (Kamlana and Holms, 1986; MacCrimmon *et al.*, 1979). A small minority of patients, especially the elderly, presents with contrasting symptoms of apathy, lethargy, depression and social isolation (Peake, 1981). Complaints of depressed mood and anxiety may occur (Wilson *et al.*, 1962).

Hyperthyroidism is diagnosed by decreased TSH (<0.2), elevated total T4 and elevated free T3. The TRH challenge test can be used to prove a hyperthyroid state, by administering 500 μg TRH intravenously, and measuring TSH levels at baseline, and every 15 minutes, for the first hour. Usually, women and men show peak values of $>6\,\mathrm{uU\,ml^{-1}}$, following the challenge test; men older than 40 years tend to exhibit a rise of $>2\,\mathrm{uU\,ml^{-1}}$. In the absence of pituitary disease, suppressed TSH response indicates thyrotoxicosis; if TSH response to the TRH challenge is within normal limits, a hyperthyroid condition is unlikely.

Joffe and Singer (1987) reported no change in thyroid function in depressed patients treated with phenelzine; however, monoamine oxidase inhibitors cause increased circulating catecholamines, and it may be suggested to avoid this class of medication in patients with a history of hyperthyroidism. Thyroid hormones activate the sympathetic nervous system, including the autonomic nervous system and central adrenergic inputs. These effects have long been recognized in the cardiovascular system (Harrison, 1964). Of note, hyperthyroidism may increase myocardial sensitivity to catecholamines, leading to cardiac toxicity in those patients with increased serum thyroid levels and elevated catecholamines (Larsen and Ingbar, 1992).

There have also been noted associations between hyperthyroidism and tricyclic antidepressant toxicity (Prange *et al.*, 1969). Hoeflich *et al.* (1992) were not able to observe adverse effects on thyroid function in depressed patients (both responders and non-responders) treated with either maprotiline or fluvoxamine for four weeks. Shelton *et al.* (1993) compared the thyroid function of depressed patients treated with desipramine or fluoxetine, reporting 26% of study patients exhibited baseline abnormalities in thyroid levels, and a small but statistically significant increase in total T4 in those treated with desipramine, and a decline in T3 in those who responded to fluoxetine but not desipramine. McCowen *et al.* (1997) found that sertraline may adversely affect thyroid function.

Mood Disorders and Thyroid Function

Depression

Although patients with unipolar major depression commonly present in euthyroid states, at ranges in both upper and lower limits of normal, various other abnormalities have also been reported. These may include elevated T4 levels, decreased serum T3, blunted TSH response to TRH challenge, and evidence of subclinical hypothyroidism (Joffe and Levitt, 1992; Sullivan *et al.*, 1997). According to Kirkegaard and Faber (1998), this normalized in responders to treatment with fluvoxamine after

only one month, suggesting interactions between the hypothalamic–pituitary–thyroid (HPT) axis and serotonin neurotransmission. Linnoila *et al.* (1979) and Kirkegaard and Faber (1981) reported increased serum levels of reverse T3 (the hormonally inactive form of T3) in acutely depressed individuals, with return to normal values upon recovery from depression, thus implying a state-dependent change.

Up to 15–20% of depressed patients exhibit subclinical hypothyroidism (Haggerty and Prange, 1995), with women patients more at risk for impaired thyroid function (Gold *et al.*, 1981). Prange and Loosen (1984) reported 25–33% of major depressive patients show blunted TSH response to TRH challenge. This could suggest a feedback mechanism involved in a chronically activated HPT axis (Banki, 1988), a response to a transient thyroxaemic condition (Loosen and Prange, 1982) or a response to hypercortisolaemia associated with depression (Re *et al.*, 1976).

Frye *et al.* (1999b) were not able to predict TRH hypersecretion and subsequent pituitary downregulation (with blunted TSH release) in patients with depression.

Of interest, there exist numerous reports of increased prevalence of anti-thyroid antibodies among depressed patients, 8–20% (Gold *et al.*, 1982; Haggerty *et al.*, 1991; Joffe, 1987; Nemeroff *et al.*, 1985) versus 5% in the general population (Tunbridge and Caldwell, 1991). The clinical significance remains unclear, since this is often accompanied by normal serum thyroid concentrations.

More recently, Joffe's group (Joffe, 1999; Joffe and Marriot, 2000) examined the relationship between basal thyroid hormone levels and the long-term course of depression and stage of treatment-resistant depression (Thase and Rush, 1995), reporting that time to recurrence of major depression was inversely related to T3 but not to T4 levels, supporting past findings that peripheral thyroid levels do not necessarily correlate with antidepressant treatment resistance (Vandoolaeghe *et al.*, 1997).

Also, the blunting of the TSH response to TRH challenge has been reported in acute and abstinent alcohol-dependent patients (Loosen, 1988; Loosen *et al.*, 1992), as well as in panic disorder patients (Fishman *et al.*, 1985; Stein and Uhde, 1988).

Bipolar Disorders

Whybrow and Prange (1981) hypothesized that mobilization of thyroid hormones in the CNS would augment recovery from depression, and, conversely, excessive mobilization would increase the risk of mania in certain individuals. In addition, thyroid hormones may have a central neuromodulatory role, through its actions on central beta-adrenergic receptors (Whybrow and Prange, 1981). However, measurements of peripherally measured hormone levels may not be an accurate reflection of central activity. Manic patients can develop a relative hyperthyroxinaemia with a slightly increased total or free T3/T4 (Bauer and Whybrow, 1988). Lee *et al.* (2000) reported on their study of 46 bipolar manic patients, followed for their thyroid indices, at baseline and one and six months after lithium treatment. Despite the absence of a placebo control group, they found that a reduction in free T4 levels and free T3 levels correlated with a drop in psychotic symptoms, as measured by the Brief Psychiatric Rating Scale (Overall and Gorham, 1962) scores, after one month of lithium treatment. In fact, a progressive reduction in TSH levels was documented throughout the six months of treatment. These observational results seem to support but not necessarily confirm Whybrow and Prange's theory.

Around 10–15% of patients with bipolar disorder are classified with the rapid-cycling subtype (experiencing four or more affective episodes yearly) (American Psychiatric Association, 1994), with women representing up to 80–95% of the patients categorized to this subtype. Various studies report both association and lack of association between hypothyroidism and rapid-cycling bipolar disorder (Cho *et al.*, 1979; Cowdry *et al.*, 1983; Joffe, 1988; Wehr, 1988). Bauer and Whybrow (1990) reported evidence of overt, mild and subclinical hypothyroidism in this sub-group. Gender (female sex) and length of treatment with lithium may be predisposing factors to developing thyroid abnormalities; Bartalena *et al.* (1990) found decreased total and free serum thyroid hormone levels in female bipolar patients, whether or not they were rapid-cyclers, and there was no difference between these two groups, in anti-thyroid antibody titres, presence of goitres and thyroid hormone indices.

Chang *et al.* (1998) reported an increase in mean TSH, and decreased T4 levels, in mixed manic patients, as compared to patients with pure mania, but no significant differences in T3 concentration or in previous exposure to lithium, agreeing with past reports that mixed mania may be more commonly associated with thyroid dysfunction. Frye *et al.* (1999a) found that lithium-treated bipolar patients, with lower free T4 concentrations, exhibited more affective episodes and greater severity of depression, but again causal links remain unclear.

Thyroid Treatment Modalities for Psychiatric Conditions

Major Depression

TRH is a neuropeptide produced by the hypothalamus, which regulates the thyroid axis by controlling the secretion of TSH and thus regulating thyroid hormone synthesis and release. Kastin *et al.* (1972) and Prange *et al.* (1972) reported antidepressant effects from TRH administered intravenously to patients with unipolar depression. However, numerous studies attempting to replicate these results demonstrated minimal or no benefit (Kiely *et al.*, 1976; Mountjoy *et al.*, 1974; Vogel *et al.*, 1977).

T3, a metabolite of T4 with a shorter half-life, appears to be the more biologically active form of thyroid hormone. It has been used as monotherapy treatment of depression, but there is little data supporting its efficacy (Feldmesser-Reiss, 1958; Flach *et al.*, 1958). Stern *et al.* (1991, 1993) performed double-blinded trials on ECT patients with unipolar, bipolar and schizoaffective depressions, who were pretreated with T3, and found improvements in mood and cognition. On the other hand, it is possible that cognitive improvements resulted from a reduction in the number of ECT treatments necessary for remission of the acute episode in those receiving T3 (Joffe and Sokolov, 1994; Sackeim, 1994).

T3 has been most studied as an adjuvant to antidepressant therapy. At doses of 25–50 μg per day, T3 was reported to accelerate the response to antidepressants (Prange *et al.*, 1969; Wheatley, 1972), and convert non-responders to responders (see Aronson *et al.*, 1996 for meta-analysis). Joffe *et al.* (1993) reported that T3 augmentation appeared equally efficacious as lithium, with both medications proving to be more useful than placebo. In fact, two-thirds of tricyclic antidepressant (TCA)-resistant patients responded successfully to T3 augmentation. Of interest, Joffe and Singer (1990) reported on a double-blind trial comparing T3 with T4 augmentation (in the absence of a placebo), and found that T3-treated patients responded at a higher rate than T4-treated individuals (53% versus 19%). Targum *et al.* (1984) reported on the use of T4 augmentation for TCA-resistant depressed patients, but also diagnosed a number of the study patients to have baseline subclinical hypothyroidism.

Case reports on the association of T3 augmentation with the development of angina exacerbation and paroxysmal atrial fibrillation in elderly patients (Cole *et al.*, 1993; Gitlin, 1986) warrant that caution be used when prescribing thyroid hormones.

Bipolar Disorders

A higher prevalence of hypothyroidism has been found in both the rapid- and non-rapid-cycling patients (Bartalena *et al.*, 1990; Joffe

et al., 1988; Wehr *et al.*, 1988), as discussed above. In a small sample sized study, Baumgartner *et al.* (1994) treated refractory bipolar patients (non-rapid-cycling) with T4, given 250–500 μg daily, and found significant response to T4, as measured by frequency of relapse and length of hospitalization. However, a significant portion of the study patients was described to also have subclinical hypothyroidism.

The use of T4 at doses up to 500 μg per day has been studied in the treatment of rapid-cycling bipolar disorder, with variable responses, from complete remission to minimal improvement (Bauer and Whybrow, 1990; Leibow, 1983; Stancer and Persad, 1982). Such therapy has been reportedly well tolerated, but there remain concerns about iatrogenically induced hyperthyroid states, and the accompanying risk, albeit theoretical, e.g. osteoporosis. Whybrow (1994) followed up patients from the 1990 study with serial bone densitometries, and discovered a net increase in bone density. However, these patients were also treated with lithium, and it is plausible that lithium may be protective against osteoporosis (Whybrow, 1994). Replication studies using larger numbers of patients, preferably with blinded and randomized controlled trials, are necessary.

GONADAL HORMONES AND MOOD DISORDERS

This topic is covered in greater detail in Chapter XVIII-12. However, this is an area that is being avidly studied in both preclinical and clinical settings. Important data continues to be published on the neurobiological mechanisms of action of these hormones — oestrogen (McEwen *et al.*, 1979), luteinizing hormone (Meller *et al.*, 2001), follicular-stimulating hormone and androgenic steroids (Weber *et al.*, 2000).

Progesterone along with its 5alpha-reduced metabolites, pregnanolone and allopregnanolone, has demonstrated anxiolytic, hypnotic and anaesthetic actions through GABA-A receptor modulation, thereby increasing chloride ion neuronal membrane permeability and reducing neuron excitability (Finn and Gee, 1994; Freeman *et al.*, 1993; Majewska, 1992; Majewska *et al.*, 1986; Wang *et al.*, 1996). Pregnenolone sulphate and dehydroepiandrosterone sulphate (DHEAS) (both 3beta-hydroxy-5-ene steroids) act on GABA-A receptors via *N*-methyl-D-aspartate (NMDA) receptor-mediated intracellular calcium influx across neuronal membranes (Bicikova *et al.*, 2000). Evidence is being gathered to support the roles of gonadal hormones in modulating dopaminergic (Woolley and McEwen, 1994), serotonergic (Biegon, 1990; Biegon *et al.*, 1983), noradrenergic (Biegon *et al.*, 1983) and other neurotransmitter systems.

Bloch *et al.* (2000) investigated the possible role of gonadal steroid level changes in post-partum depression (PPD). Hormonal changes in pregnancy and parturition were artificially simulated in healthy, euthymic, non-pregnant women, with and without history of PPD. Findings suggested a differential sensitivity to the mood-destabilizing effects of gonadal steroids in women with a history of PPD. A year earlier, Buckwalter *et al.* (1999) published their findings on 19 women who were followed during and after pregnancy. Steroid hormone levels were compared to patients' cognitive functioning and mood symptoms. During pregnancy, higher progesterone levels seemed to be associated with greater mood disturbances (particularly with obsessive-compulsive type symptoms), and higher DHEAS with better mood. Two months after delivery, higher testosterone levels was more associated with greater mood disturbances. While cognitive changes (particularly, verbal memory) were documented during pregnancy, they occurred independent of mood changes. Young *et al.* (2000) found that in comparing a sample of 25 depressed with 25 non-depressed women (all premenopausal, aged 20–49), the depressed women exhibited lower oestradiol levels, and a shorter half-life of the luteinizing hormone. Study limitations included a small sample size, and the fact that patients were enrolled at random phases of the menstrual cycle. Attempts were made to control for cycle variability by matching each depressed patient with a control patient, who was similar in age and in menstrual cycle day. This is one example of the intrinsic difficulty facing researchers investigating gonadal hormones and their links to various psychiatric and cognitive conditions.

Further literature has been published on the use of gonadal steroids in treating mood and anxiety disorders, focusing on their efficacy in treating post-partum affective disorders (Gregoire *et al.*, 1996; Sichel *et al.*, 1995), premenstrual syndrome and premenstrual dysphoric disorders (Magos *et al.*, 1986; Watson *et al.*, 1989), and perimenopausal and menopausal depressions (Best *et al.*, 1992; Ditkoff *et al.*, 1991; Limouzin-Lamothe *et al.*, 1994; Oppenheim, 1983; Schneider *et al.*, 1977; Wiesbader and Karzrok, 1938). Lastly, a body of data is accumulating on the effects of the gonadal steroids on mood and aggressive disorders in men (Pope *et al.*, 2000).

Soares *et al.* (2001) reported on their 12-week, double-blind, randomized, placebo-controlled trial of 17beta-oestradiol transdermal patch (100 μg) for the treatment of perimenopausal women with major depression, dysthymic disorder or minor depression for 50 women. Remission from depression was reported in 68% of treated women, versus 20% in the placebo-treated group. Results may be considered difficult to interpret, due to small sample size, broad diagnostic entry criteria (both minor and major depressive disorders) and vague exclusion criteria. The study did attempt to address safety concerns of the transdermal oestrogen patch, referring to published studies of the use of this patch in women with severe PMS (Smith *et al.*, 1995) and post-partum depression (Ahokas *et al.*, 1999). On the other hand, they did not refer to safety data on the use of the patch for perimenopausal women. A last possible concern would be the use of unopposed oestrogen therapy in patients with intact uteri. Albeit the study was limited to only 12 weeks, the risk of endometrial cancer increases by a factor of eight to ten in women who use unopposed oestrogen for at least ten years (Grady *et al.*, 1995).

Among the postmenopausal population in the United States, approximately 38% use hormone replacement therapy (Keating *et al.*, 1999). Manson and Martin (2001) provided an excellent review on this topic, addressing the conundrum of such widespread use of HRT, despite the lack of conclusive data regarding benefits and risks of such treatment, since much collected data were from observational studies. The safety issues of prescribing hormone replacement are closely following along, with recent literature seeking to answer concerns of increased risk for venous thrombosis in women on low-dose oral contraceptives (Vanderbroucke *et al.*, 2001), risk of endometrial cancer recurrence in HRT patients (Suriano *et al.*, 2001), safety of HRT in breast cancer survivors (O'Meara *et al.*, 2001) and possible risk for developing ovarian cancer in women already on HRT (Rodriguez *et al.*, 2001). All of these issues must be carefully considered in the face of potentially increased use of gonadal steroids in the treatment of psychiatric disorders. However, literature on the use of oestrogen and progesterone for treatment of perimenopausal and postmenopausal symptoms is now reporting that a lower dose of combination therapy is equally efficacious (Archer *et al.*, 2001; Utian *et al.*, 2001).

The cognitive benefits of oestrogen and oestrogenic-receptor modulators are also being actively studied. LeBlanc *et al.* (2001) performed a systematic review and meta-analysis on 29 studies and reported difficulty in combining studies on cognition, due to the heterogeneous nature of the study designs. They did perform a meta-analysis on the observational studies, finding that HRT may decrease the risk of dementia (summary odds ratio of 0.66), but reiterated concerns of methodological limitations in most studies. Mulnard *et al.* (2000), of the multi-centred Alzheimer's Disease

Cooperative Study, reported on their randomized, double-blinded, placebo-controlled trial conducted between 1995 and 1999, on 120 women with mild to moderate AD. It was found that oestrogen replacement given for a one-year period did not slow disease progression, nor did it improve global, cognitive or functional outcomes of patients in the study, and acknowledged the need for much more research. Yaffe *et al.* (2001) reported that use of the selective oestrogen-receptor modulator raloxifene (for treatment of postmenopausal women with osteoporosis), over a three-year period, did not improve cognitive scoring.

Gender Differences and Age-Related Changes in the HPA Axis

Seeman *et al.* (2001) tested the hypothesis that women exhibit greater age-related HPA axis reactivity to challenge (using a standardized 30 minute cognitive challenge). A group of 26 younger subjects (9 men and 17 women), in their 20s were compared to 14 older subjects (7 men and 7 women) in their 60s. Baseline salivary cortisol levels were measured and repeated during and following a battery of cognitive tests. These tests were administered in a fast-paced, time-limited fashion, and designed to increase both HPA and sympathetic nervous system activity. Results from previously published data showed that older age was associated with higher baseline cortisol (Deuschle *et al.*, 1997; van Cauter *et al.*, 1996); women exhibited shorter nocturnal nadirs, higher morning cortisol levels, and higher mean 24-hour readings (van Cauter *et al.*, 1996). Postmenopausal women showed greater HPA reactivity to CRH challenge (Heuser *et al.*, 1994) and less feedback inhibition (blunted ACTH decline) in response to cortisol infusion, at the level of the pituitary. In the younger age group, some studies reported that men tended to exhibit greater cortisol response (Kirshbaum *et al.*, 1992), while others found no significant gender difference (Gallucci *et al.*, 1993; Stoney *et al.*, 1987; Streeten *et al.*, 1984).

The question of the possible role gonadal hormones may play in shifting the HPA axis of older women to greater reactivity was raised, since higher oestrogen levels have been linked to decreased blood pressure (von Eiff, 1971) and decreased HPA reactivity (Bonen *et al.*, 1991; Marinari *et al.*, 1976). This may be counteracted in settings of higher progesterone levels (Kirshbaum *et al.*, 1999). On the other hand, hormone replacement therapy has been linked to blunted cortisol responses to cognitive and physical stress tests in postmenopausal women (Lindheim *et al.*, 1992, 1994). In addition to the lack of feedback inhibition to cortisol in older women, a greater increase in the ACTH response to CRH or vasopressin challenge has also been observed (Born *et al.*, 1995). Preclinical studies have suggested oestrogenic regulation of the hippocampus and hypothalamus, due to the presence of oestrogen receptors in these regions (Teyler and DiScenna, 1986), oestrogenic regulation of synaptogenesis in the CA1 region of the hippocampus (McEwen *et al.*, 1994) and induction of other electrical activity and membrane changes in the hippocampus and hypothalamus by oestrogen (Foy *et al.*, 1982; Minami *et al.*, 1990; Wong and Moss, 1991).

SUMMARY

Numerous factors may contribute to the pathophysiology of mood disorders, including the pathophysiology of the brain's adaptation to stress, (allostatic load) (McEwen and Stellar, 1993; Sterling and Eyer, 1988), the possible role of chronic stress on hippocampal damage and dysfunction (which may result in psychiatric symptoms) (McEwen, 2000), as well as the effects of genetic predisposition and developmental stresses (Liu *et al.*, 1997). It remains challenging and at times, controversial, to draw firm conclusions from observations of the endocrine abnormalities associated with mood disorders. The stress effect arising from these psychiatric conditions can precipitate significant and widespread metabolic/endocrine changes ('state' markers). Researchers are now attempting to differentiate this from causal factors ('trait' markers) for such endocrine changes, to determine which are associated with the pathophysiological mechanisms of mood disorders.

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XVIII-6

Psychophysiology of Mood Disorders

Silvana Galderisi

INTRODUCTION

Mood disorders are markedly heterogeneous with respect to signs, symptoms, course, response to therapeutic interventions and outcome. Attempts at reducing heterogeneity by subgrouping subjects on the basis of clinical aspects is a valuable strategy; however, the scarce impact on everyday practice of most clinical subtypes underscores its limitation. External validation of clinical subtypes (demonstrating that they are characterized by different psychophysiological patterns) has been an important goal of psychophysiological research in mood disorders. Several other conceptual strategies can be identified throughout the reviewed literature: the search for trait or state markers, the attempt at clarifying pathogenetic mechanisms of the observed dysfunctions, and the identification of early predictors of course, outcome or response to different therapeutic interventions.

On the whole, however, the field has suffered from the lack of unifying hypotheses and systematic research, which is reflected in the fact that relevant reviews, including the present one, are organized according to the different techniques applied in the investigations. Moreover, evaluation and comparison of different findings is often hampered by poor characterization of studied populations with respect to relevant or confounding variables such as symptoms, clinical subtypes, medication (or other therapeutic intervention) history, or substance abuse or dependence.

This chapter is organized in two main sections, peripheral and central measures, each including several subsections dealing with specific measures or techniques.

As previous excellent reviews of the topic are available (Goodwin and Jamison, 1990; Henriques and Davidson, 1989; Zahn, 1986), an attempt has been made to cover the relevant literature with particular attention to more recent findings. Furthermore, since some readers might be unfamiliar with specific technical issues, when deemed appropriate a brief definition and description of the psychophysiological measures or techniques discussed in the section is provided.

PERIPHERAL PSYCHOPHYSIOLOGICAL INDICES IN RESEARCH ON MOOD DISORDERS

Disturbances of several physiological functions, such as sleep, appetite, sexual drive, sweat secretion and chronobiological rhythms, are commonly found in mood disorders and indicate a dysregulation of the autonomous system in affected subjects. Despite the large number of studies focusing on autonomic measures, much remains to be done to characterize this dysregulation. Investigations based on the concomitant evaluation of several autonomic parameters are promising; their integration with central measures, as well as with neuroendocrine and neurochemical measures, may contribute greatly to progress in this field of research.

Electrodermal Activity

Electrodermal activity (EDA) has been largely investigated as a measure of tonic and phasic arousal. Different variables can be evaluated, including the skin conductance level (SCL; the tonic level, generally measured after a resting period, before the first stimulus presentation), the spontaneous skin conductance responses (SSCRs; nonspecific fluctuations of activity independent of external stimuli), the skin conductance orienting responses (SCORs; related to external stimuli) and habituation (reduction in response to repeated stimulation). For each of these indices the eventual asymmetry pattern can be assessed.

Low baseline EDA has been frequently found in depressed patients when compared with healthy subjects. Such a reduction has been observed for both SCL and SCORs (Dawson *et al.*, 1977, 1985; Iacono *et al.*, 1983; 1984; Schnur *et al.*, 1995; Storrie *et al.*, 1981; Ward *et al.*, 1983; Zahn, 1986). Negative or discrepant findings have also been reported: Toone *et al.* (1981) found no difference between unmedicated subjects with depression and healthy comparison subjects; Albus *et al.* (1982) found higher SCL in drug-free depressed patients as compared with controls; Levinson (1991) reported higher SCL and a trend toward more SSCRs in a group of depressed patients (including schizoaffective depressed subjects), and interpreted the finding as the result of a hyperarousal condition. Faster habituation of the SCOR has been demonstrated in depressive patients (Frith *et al.*, 1982; Thorell, 1987). However, findings have been inconsistent (Miquel *et al.*, 1999; Storrie *et al.*, 1981). Methodological issues might partly account for the observed discrepancies, while the confounding role of clinical heterogeneity in tested populations is not strongly supported by available findings. In fact, the reduction of EDA activity and reactivity in subjects with mood disorders appears rather independent of subjects' clinical characteristics. Low SCL has been found in both medicated and unmedicated depressed subjects (Iacono *et al.*, 1983; Storrie *et al.*, 1981; Thorell *et al.*, 1987; Ward and Doerr, 1986), in unipolar and bipolar patients (Williams *et al.*, 1985) and in acutely ill depressed and manic patients, as well as after clinical recovery associated with antidepressant treatment or electroconvulsive therapy (Dawson *et al.*, 1977; Storrie *et al.*, 1981), in both suppressors and non-suppressors to the dexamethasone suppression test (DST) (Williams *et al.*, 1985), and in subclinically depressed versus non-depressed college students (Gehricke and Shapiro, 2001). However, EDA reduction seems to be even more pronounced in endogenous patients and in patients with symptoms of inhibition (Dawson *et al.*, 1977; Lader and Wing, 1969; Thorell *et al.*, 1987; Williams *et al.*, 1985).

EDA stability across different clinical subtypes of affective disorders has prompted research on its potential diagnostic utility. Reduced SCL as a diagnostic index of depression was investigated by several authors (Dawson *et al.*, 1985; Iacono *et al.*, 1983; Ward

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et al., 1983), who reported a sensitivity ranging from 65% to 96%, a specificity from 46% to 89% and an efficiency from 64% to 88%. Dawson *et al.* (1985) also investigated SCOR as a diagnostic marker and reported a sensitivity of 85%, a specificity of 75% and an efficiency of 80%. In an attempt to improve discrimination among different diagnoses, some authors have used multi-component recording to measure the orienting response (OR). In particular, the finger pulse amplitude response (FPAR) has often been used in association with the SCOR, as it represents the OR of an autonomic system component which is physiologically and biochemically independent of the EDA system (Bernstein *et al.*, 1981; Fowles, 1986). The use of multi-component recording to measure OR might also foster understanding of the pathophysiological mechanisms of abnormal OR in psychiatric disorders. Empirical findings suggest that when OR is jointly indexed by both SCOR and FPAR, subjects with depression can be differentiated much better from subjects with schizophrenia (Bernstein *et al.*, 1988): in fact, while the latter exhibit reduced responding across all OR components, suggesting a central OR deficit related to attentional dysfunction, the former showed a high rate of non-responding only in the electrodermal component, interpreted by Bernstein *et al.* (1988) as related to a peripheral deficit involving the EDA system. More recently the authors confirmed previous findings (Bernstein *et al.*, 1995) and proposed that a reduction in cholinergically mediated activity, rather than a central deficit of OR, may be implicated in autonomic findings in depression. The hypothesis is based on the fact that, besides FPAR, other aspects of the OR component have been found unimpaired in depressives (e.g. pupillary dilatation response or P300), while other cholinergically mediated systems appear dysfunctional (e.g. salivation reduction, reduced blood flow response in the forearm during mental tasks, reduced variability of the respiratory sinus arrhythmia in the resting ECG). The hypothesis remains difficult to reconcile with evidence for a functional excess of central cholinergic activity in depression. It should be mentioned, however, that measuring SCOR and FPAR in concert, Levinson (1991) failed to differentiate subjects with schizophrenia from those with depression, while Schnur *et al.* (1999) could not differentiate patients with schizophrenia from those with mania.

The possibility that different pathophysiological mechanisms underlie EDA reduction in subjects with schizophrenia and those with mood disorders is also suggested by the findings of Katsanis and co-workers, showing that in subjects with mood disorders, in contrast with what is observed in those with schizophrenia, there is no relationship between season of birth and EDA reduction (Katsanis *et al.*, 1992). Similarly, Bernstein *et al.* (1988) pointed out that in schizophrenics the frequency of electrodermal responding varies with the task relevance of the stimulus, while in depression no relationship is observed, suggesting that SCOR deficit is centrally determined in schizophrenia but not in depression, where a peripheric cholinergic defect might be implicated.

Bilateral recordings of EDA in subjects with mood disorders have shown higher activity from the left than from the right hand (Gruzelier and Venables, 1974; Myslobosky and Horesh, 1978; Schneider, 1983). The asymmetry pattern has been interpreted as the result of either a hypoactivation or a hyperactivation of the right hemisphere in depressive disorders. Conflicting interpretations originate from the difficulty of disentangling the relative activating or inhibitory contribution of each hemisphere to EDA from either hand. Lenhart and Katkin (1986) recorded, in adjunct to bilateral EDA, conjugate lateral eye movements in a group of college students with subsyndromal depression. They confirmed the previously reported pattern of EDA asymmetry (L > R) and found a bias toward left-tending conjugate lateral eye movements. Findings were interpreted as reflecting right-hemisphere hyperexcitability in affective illness.

The EDA asymmetry pattern L > R was also found in high-risk subjects, the offspring of parents with bipolar affective disorders (Zahn *et al.*, 1989). However, in the latter study it was limited to the recording task period and concomitant with high self-ratings of depressed mood, which led the authors to conclude that, rather than being a stable property of the nervous system, it should be considered as a state marker of depression. Several studies failed to find any asymmetry in bilateral EDA recordings of patients with affective disorders (Iacono and Tuason, 1983; Storrie *et al.*, 1981; Toone *et al.*, 1981).

Cardiovascular Measures

Heart rate (HR), heart rate orienting responses (HRORs) and heart rate variability (HRV) have been the most frequently used indices. Systolic, diastolic and mean blood pressure, as well as forearm blood flow, have also been investigated in some studies.

The main findings in subjects with depression included an elevated resting heart rate and/or HRORs (Carney *et al.*, 1988; Dawson *et al.*, 1977, 1985; Lahmeyer and Bellur, 1987; Lehofer *et al.*, 1997) and a reduced HR variability (Carney *et al.*, 1995a; Dallack and Roose, 1990). Discrepant findings have also been reported (Jacobsen *et al.*, 1984; Lader and Wing, 1969; Yeragani *et al.*, 1991). Medication status, concomitant anxiety and failure to adequately match subjects for age and sex might account for the discrepancies.

Research on HRV has received particular attention, due to its medical implications: reduced HRV as a result of diminished cardiac vagal tone was shown to be a strong predictor of sudden death in subjects with myocardial infarction; subjects with depressive disorders have an increased incidence of mortality for cardiovascular diseases, and reduced HRV might represent a predisposing factor (for review see Dalack and Roose, 1990).

As a measure of HRV the respiratory sinus arrhythmia (RSA; the variation in heart rate associated with respiratory cycles) has been employed in some studies. It is used to assess cardiac parasympathetic tone. Spectral analysis of beat-to-beat variations in HR and blood pressure (BP) has also been applied to describe sympathetic and parasympathetic contributions to cardiovascular regulation (Malliani *et al.*, 1991; Tulen, 1993). Three spectral peaks are identified: a low-frequency peak (around 0.04 Hz), which for HR is associated with both sympathetic and parasympathetic activity, while for BP reflects variations in peripheral vasomotor activity; a mid-frequency peak (around 0.1 Hz), associated with the baroreflex response (and reflecting mainly sympathetic activity, although for HR the parasympathetic contribution cannot be excluded); and a high-frequency peak (around 0.20–0.35 Hz), which for HR reflects the RSA and therefore the parasympathetic tone, while for BP mainly results from mechanical effects of respiration.

Tulen *et al.* (1996) investigated autonomic regulation in 16 drug-free female depressed patients, divided into high and low on trait anxiety (HTA, LTA), during supine rest, orthostatic challenge, and post-orthostatic challenge supine rest. Spectral analysis of fluctuations in HR and BP was employed. They found that patients did not differ from controls during supine rest; during the orthostatic challenge they did not show the normal increase of BP mid-frequency band fluctuations, suggesting a reduced sympathetic activation, and exhibited a reduction of HR variations, pointing to a strong vagal inhibition. The latter effect was more marked in HTA patients; during the post-orthostatic supine rest patients showed a significant increase in HR variability, in the absence of clear changes in BP variability, suggesting a predominant cardiac vagal increase. The authors conclude that an impairment of orthostatic reflex in depressed patients is suggested, with a prevalence of parasympathetic over sympathetic dysfunction, especially in HTA patients.

Lehofer *et al.* (1997) studied both RSA and HR in 23 unmedicated and 23 medicated DSM-III depressed subjects, melancholic subtype, and 46 healthy comparison subjects. HR was higher in both medicated and unmedicated depressed subjects, while RSA was lower only in medicated patients versus comparison subjects. They concluded that there is no difference in vagal tone between unmedicated depressives and healthy controls, and therefore higher HR in unmedicated patients might be due to either an increased sympathetic tone or an increased 'autonomous' heart rate (which, together with the vagal and sympathetic activity, contributes to the heart frequency). It is independent of autonomic nervous system control, is about 100 beats per minute and is found in transplanted heart patients (Bernardi *et al.*, 1989). Moser *et al.* (1998) came to a similar conclusion measuring heart rate, RSA, pulsewave velocity (a measure of the sympathetic tone in the cardiovascular system) and BP in 26 patients suffering from major depression, melancholic type, who had been unmedicated for at least three months, and 26 comparison healthy subjects. Patients exhibited a higher HR, and no difference in cardiac vagal tone or sympathetic cardiovascular tone. Taken together, the results indicate that autonomous heart rate is higher in subjects with depression; whether this is due to a less well-trained heart resulting from reduced physical exercise or is a physiological state or trait marker of depression is left open by the authors.

Austen and Wilson (2001) measured RSA in unmedicated subjects with subsyndromal seasonal affective disorder (SAD) and healthy comparison subjects in both winter and non-winter periods. They found a significant group−season interaction due to significant higher RSA scores in winter only in subjects with SAD. For HR, SCL and diastolic blood pressure, instead, only an effect of the season was found: in both SAD and control subjects HR decreased while SCL and blood pressure increased during winter. According to the authors, increased RSA, due to an increased vagal tone, may parallel the sleep symptom observed in SAD (increased sleep), thus suggesting a similarity with the hibernation process and different underlying mechanisms with respect to non-seasonal depression. As also proposed by the authors, further research across a year-long period is needed to confirm and extend these observations across groups, and eventually confirm that SAD is a different disorder from non-seasonal depression.

Dysregulation of the autonomic nervous system has been considered a leading candidate mechanism for the association between depression and increased medical morbidity and mortality in patients with coronary heart disease (CHD) (Carney *et al.*, 1999). In a group of 50 depressed and 39 medically comparable CHD non-depressed subjects, Carney *et al.* (1999) found an elevated resting HR and an exaggerated HR response to orthostatic challenge in the former group. However, the mechanism underlying the observed autonomic dysfunction remains unclear: in fact, the two groups did not differ in resting or standing plasma levels of norepinephrine, excluding an increased sympathetic activity; at the same time, studies of HRV in depressed CHD patients have found no support for lower vagal tone in these subjects (Carney *et al.*, 1995b).

Eye Movements

Although included in the section on peripheral measures, the study of eye movements is not relevant to autonomic dysregulation. Eye movement investigations, in fact, provide important clues to central nervous system functioning. Increasingly sophisticated studies enable attempts to relate the results of electrophysiological recordings to the topology of brain function, as well as to brain imaging findings.

The majority of eye movement studies carried out in subjects with affective disorders have focused on the smooth pursuit eye movement system; a minority have investigated saccadic eye movements.

Smooth pursuit eye movements (SPEMs) occur when a moving object (target) is fixated and followed by the eyes. More often visual targets oscillating in a uniform sinusoidal or pendular manner have been used. Saccades are the brief rapid movements of the eyes aimed at bringing the eye focus from one point to another of the visual field.

SPEMs have been largely investigated in subjects with schizophrenia, in which their dysfunction is regarded as a promising biological marker of the disorder (Holzman, 1987; Holzman *et al.*, 1973, 1974; Iacono and Clementz, 1992). Several studies have also found SPEM abnormalities in subjects with affective disorders, calling into question the diagnostic specificity of SPEM dysfunction.

Studies contrasting subjects with affective disorders to healthy comparison groups have reported abnormal SPEMs in patients (Holzman, 1985; Iacono *et al.*, 1982; Klein *et al.*, 1976; Levin *et al.*, 1981; Salzman *et al.*, 1978; Shagass *et al.*, 1974). Several studies have reported no striking difference between subjects with mood disorders (both bipolar and unipolar) and those with schizophrenia on eye-tracking performance (Amador *et al.*, 1991; Friedman *et al.*, 1992; Iacono *et al.*, 1992; Küfferle *et al.*, 1990; Levin *et al.*, 1981; Lipton *et al.*, 1980; Sweeney *et al.*, 1999; Yee *et al.*, 1987), while others did find significant differences (Cegalis and Sweeney, 1981; Iacono and Koenig, 1983; Iacono *et al.*, 1982; Muir *et al.*, 1992). Heterogeneity in recording techniques, experimental paradigms, evaluated measures, patients' diagnosis or medication status may account for discrepancies.

Some studies used global performance measures (qualitative ratings of eye tracking or quantitative global evaluation, which assess the similarity between the target trace and the eye movement trace), while others used quantitative measures of SPEM-specific characteristics, such as gain (eye velocity/target velocity), anticipatory saccade (AS) rate (frequency of large saccades that take the eyes ahead of target), square wave jerk (SWJ) rate (frequency of pairs of small saccades, in opposite directions, separated by approximately 200 ms) or corrective catch-up saccade (CUS) rate (frequency of saccades occurring when a low pursuit gain causes the eyes to fall behind the target in order to quickly take the eyes to the target). ASs and SWJs are intrusive saccades, while CUSs are corrective saccades.

The increasing use of more sophisticated methods is relevant to the diagnostic issue: global measures might be less sensitive than specific quantitative measures. Patients in different diagnostic categories might have globally abnormal eye tracking; however, the specific pattern of abnormalities might help discriminating among them.

Friedman *et al.* (1995), in a study including 26 unmedicated schizophrenics, 14 unmedicated subjects with affective disorders (mostly unipolar), never treated with lithium, and 45 healthy subjects, used two global measures — qualitative rating and root mean square (RMS), which is a measure of the extent to which the eye position and target position differ over time — and six quantitative measures — CUS rate, CUS amplitude, gain, SWJ rate, AS number, and total time scored. They found that (1) RMS error and qualitative measures did not differentiate subjects with schizophrenia or affective disorders from healthy comparison groups, and (2) patients with affective disorders were not impaired on eye-tracking measures, whereas patients with schizophrenia were impaired in terms of gain, CUS rate and total time scored. They concluded that unmedicated subjects with affective disorders never exposed to lithium treatment perform an easy SPEM task as well as comparison subjects, that SPEM abnormalities are specific to schizophrenia and that specific quantitative ratings are more sensitive than global ones.

Friedman *et al.* (1992) reported that subjects with schizophrenia and those with affective disorders did not differ on SWJ and AS rate; the same group (Abel *et al.*, 1991) had previously found that CUS rate at the target velocity of 20°/s differentiated

the two diagnostic groups, being significantly higher in subjects with schizophrenia. Each diagnostic group showed a different abnormality pattern when compared with healthy subjects. Similar findings were reported by Flechtner *et al.* (1997), who studied 43 subjects with schizophrenia, 34 with major depression and 42 healthy comparison subjects, and found that the two patient groups did not differ on gain, intrusive saccades and AS, while CUS rate was significantly higher in subjects with schizophrenia than in those with depression.

Sweeney *et al.* (1999) have recently stressed the importance of studying eye tracking by using experimental paradigms providing assessment of the pursuit across different target velocities and evaluating other important SPEM components, such as pursuit initiation or performance in conditions of low stimulus position predictability. In a carefully designed study, they tested 32 subjects with schizophrenia (20 drug-naïve, 12 drug-free for 28 days), 35 with affective disorders (26 unipolar, 9 bipolar, all drug-free for 28 days) and 24 comparison healthy subjects. All patients were tested during acute episodes of illness. No abnormality showed a diagnostic specificity.

As mentioned above, several variables may affect eye movements. Substances with a depressant effect on the central nervous system (such as barbiturates, chloral hydrate, alcohol and benzodiazepines) may either superimpose nystagmus or increase saccadic events during pursuit or fixation, whereas neuroleptics, tricyclics and monoamine oxidase inhibitors do not seem to influence SPEMs (Holzman *et al.*, 1975, 1991; Levy *et al.*, 1984). In studies involving subjects with affective disorders, lithium treatment in particular has been regarded as an important confounding variable (Iacono *et al.*, 1982; Levy *et al.*, 1985). According to Corbett *et al.* (1989) the oculomotor effects of lithium include nystagmus, saccade pursuit, saccadic dysmetria and oculogyric crises. Holzman *et al.* (1991), in a study in which subjects with affective disorders were tested prior to starting lithium and then at weekly intervals after beginning treatment, reported that, in the context of clinical improvement, lithium administration degraded SPEMs and the nature of the degradation was idiosyncratic, involving different changes in different subjects and including gain decrements, increase in total number of saccades (but not AS) and fixation instability. Gooding *et al.* (1993), however, in a carefully designed study, found no lithium effect on SPEMs.

Eye-tracking abnormalities were also investigated in the offspring of probands with either unipolar or bipolar depression. In the unaffected offspring of subjects with bipolar disorder no impairment of eye tracking was found (Holzman *et al.*, 1984; Iacono *et al.*, 1992; Levy *et al.*, 1983), suggesting that the dysfunction represents a trait marker in schizophrenia and a state-related measure in mood disorders. Such a conclusion is not completely supported by a study carried out in the offspring of schizophrenic, unipolar depressed and bipolar probands from the New York High-Risk Project (Rosenberg *et al.*, 1997). In that study, in fact, a significant global performance deficit was demonstrated in the offspring of depressed probands and of schizophrenic probands, but not in those of bipolar patients. However, only the offspring of probands with schizophrenia had a higher mean frequency of anticipatory saccades. The authors suggested that the familial specificity of global eye-tracking deficit to schizophrenia might be limited with respect to unipolar major depression.

Visual fixation — that is, the ability to maintain gaze on a stationary target — has also been investigated in subjects with affective disorders. Amador *et al.* (1991, 1995) found fixation abnormalities in subjects with schizophrenia and their first-degree relatives, but not in those with depression. Gooding *et al.* (2000) did not find any fixation abnormality in either subjects with schizophrenia or those with bipolar I disorder. The latter study used a different recording technique (infrared scleral reflection instead of electro-oculogram recording) and added quantitative to qualitative evaluation.

Several studies showed no latency or accuracy abnormality of saccadic eye movements in subjects with bipolar affective disorders versus healthy comparison groups (Crawford *et al.*, 1995; Sereno and Holzman, 1995; Tien *et al.*, 1996). Some other studies, while confirming saccade accuracy, reported increased saccadic latency in bipolar versus healthy subjects (Park and Holzman, 1992; Yee *et al.*, 1987). An increased error rate on antisaccade tasks has also been reported in bipolar versus healthy comparison subjects (Sereno and Holzman, 1995; Tien *et al.*, 1996). Relatively few studies have investigated saccadic eye movements in depressed subjects. Fukushima *et al.* (1990) reported that medicated depressed patients had a normal saccade latency and were able to suppress reflexive saccades during an antisaccade task. Done and Frith (1989) found a normal saccade latency during a visually guided saccade task in a small group of drug-free depressed subjects, but a prolonged latency during a voluntary saccade task. Sweeney *et al.* (1998) administered a battery of oculomotor tasks (selected to assess the functional integrity of frontostriatal circuitry and of the cerebellar vermis) to 29 unmedicated inpatients with unipolar major depression and 19 comparison subjects. In the patient group, in comparison with healthy subjects, they found no abnormality of saccade latency, an impaired ability to suppress saccades to peripheral targets in the antisaccade task, more saccadic intrusions during fixation and a mild saccadic dysmetria during sensorially guided eye movements. Since lesions of the cerebellar vermis disrupt saccade accuracy but not necessarily latency or velocity, the impaired accuracy observed in depressed patients is compatible with a dysfunction of the cerebellar vermis. The remaining abnormalities are interpreted as the result of prefrontal dysfunction. The authors conclude that a functional disturbance of the dorsolateral prefrontal cortex may be common to both bipolar and unipolar patients, whereas the cerebellar vermis dysfunction seems characteristic of unipolar patients. It is worthy of notice that abnormalities of the cerebellar vermis have been reported by both structural and functional brain imaging studies in depressed patients (Dolan *et al.*, 1992; Shah *et al.*, 1992).

Bipolar patients receiving neuroleptic treatment were less accurate than neuroleptic-free patients on saccades directed towards remembered or predicted targets, which presumably require an internal representation of the stimulus (Crawford *et al.*, 1995).

CENTRAL PSYCHOPHYSIOLOGICAL INDICES IN RESEARCH ON MOOD DISORDERS

Central indices have been extensively used in the psychophysiological research on mood disorders. The huge literature on the EEG, ERPs and sleep parameters in affective disorders cannot be exhaustively reviewed for obvious space limitations. In the following section, the main results will be summarized and discussed in the light of their contribution to the generation of hypotheses on the pathophysiology of these disorders.

Quantitative Electroencephalogram (QEEG)

QEEG in Depressive Disorders

QEEG research in depressive disorders did not report consistent findings. Most replicated results include an increase of alpha (Brenner *et al.*, 1986; John *et al.*, 1988; Schaffer *et al.*, 1983; von Knorring, 1983) and/or beta (Flor-Henry *et al.*, 1979; John *et al.*, 1988; Knott and Lapierre, 1987a) activity in depressed patients, when compared with healthy subjects. Increased slow-wave activity, generally observed in subjects with dementia, has

been found in elderly depressed patients by some authors, though to a lesser degree than in dementia (Brenner *et al.*, 1986; Have *et al.*, 1991; Nystrom *et al.*, 1986). Studies investigating early- and late-onset depressed patients failed to find differences between the two groups, but reported a relationship between increased delta wave activity and poor performance on several neurocognitive tests in patients with late-onset depression (Dahabra *et al.*, 1998; Visser *et al.*, 1985).

Lateralized findings have also been inconsistent. An increase of beta activity, predominantly over the left side, was reported in depressed when compared to healthy subjects, suggesting left hemisphere overactivation (Flor-Henry and Koles, 1984; Kemali *et al.*, 1981; Matousek *et al.*, 1981). Some authors reported an increase of alpha activity and a polymodal distribution of the mean alpha amplitude over the right hemisphere in depressed compared to healthy subjects, suggesting a reduced activation and a disorganization of this hemisphere (von Knorring, 1983; von Knorring *et al.*, 1983).

An asymmetric increase of the alpha activity over the left frontal regions was reported by some studies in currently or previously depressed patients (Baehr *et al.*, 1998; Gotlib *et al.*, 1998; Henriques and Davidson, 1990, 1991) and in subclinically depressed students (Davidson *et al.*, 1987; Schaffer *et al.*, 1983). Children of depressed mothers, who might be at risk for affective disorders, were shown to have greater alpha activity over the left than over the right frontal regions (Tomarken *et al.*, 1994). The increase of alpha was interpreted as a sign of decreased left frontal activation with a deficit in approach-related behaviours (Davidson, 1992). The opposite asymmetry pattern was observed for the parietal regions, with an increase of alpha power over the right-hemisphere leads, in currently or previously depressed subjects (Davidson *et al.*, 1987; Henriques and Davidson, 1990). The increase of alpha over the right parietal regions might be associated with cognitive deficits, suggesting right posterior dysfunction in depression (Davidson *et al.*, 1987; Tucker *et al.*, 1981). For the parietal asymmetry pattern, however, conflicting results were reported by some studies in either depressed students or subjects with major depression. Some of them failed to find any abnormality in parietal regions (Henriques and Davidson, 1991; Schaffer *et al.*, 1983), while others found a bilateral increase of alpha (Pollock and Schneider, 1989, 1990) or a right posterior alpha reduction (Pozzi *et al.*, 1995; Suzuki *et al.*, 1996). According to Bruder *et al.* (1997) the presence of anxiety in the clinical picture might influence the laterality pattern observed over the posterior regions. In fact, non-anxious depressed patients showed more alpha activity (less activation) over the right than over the left posterior leads, whereas anxious depressed patients showed the opposite pattern. Two studies reported a failure to suppress alpha activity over the right posterior regions in depressed subjects during the performance of a spatial task (Henriques and Davidson, 1997; Reid *et al.*, 1995), suggesting that a cognitive challenge might be necessary to disclose the right-hemisphere deficit in posterior regions. Suicidal ideation might also influence the posterior asymmetry pattern. In fact, preliminary findings suggest that adolescent depressed and non-depressed suicide attempters have more alpha activity over the left than the right frontal regions, but differ in the direction of the asymmetry over the posterior regions: a greater alpha activity over the left than the right posterior leads was related to suicidal intent, but not to depression severity (Graae *et al.*, 1996).

So far the topographic analysis of the brain electrical activity from multilead recordings by means of the identification of the brain electrical microstates (BEMs) has not received great attention in relation to mood disorders. BEMs are stable segments of scalp electric fields in the sub-second range, and reflect the coordinated activation of neuronal circuits involved in mentation (Koenig and Lehmann, 1996; Lehmann *et al.*, 1987). The main quantitative parameters of the BEMs include the duration, the spatial variance (i.e. spatial changes per time unit), the topographic characteristics of the field, as assessed by the locations of the positive and negative centroids (the centre of gravity of the positive and negative areas, respectively), and the field strength. With respect to healthy comparison subjects, depressive patients showed a reduced duration of the brain electrical microstate more represented in subjects' mentation and a larger spatial variance. However, the topographic characteristics of the identified microstates were similar in the two groups (Strik *et al.*, 1995). The findings were interpreted as the electrophysiological correlates of impaired attention and automatic processing in depressive patients.

QEEG in Bipolar Disorder

Only a few QEEG studies have been carried out in acute, drug-free subjects during a manic episode, probably because of the difficulty of obtaining cooperation from these patients. Flor-Henry and Koles (1984) found a decrease of alpha power in manic patients with respect to healthy subjects, suggesting an overarousal in the former group. Shagass *et al.* (1984) found that patients with mania have higher EEG frequencies and greater variability than those with depression, and interpreted the results as a sign of overarousal in mania. In bipolar subjects left hemisphere abnormalities have been reported, akin to those in schizophrenia (Davidson, 1987; Flor-Henry, 1987).

A study on the adult offspring of subjects with bipolar disorder, comparing the ill siblings (subjects who met the DSM-III criteria for bipolar disorder) with those who were well, showed a reduction of alpha activity in the ill group (Knott and Lapierre, 1987b). However, in the ill-sibling group, all subjects were asymptomatic at the moment of assessment, some were on maintenance treatment or had been treated with antidepressants and observed a very short wash-out period (72 hours) before assessment, leaving open the possibility that study findings were influenced by drug treatment.

QEEG and Treatment Responsiveness

A few investigations were aimed at assessing the usefulness of QEEG in the prediction of response to antidepressant drugs. Knott *et al.* (1985) with an open-label paradigm found a lower relative theta power in the baseline recording of depressed subjects with a favourable clinical response to imipramine, with respect to non-responders to the same drug. Cook *et al.* (1999) used a new measure, combining relative and absolute power, the cordance, in the theta band to investigate QEEG correlates of response to fluoxetine in unipolar depression, with a double-blind, placebo-controlled paradigm. Significantly more depressed subjects with high cordance in theta band were responders to fluoxetine in comparison to those with low cordance. Since previous studies showed that cordance correlates with brain metabolism (Leuchter *et al.*, 1999), the results were interpreted as suggesting that only depressed subjects with low baseline dysregulation respond to treatment.

Galderisi *et al.* (1996) used a test-dose procedure to study the QEEG changes associated with clinical response to moclobemide in unipolar depressed patients. A single dose of 200 mg moclobemide induced a transient increase of theta activity (observed up to the third hour after drug administration), a slight augmentation of alpha and a sustained increase of beta activity (observed up to the sixth hour after drug administration). Drug-induced increase of beta was found to correlate with the decrease of depression psychopathological ratings observed after 42 days of treatment.

In a group of drug-free, acute patients with mania, Small *et al.* (1999) reported more baseline fast theta activity (6–8 Hz) in non-responders to pharmacotherapy than in responders. After treatment, non-responders had more delta and theta over frontal and temporal regions, as well as more beta1 activity over left temporal leads.

Event-Related Potentials (ERPs)

ERPs in Depressive Affective Disorders

For nearly all ERP components discrepant findings were reported in depressed subjects (Bruder *et al.*, 1995, 1998; el Massioui and Lesevre, 1988; Kayser *et al.*, 2000; Ogura *et al.*, 1993; Pierson *et al.*, 1996; Zahn, 1986).

The contingent negative variation (CNV) is a slow negative potential occurring within the interval between a warning stimulus and an imperative signal which prompts the subject's response. The CNV has generators in the prefrontal cortex and shows a brainstem cholinergic modulation (Halgren, 1990). It is thought to be related to the orientation, expectation and preparation for the motor act (Wascher *et al.*, 1997).

The CNV was found to be reduced in depressed when compared with healthy subjects (Ashton *et al.*, 1988; Sartory, 1985; Shagass, 1983). However, other studies failed to find the CNV reduction (Elton, 1984; Knott and Lapierre, 1987a). A study subdividing depressed patients into two groups, one with retardation/blunted affect and the other with anxiety/impulsiveness, found a reduced CNV only in the former group versus healthy subjects (Pierson *et al.*, 1996). An augmented CNV amplitude was demonstrated in a subgroup of depressed patients with increased reactivity to apomorphine, underscoring the clinical-biological heterogeneity of depressive disorders (Timsit-Berthier, 1986). A reduction of the P1-N1 component amplitude was reported in depressed compared to healthy subjects (el Massioui and Lesevre, 1988; Pierson *et al.*, 1996), suggesting a disturbance in early sensory processing. The finding was not replicated by other studies (Bruder *et al.*, 1995; 1998).

For N2, an ERP component thought to index both automatic orienting and initial effortful stimulus evaluation (Bruder *et al.*, 1998), a reduced amplitude was observed in depressed versus healthy subjects, when using emotional faces; differences were maximal over right parietal regions, indicating a posterior right-hemisphere deficit (Deldin *et al.*, 2000).

Several studies examined the P3 component of the ERPs in depression. This component includes (1) the P3a subcomponent, with a centro-frontal amplitude maximum, which represents an orienting response, and (2) the P3b subcomponent, with a centro-parietal maximum, which is related to the categorization and completion of the task. The P3 amplitude was found to be reduced in depressed versus healthy subjects (Bruder *et al.*, 1998; Pfefferbaum *et al.*, 1984; Roth *et al.*, 1981; Thier *et al.*, 1986). However, according to some studies (Gangadhar *et al.*, 1993; Picton, 1992), the reduction was not seen during episode remission. Other studies reported a normal P3 amplitude in depressives (Bange and Bathien, 1998; Giedke *et al.*, 1980; Sara *et al.*, 1994). Depressed subjects with retardation/blunted affect presented a reduced P3a amplitude, while those with agitation/impulsiveness did not (Partiot *et al.*, 1993). Since P3a is related to the orienting, the authors interpreted the results as evidence of disturbed automatic processing in depression with retardation and blunted affect. In a subsequent study, in which a more complex task was used, a group of retarded/blunted depressives showed a reduction of both P3a and P3b amplitude with respect to anxious/impulsive patients and healthy subjects (Pierson *et al.*, 1996). Actually, in the anxious/impulsive group the P3b amplitude was even larger than in healthy subjects, suggesting that the combination of the two subgroups may cancel out differences between patients and comparison groups. Kayser *et al.* (2000) investigated the P3 during passive viewing of faces with negative and neutral emotional valence in depressed and healthy subjects. The P3b showed an amplitude reduction in depressed compared to healthy subjects. Furthermore, the P3b showed an amplitude enhancement over right posterior leads for negative as compared to neutral faces, only in healthy subjects. Depressed patients presented a similar effect for the P3a component. The authors hypothesized that an early stimulus categorization (indexed by the P3a) was followed by inhibition of further affective processing (indexed by the P3b) in depressed subjects. The study findings gave further support to the presence of a reduced activation of right posterior areas in depressed subjects.

Discrepant findings were reported for P3 latency increase (which might index cognitive deterioration) in depressed subjects (Blackwood *et al.*, 1987; Dahabra *et al.*, 1998; Pfefferbaum *et al.*, 1984; Roth *et al.*, 1991). Bruder (1995) criticized the use of very easy tasks in studies reporting negative results and demonstrated a longer P3 latency in depressed subjects, with respect to a comparison group, only for a right-hemisphere task (Bruder, 1995). In a previous study, comparing subgroups of depressed subjects, Bruder *et al.* (1991) reported an increase of P3 latency in subjects with typical as compared with those with atypical depression only during the performance of a right-hemisphere task. Thus, discrepancies concerning P3 latency might be related to the use of tasks of different complexity and to the heterogeneity of depressive disorders.

ERPs in Bipolar Disorder

A reduced sensory gating, as measured by the suppression of the amplitude of the auditory P50 in response to the second of paired stimuli, was observed in manic patients, akin to findings in schizophrenia. However, in mania the abnormality is limited to acute symptomatic phases, while in schizophrenia it is a trait characteristic (Franks *et al.*, 1983; Freedman *et al.*, 1987). A study found that although the abnormality was present in bipolar subjects with manic or depressive episodes as well as in schizophrenics, only in subjects with mania it was associated with increased plasma levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (Baker *et al.*, 1990). Knott and Lapierre (1987b) reported an increased amplitude of the auditory P1 in the ill group compared with the well group in the adult offspring of bipolar subjects, suggesting an increased arousal in the ill group. The P200 component, thought to reflect the evaluation of stimuli physical characteristics and with generators in modality-specific cortical areas, was found to be reduced in bipolar I and schizophrenic patients with a negative family history compared to those with a positive family history for psychotic disorders (Tabarés-Seisdedos *et al.*, 2001). The two groups also differed in the presence of a larger right Sylvian fissure in patients with a negative family history. All patients were treated at the moment of testing.

Asymmetry indices might be useful in discriminating bipolar depressed from both unipolar depressed and healthy subjects (Bruder *et al.*, 1992). As a matter of fact, bipolar depressed subjects had a lower N1 amplitude for stimuli presented to the left than for those presented to the right visual hemifield. The same asymmetry was not found in either unipolar depressed or healthy subjects. In the same study, bipolar subjects were tested in a dot enumeration task and showed a reduced left visual field accuracy which was responsible for the absence of the visual field asymmetry found in both unipolar depressed and comparison subjects. The findings were interpreted as signs of reduced right-hemisphere arousal due to a dysfunction of the arousal/attentional system in the same hemisphere.

A few studies examined the P3 component in patients with mania. Salisbury *et al.* (1999) investigated P3 in treated patients with schizophrenia and bipolar disorder with psychotic features, and in healthy comparison subjects. They found a P3 amplitude reduction in both patient groups, with respect to healthy subjects. Maximum reduction of P3 amplitude was found over the left temporal lead in patients with schizophrenia and over the midline frontal lead in those with bipolar disorder. A study comparing

bipolar and healthy subjects reported an amplitude reduction over the frontal regions and a more posterior location of the P3 topography in the patient group, suggesting a reduced inhibitory frontal control in mania (Strik *et al.*, 1998). In line with a frontal deficit are recent findings of a functional imaging study showing a reduction of frontal activity in mania (Blumberg *et al.*, 2000).

ERPs and Treatment Responsiveness

The intensity dependence of the N1/P2 component of the auditory ERPs has been used to predict clinical response to treatment with lithium or antidepressants in subjects with mood disorders. The slope of the amplitude/stimulus intensity function (ASF) is an index of the amplitude changes due to increasing stimulus intensity: the steeper the slope, the greater the amplitude changes of the studied ERP components due to increasing stimulus intensity. The concept of intensity dependence has substituted that of augmenting/reducing introduced by Buchsbaum and Silverman (1968). Several authors reported that subjects showing a steeper pre-treatment ASF exhibited a favourable response to lithium treatment (Baron *et al.*, 1975; Buchsbaum *et al.*, 1971, 1979; Hegerl *et al.*, 1986, 1987; Nurnberger *et al.*, 1979). Hegerl and Juckel (1993) argued that the intensity dependence of the N1/P2 auditory ERP component is modulated by the serotonergic system: a strong intensity dependence of this component indicates a low central serotonergic activity. Among the findings supporting this hypothesis, the relationship between ASF slope and response to fluvoxamine is relevant to the present review. The effects of a fluvoxamine test dose on the ASF slope was related to clinical response to the same drug: patients with a flattening of the ASF slope induced by the fluvoxamine test dose were responders to the subsequent fluvoxamine therapy (Hegerl and Juckel, 1993). Subsequent research by Hegerl's group confirmed the role of serotonin in the modulation of ASF (Hegerl and Juckel, 1993; Juckel *et al.*, 1997, 1999). They showed that a strong pre-treatment ASF of the N1/P2 predicts a favourable clinical response to specific serotonin reuptake inhibitors (SSRI) in depressed patients (Gallinat *et al.*, 2000; Hegerl *et al.*, 2001).

A study showed an increase in the amplitude of the visual P3b and a significant decrease in its latency in depressed patients, after 6 weeks of treatment with moclobemide with respect to baseline (Galderisi *et al.*, 1996). The latency modification of the component was independent of changes in psychopathological scores.

Ancy *et al.* (1996) showed a larger baseline P300 amplitude in responders than in non-responders to ECT.

Sleep Studies

Sleep disturbances are common in affective disorders, with a prevalence of insomnia in the majority of subjects and hypersomnia in some subtypes of affective disorders (Armitage and Hoffmann, 1997; Reynolds and Kupfer, 1987; Riemann *et al.*, 2001). Insomnia represents a risk factor for depression (Ford and Kamerow, 1989; Livingston *et al.*, 1993; Schramm *et al.*, 1995) and increases the risk of suicide (Wingard and Berkman, 1983). Sleep deprivation may alleviate depressive symptoms (Wirz-Justice and Van der Hoofdakker, 1999; Wu and Bunney, 1990) and trigger mania in bipolar subjects (Wehr, 1991). In line with the latter observation, insomnia was shown to be a risk factor for mania (Barbini *et al.*, 1996). These data underscore the importance of sleep to mood disturbances.

Normal sleep includes alternating episodes of rapid eye movement (REM) sleep and non-REM (NREM) sleep. The latter includes four stages of progressively deeper sleep (stages 1–4). The deepest stages (3 and 4) represent the so-called slow-wave sleep (SWS). A sleep cycle of about 90 minutes includes both NREM and REM episodes. Within the cycle, SWS predominates during the first part of the night, while REM sleep predominates during the last part of the night.

An endogenous circadian clock, involved in biological circadian rhythms (such as body temperature and melatonin secretion) and thought to be located in the suprachiasmatic nucleus of the hypothalamus, regulates REM sleep and sleep spindles (12–15 Hz EEG oscillations with prevalent occurrence in stage 2 of the non-REM sleep). The circadian clock is entrained by time cues so that the sleep–wake cycle is synchronized to the day–night cycle and biological rhythms are synchronized with one another. A forced desynchronization in healthy subjects between the circadian internal clock and day–night cycle influences mood (Boivin *et al.*, 1997). There is also strong evidence that the balance between cholinergic and aminergic neurotransmitter systems regulates REM propensity. In particular, serotonergic tone regulates the level of activity of the cholinergic pontine neurons that stimulate REM sleep (Rye, 1997). Sleep spindles are associated with low levels of hyperpolarization of thalamocortical GABAergic neurons, while SWS is associated with high levels of hyperpolarization of the same neurons and a complete inhibition of the thalamic relay (McCormick and Bal, 1997; Steriade *et al.*, 1993). Homeostatic mechanisms (e.g. enhancement of the propensity to sleep by prolonged wakefulness) determine the amount of SWS and contribute to the regulation of sleep spindles and the progression from spindles to SWS (Borbely, 1998; Dijk and Czeisler, 1995).

Power spectral analysis of the sleep EEG demonstrated that SWS sleep is characterized by high amplitude delta, which is inversely related to beta activity (Merica and Fortune, 1997; Portas *et al.*, 1997). The increase of low-frequency (in the theta-alpha range) power during extended wakefulness might be associated with the homeostatic process of sleepiness promotion (Cajochen *et al.*, 1999). An association between beta frequency and cortisol secretion was demonstrated (Chapotot *et al.*, 1998), suggesting a relationship between arousal and HPA axis function. Beta activity during non-REM sleep was found to be inversely correlated with subjective sleep quality (Nofzinger *et al.*, 2000).

It has been recently hypothesized that non-REM sleep involves the deactivation of the ventromedial prefrontal cortex, mediating a dissociation of higher order cortex from sensory cortex, which might be essential in restorative sleep (Nofzinger *et al.*, 2000).

Sleep Disturbances in Unipolar Depression

Sleep alterations in depression include reduced sleep time and sleep fragmentation, reduced REM latency (<65 min), increased percentage of time spent in REM sleep in the first part of the night and increased REM density (i.e. density of rapid eye movements during REM sleep), and reduced SWS, particularly during the first sleep cycle (Benca *et al.*, 1992; Lauer *et al.*, 1991; Reynolds and Kupfer, 1987; see also Brunello *et al.*, 2000; Riemann *et al.*, 2001, for reviews).

Most of these sleep abnormalities, probably with the only exception of increased REM density, are not specific to depressive patients (Benca *et al.*, 1992; Lauer *et al.*, 1991; Reynolds and Kupfer, 1987), and are more prominent in older than in young depressed subjects (Riemann *et al.*, 2001). The relationships of sleep abnormalities with severity of depression, endogenous or psychotic features, and the course of the illness remain controversial (Buysse *et al.*, 1994; Frank *et al.*, 1992; Kerkhofs *et al.*, 1988; Hubain *et al.*, 1995, 1996; Kupfer *et al.*, 1984, 1990; Riemann and Berger, 1989; Rush *et al.*, 1986; Thase *et al.*, 1986, 1995). Patients with seasonal affective disorders, which often present daytime sleepiness and fatigue, do not show the same sleep abnormalities reported in those with non-seasonal major depression (Anderson *et al.*, 1994; Brunner *et al.*, 1996; Palchikov *et al.*, 1997; Partonen *et al.*, 1993). However, they might have a disturbance in

homeostatic processes as expressed by a lower increase of theta-alpha power during extended wakefulness, with respect to healthy controls (Cajochen *et al.*, 2000).

It is not clear whether some of the described sleep abnormalities might be present during phases of remission (Riemann and Berger, 1989; Rush *et al.*, 1986; Steiger *et al.*, 1989). Discrepancies in the findings might be related to the medication status of the subjects (REM sleep is likely to rebound at initial withdrawal from antidepressants) or to the definition of remission adopted by the different investigations. A reduced REM latency in remitted depressed subjects was found to be related to the risk of relapses (Buysse *et al.*, 1997; Giles *et al.*, 1987).

Cholinergic stimulation by arecoline administration was found to induce shorter latency REM episodes in remitted depressed subjects than in healthy comparison groups (Nurnberger *et al.*, 1989; Sitaram *et al.*, 1980). Relatives of depressed subjects without a personal lifetime history of affective disorders showed the same response to the cholinergic challenge (Krieg *et al.*, 2001; Schreiber *et al.*, 1992; Sitaram *et al.*, 1982); however, a study found that affectively ill relatives had shorter latency REM episodes than well relatives (Sitaram *et al.*, 1987), suggesting that the cholinergic hypersensitivity is both a trait and a state marker.

Increased REM density and reduced SWS were also reported in non-depressed subjects with a family history of affective disorders, suggesting that these abnormalities represent a vulnerability marker (Giles *et al.*, 1989; Krieg *et al.*, 2001; Lauer *et al.*, 1995).

Studies using power spectral analysis have reported that high pretreatment delta activity, irrespective of sleep stage, is associated with clinical responsiveness (Kupfer *et al.*, 1994). The decrease of slow-wave activity in the first with respect to the second non-REM episode was found to be related to poor clinical response to both pharmacotherapy and psychotherapy (Ehlers *et al.*, 1996; Thase *et al.*, 1998).

Buysse *et al.* (1997) found that the upper frequency part of the delta activity (2–3 Hz) is related to the acute depressed state, while the lower part (0.5–1 Hz) is related to the risk of recurrence.

A study has reported an increase of beta activity in non-REM sleep in depressed as compared with healthy subjects, which was related to increased relative glucose metabolism in ventromedial prefrontal cortex (Nofzinger *et al.*, 2000).

Period analysis of the EEG has also been used to study sleep dysregulation in depression, particularly disturbances of ultradian rhythms (90 minutes, which is approximately the period of a sleep cycle). The main findings include a lower interhemispheric coherence in the beta and theta frequency range and reduced intrahemispheric coherence between delta and beta bands (Armitage *et al.*, 1992, 1993, 1995). The same abnormalities were found in remitted, previously depressed, drug-free patients (Armitage *et al.*, 1993), as well as in healthy relatives of affective patients (Armitage *et al.*, 2000). The authors interpret the data as evidence of dysregulated ultradian rest–activity cycle as a vulnerability marker of depression (Armitage *et al.*, 2000).

Sleep Disturbances in Subjects with Bipolar Affective Disorders

Several studies comparing age-matched unipolar and bipolar depressed subjects did not find any difference in sleep abnormalities (Duncan *et al.*, 1979; Feinberg and Carrol, 1984; Kerkhofs *et al.*, 1988; Lauer *et al.*, 1992).

Sleep architecture in bipolar depressed subjects with hypersomnia was found to be normal (Thase *et al.*, 1989).

Manic patients have severe sleep disorders but are difficult to investigate and only a few studies were carried out. Some of them reported a normal sleep architecture, while others found reduced REM latency and increased REM density with respect to healthy comparison groups (Gann *et al.*, 1993; Goodwin and Jamison, 1990; Hudson *et al.*, 1988, 1992). Unlike depressed patients, manic patients do not show reduced SWS (Hudson *et al.*, 1988, 1992).

CONCLUSIONS

In conclusion, a great amount of work has been carried out, but few questions have been clearly addressed and answered. Several limitations in many of the reviewed studies have hampered the possibility of fully exploiting the potential of such valuable work; the most important among them are the lack of strong hypotheses, the poor characterization of populations under investigation and the application of experimental paradigms using tasks which are far too simple to disclose cognitive-emotional dysfunctions specific to these disorders.

Some efforts must be acknowledged, such as the identification of psychophysiological markers of vulnerability to mood disorders and predictors of illness course, response to treatment and outcome. Vulnerability markers have been identified by research on eye tracking, quantitative EEG asymmetry and sleep architecture, while ERP studies have found predictors of response to SSRI drugs and sleep studies have characterized predictors of both course and treatment responsiveness. The clinical relevance of these research findings urges further investigation to confirm and extend the available evidence.

Much work has also been devoted to the search for a diagnostic marker, but no conclusion can be drawn. EDA reduction, REM dysregulation and the reduction of SWS are examples of proposed diagnostic markers for affective disorders. However, their efficiency was not systematically assessed and, when evaluated, has not always appeared satisfactory; according to some data it might be improved by the combination of multiple indices. Attempts at using psychophysiological indices to validate clinical subtypes (e.g. unipolar/bipolar, typical/atypical, endogenous/non-endogenous) have not been systematic, preventing clear conclusions. The use of psychophysiological indices to identify more homogeneous phenotypes might be a promising alternative strategy, which has not been adequately explored so far.

A few studies (e.g. those investigating ERP asymmetry during emotional activation) have designed experimental paradigms using tasks aimed at disclosing specific cognitive-emotional dysfunctions of affective disorders, yielding consistent findings. A larger application of these experimental paradigms is highly advisable.

Findings provided by EEG asymmetry studies in depression deserve special attention for their internal consistency and coherence with both neuropsychological and brain imaging findings (Bench *et al.*, 1992; 1993; Delvenne *et al.*, 1990; Fox, 1994; Passero *et al.*, 1995; Schlegel *et al.*, 1989). Future EEG and ERP studies in mood disorders might take advantage from the application of advanced electrophysiological topographic and tomographic techniques, such as the brain electrical microstates and low resolution electromagnetic tomography (LORETA) (Pascual-Marqui *et al.*, 1994).

An important limitation of psychophysiological research in mood disorders has been the poor control of confounding variables, such as medication status, age, gender, handedness, state or trait anxiety. This limitation has plagued early investigations in this field, and, surprisingly, can still be found in more recent work, despite clear demonstrations of the strong influence that such confounders exert on psychophysiological indices.

A task of future research should be the disambiguation of important empirical findings, such as those concerning EDA asymmetry, interpreted as either a hypoactivation or a hyperactivation of the right hemisphere.

Further progress of the whole research field might be fostered by studies carried out in a more comprehensive perspective — that

is, combining, for example, clinical, psychophysiological, neuroendocrinological and brain imaging techniques.

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The Neuropsychology of Mood Disorders: Affect, Cognition and Neural Circuitry

Aprajita Mohanty and Wendy Heller

Research in the past decade has greatly enhanced our understanding of the neural processes that implement cognitive, emotional and physiological functions in mood disorders. Abnormalities or disturbances in these functions have been shown to be associated with corresponding abnormalities in regional brain functions (Heller and Nitschke, 1998). This has led to increased research efforts focused on linking theories of cognitive neuropsychology to the anatomy and physiology of related brain function in mood disorders.

Despite extensive evidence indicating impairment of cognitive functioning in depression, this area is largely ignored, especially in diagnostic evaluation (Austin *et al.*, 1992). The current *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV; American Psychiatric Association, 1994) identifies cognitive factors (e.g. indecisiveness, difficulties in thought and concentration) as fundamental components of depressive episodes and dysthymia. Neuropsychological studies have identified a variety of other cognitive characteristics associated with depressed moods. For example, depression is associated with deficits in executive functioning (Channon and Green, 1999; Freidman, 1964; Goodwin, 1997; Raskin *et al.*, 1982; Silberman *et al.*, 1983), memory (Burt *et al.*, 1995), attention (Mialet *et al.*, 1996), and visuospatial processing (Asthana *et al.*, 1998). Depression-related cognitive deficits range in severity from mild subclinical impairments to pervasive global deficits, often referred to as pseudodementia (Abrams and Taylor, 1987; Golinkoff and Sweeny, 1989; Watts *et al.*, 1990). The term pseudodementia was coined to describe patients with affective disorders who display an unusually large number of cognitive deficits that are typically associated with organic brain disease (Kiloh, 1961). The conjunction of depression and cognitive impairments of various types poses a clinical diagnostic problem because it increases the likelihood of a variety of misdiagnoses, including degenerative dementia, stroke or learning disability (Marsden and Harrison, 1972).

Abnormal patterns of brain activity and function associated with depression have been demonstrated in research using a variety of techniques, such as neuropsychological testing (Miller *et al.*, 1995; Rubinow and Post, 1992; Silberman and Weingartner, 1986), lesion techniques (Lipsey *et al.*, 1983; Robinson and Price, 1982; Robinson *et al.*, 1984), electrophysiological techniques (Deldin *et al.*, 2000; Heller *et al.*, 1995; Henriques and Davidson, 1990), and haemodynamic techniques (Baxter *et al.*, 1989; Bench *et al.*, 1992, 1993; George *et al.*, 1994a). Parallel findings to those obtained in depressed populations have been described in normal individuals following induction of depressed mood (Davidson *et al.*, 1979, 1985) as well as individuals scoring high on measures of depressed affect (Tomarken *et al.*, 1992). Despite substantial evidence indicating that the same brain regions implicated as abnormal in depression are also fundamental for various aspects of cognitive processing, the implications of these neuropsychological findings for cognitive processing in depression have rarely been studied (Rubinow and Post, 1992).

In this chapter, we review the evidence and describe the nature of the cognitive characteristics that have been identified in depression. In addition, we provide a brief discussion of the neural mechanisms likely to be associated with these cognitive characteristics. Before discussing the nature of cognition in depression we review a number of important factors that are likely to impact cognitive function in depression. These factors can introduce important methodological confounds in studies examining the relationship between depression and cognitive impairment, thus weakening the conclusions drawn from the results of clinical neuropsychological studies (Murphy and Sahakian, 2001).

GENERAL COGNITIVE DEFICITS AND CHARACTERISTICS OF DEPRESSION

Cognitive deficits in depression are influenced by the clinical characteristics of the disorder, such as the presence of specific symptoms, depression subtype and symptom severity. For example, cognitive deficits in depression have been found to vary with the presence of psychotic symptoms.

Cognition and Psychosis

Studies show that depressed patients with psychotic features such as delusions and hallucinations have more structural brain abnormalities than normal controls (Lesser *et al.*, 1991), show neuropsychological performance comparable to that of patients with schizophrenia (Jeste *et al.*, 1996; Nelson *et al.*, 1998) and are more impaired than patients with non-psychotic depression and normal controls (Basso and Bornstein, 1999; Jeste *et al.*, 1996; Lesser *et al.*, 1991; Nelson *et al.*, 1998) on a broad range of neuropsychological measures such as attention, response inhibition, verbal declarative memory and visuospatial abilities. It is important to keep in mind that drug effects can be a possible confounding factor in these studies as the selected clinical groups differed in the types and dosages of psychotropic medications with which they were being treated. Studies have shown that some psychotropic medications can impair or, conversely, improve neuropsychological test performance (Spohn and Strauss, 1989). Furthermore, individuals with psychotic depression may have more severe depressive symptoms, more frequent recurrence of depressive episodes and longer episodes, poorer response to pharmacotherapy, more hyperactive hypothalamus–pituitary–adrenal (HPA) activity (Coryell *et al.*,

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1996) and more structural brain abnormalities such as cortical atrophy (Gewirtz *et al.*, 1994). To the degree that these factors have a detrimental influence on cognitive function, individuals with psychotic features will present with more deficits.

Cognition and Subtypes of Depression

Cognitive deficits have also been found to vary with subtypes of depression. For example, Austin *et al.* (1999) reported that although depressed individuals were impaired on most mnemonic tasks, on simple reaction time and on Trails B of the Trail Making Test, melancholic patients defined by narrower criterion were additionally impaired on the Wisconsin Card Sorting Task (WCST) and on digit symbol substitution. In contrast, the cognitive performance of the more narrowly defined non-melancholic patients was largely unimpaired. Silberman *et al.* (1985) reported that patients exhibiting an abnormal dexamethasone suppression test (DST) response, usually seen in endogenous depression, showed more cognitive impairment than those with less evidence of physiological dysfunction. Using the Newcastle scale to define subjects with endogenous and non-endogenous depression, Cornell *et al.* (1984) reported impairment of complex reaction time only in subjects with endogenous depression.

Cognition and Severity of Depression

The relationship between depression subtype and cognitive deficits is confounded by depression severity, which can also affect cognitive functioning. For example, while Austin *et al.* (1992, 1999) reported frontal deficits only in individuals narrowly diagnosed with endogenous or melancholic depression, the differences disappeared after controlling for depression severity (Austin *et al.*, 1999). Individuals with melancholia showed poorer performance on cognitive tasks in the morning, when mood is reported to be more depressed, compared to evening — whereas controls displayed the opposite pattern (Moffoot *et al.*, 1994). The impact of severity has also been studied by correlating scores on clinical scales such as the Hamilton Depression Scale (Hamilton, 1960) with cognitive performance scores (for review, see Austin *et al.*, 2001). Using this method, studies have shown that severely depressed patients, especially those with significant psychomotor impairment, are more cognitively impaired (Austin *et al.*, 1992; Tarbuck and Paykel, 1995). Thus, depression severity appears to contribute to the neuropsychological deficits seen in individuals with depression. However, this does not imply that cognitive deficits occur only in acute stages; some research has shown that cognitive deficits might persist even after symptom remission (Trichard *et al.*, 1995).

Cognition and Treatment of Depression

Individuals with depression generally receive a combination of medications — including mood stabilizers, antidepressants, neuroleptics and benzodiazepines — which could have an impairing effect on neuropsychological performance. It is possible that differences observed between patients and controls, or patients in different stages of bipolar illness, are confounded by different medication regimens (Murphy and Sahakian, 2001). There is some evidence to suggest that different antidepressants may exert different effects on cognitive functioning. For example, amitriptyline, mianserin and trazodone have been reported to have adverse effects on a range of cognitive processes, including memory. The detrimental impact of these drugs was found to be more marked immediately following administration, and the effects wore off with time (Thompson, 1991). Studies have also shown that compared to depressed patients not treated with ECT, patients treated with ECT showed explicit memory deficits (Squire *et al.*, 1984). In general, however, prolonged antidepressant treatments appear to be associated with improved cognitive function as depressive symptoms remit (Frith *et al.*, 1983).

Cognition and Motivation

Some studies have argued that cognitive deficits in depression reflect motivational impairments (Hockey, 1986). In support of this notion, depressed patients show deficits in effortful as compared to automatic cognitive tasks, including problem solving, explicit memory, general learning and reading (for review, see Hartlage *et al.*, 1993; see also Klein and Seligman, 1976; Klein *et al.*, 1976; Price *et al.*, 1978). For example, depression has been found to be associated with impaired performance on verbal recall but unimpaired performance on verbal recognition (Roy-Byrne *et al.*, 1986) and with impaired explicit memory performance but intact implicit memory performance (Danion *et al.*, 1995; Ilsley *et al.*, 1995).

Several hypotheses have been proposed to explain the impairment of controlled operations in depression. These include lack of availability of cognitive resources due to decreased arousal (Gjerde, 1983), inadequate allocation of available resources due to an emphasis on irrelevant thoughts (Ellis and Ashbrook, 1988) or self-focused worries (Ingram, 1990), lack of initiative in the use of strategies required for information processing (Hertel and Hardin, 1990), or activation of automatic processes in depression (such as depressive ideation and depressive biases) which interfere with controlled processes (Mialet *et al.*, 1996).

Some researchers have suggested that abnormal responsivity to negative feedback, a commonly reported phenomenon in depression, may contribute to poor performance on some cognitive tasks. They (e.g. Elliott *et al.*, 1996) have argued that deficits in executive functions in depression are mediated by a highly specific form of motivational deficit involving the response of patients to perceived failure. Using conditional probability analyses, their study demonstrated that failure on one item of a test dramatically increased the chance of failure on the subsequent item in patients with depression. On both the Tower of London test and matching to sample tests, both of which provide feedback on performance, depressed patients showed a significant tendency to respond incorrectly after negative feedback. According to Elliott *et al.* (1996) the over-sensitivity to negative feedback is a highly specific form of motivational impairment that interacts with and exacerbates the neuropsychological deficits seen in depression. This impairment in the ability to alter behaviour in response to feedback could also account for the higher rates of perseverative errors seen in the performance of depressed individuals on executive functioning tasks (Channon, 1996).

Although motivational factors are likely to play a role in most aspects of performance, current perspectives do not support the notion that deficits in motivation alone can account for the patterns of cognitive deficit in depression (Heller and Nitschke, 1998; Hertel and Rude, 1991; Richards and Ruff, 1989). Heller and Nitschke (1998) argued that most of the data can be accounted for by impairments in functions of the prefrontal cortex (to be reviewed in more detail in subsequent sections of this chapter).

Cognition and Anxiety

A great deal of research has suggested that depression and anxiety are accompanied by distinct physiological, cognitive and emotional characteristics that appear to reflect the activity and function of different brain regions (Heller and Nitschke, 1997, 1998; Heller *et al.*, 1995; see also Chapter XIX-8). An important implication of these findings would be that a number of neuropsychological and neurophysiological studies may have been compromised by a failure to account for the substantial comorbidity of depression

and anxiety (Keller *et al.*, 2000). In light of these findings, it is important to consider the comorbidity of anxiety and depression in studies of cognition as disparate patterns of activation in anxiety and depression may differentially affect cognitive functioning.

From this review it is evident that differences observed between patients and controls, or between patients in different stages of depression, may be confounded by different factors such as clinical features of depression, medication regimens and comorbidity of depression with anxiety. Although these methodological problems bring unavoidable complications into investigations of disordered mood and cognition, it is important to adopt measures that control for potential confounds (Murphy and Sahakian, 2001). These measures include careful choice of controls, matching patients and controls on age and premorbid intelligence, and matching patients on measures of phase of illness, severity of depression, treatment regimen, comorbid conditions, etc.

NEUROPSYCHOLOGICAL FUNCTIONING IN DEPRESSION

Executive Functioning

Executive functions are known to rely on anterior regions of the brain (Banich, 1997; Heller and Nitschke, 1997) and include judgement, planning, abstract thinking, metacognition (i.e. 'thinking about thinking'), cognitive flexibility (i.e. flexibility in strategy use), verbal fluency, initiative, and the ability to generate alternate strategies and to direct behaviour in a goal-directed manner. Examples might include the ability to shift response set and modify strategies in task performance (Cicerone *et al.*, 1983), the evaluation of a situation and the use of cues and extra information in the environment to guide behaviour (Alivisatos and Milner, 1989), and the ability to monitor behaviour or performance accurately (Luria, 1966).

Miyake *et al.* (2000) argued that executive functions involve three important target functions — shifting, updating and inhibition — which are clearly distinguishable but are also moderately correlated constructs. Shifting involves shifting back and forth between multiple tasks, operations or mental sets (Monsell, 1996), updating requires monitoring and coding incoming information for relevance to the task at hand and revising the items held in working memory by replacing old, irrelevant information with new, relevant information (Morris and Jones, 1990), and inhibition involves the ability to deliberately inhibit dominant, automatic or prepotent responses when necessary (Miyake *et al.*, 2000). Furthermore, these executive functions were seen to contribute differentially to performance on specific tasks. More specifically, WCST performance was related most to shifting, Tower of Hanoi to inhibition, random number generation to inhibition and updating, and operation span to updating.

Individuals with depression display deficient performance on a number of set-shifting tasks such as the WCST and 'Go–No Go' tasks (Jones *et al.*, 1988). Deficits on the WCST were correlated with the severity of depression and were less severe than those demonstrated by patients with schizophrenia (Merriam *et al.*, 1999). However, another study showed that both schizophrenic and depressed individuals showed very little difference in their deficient performance on frontal lobe tasks like WCST, the Trail Making Test, and a verbal fluency test (Franke *et al.*, 1993). Channon (1996) reported that dysphoric subjects were poorer than controls in sorting to correct categories and had higher rates of both perseverative and non-perseverative errors on the WCST. The dysphoric subjects also had greater difficulties in shifting sets correctly. Unipolar depressed patients have been seen to show deficits on other attentional set-shifting tasks such as intradimensional/extradimensional (ID/ED) set shift (Purcell *et al.*, 1997). Thus, the majority of evidence points towards deficits on set-shifting tasks in depression.

A number of studies have also shown that depression is associated with impairment in working memory (Elliott *et al.*, 1996). Working memory is conceptualized as a limited capacity mental workspace involving holding task-relevant information online as well as simultaneous manipulation and updating of its contents by executive processes. Thus, working memory is presumed to be supported by an attentional control system, or 'central executive', probably localized to dorsolateral prefrontal cortex (Nitschke *et al.*, 2000; Pelosi *et al.*, 2000). Measuring event-related potentials (ERPs) as a neurophysiological correlate of working memory impairment, Pelosi *et al.* (2000) reported that individuals with depression showed deficits on a working memory task that were accompanied by ERP abnormalities consistent with dysfunction of a central executive system.

Individuals with depression also show impaired performance on tasks that test inhibitory aspects of executive functioning such as the Tower of London planning test (Elliott *et al.*, 1996, 1997). Similar results have been reported for the Stroop colour-word test (Degl'Innocenti *et al.*, 1998; Trichard *et al.*, 1995). In summary, findings from a wide variety of neuropsychological tasks indicate that depression is associated with deficits on important executive functions including set shifting, inhibition and updating.

Researchers have also investigated whether executive functioning deficits are a persistent feature of depression. A residual deficit in executive function appears to be seen in some patients with a history of depression. Paradiso *et al.* (1997) reported neurocognitive impairment in individuals after recovery from unipolar depression which was most noticeable on set-shifting tasks and was not related to medication status. Beats *et al.* (1996) also showed that deficits such as slowed simple and choice reaction times, perseveration on the set-shifting task and impaired verbal fluency did not fully remit upon recovery. Similarly, Trichard *et al.* (1995) in a study of executive functioning in middle-aged individuals with severe depression reported improved performance on the verbal fluency task but not the Stroop task upon recovery. Currently, it appears that residual deficits in mnemonic and executive functions persist in some patients with a history of depression. However, the relationship of these deficits to epidemiological variables such as age, treatment, duration and chronicity of illness, and number of episodes remains to be more clearly determined (Austin *et al.*, 2001).

Neural Correlates of Executive Function

The cognitive findings described above showing deficits in multiple domains of executive function are consistent with evidence from a variety of neuropsychological techniques indicating abnormal function of anterior brain regions in depression. Studies have identified abnormal patterns of asymmetric brain activity as well as abnormal levels of brain activity in a number of regions in prefrontal cortex, including dorsolateral, medial, orbitofrontal and anterior cingulate regions.

Abnormal patterns of asymmetric activity in anterior brain regions are demonstrated by lesion studies showing that left hemisphere strokes are significantly associated with the development of symptoms of depression (Lipsey *et al.*, 1983; Robinson *et al.*, 1983). Furthermore, significant correlations have been reported between the severity of depression and proximity of the lesion to the anterior pole of the left hemisphere (Lipsey *et al.*, 1983; Robinson and Price, 1982; Robinson *et al.*, 1984, 1985). The finding of asymmetric activity in anterior brain regions in depression has been substantiated by psychophysiological studies. In studies of EEG alpha, depression has been associated with less left than right anterior activity (Henriques and Davidson, 1990, 1991;

Schaffer *et al.*, 1983). Similarly, evidence from a variety of resting and cognitive activation paradigms has shown that depression is associated with altered regional cerebral blood flow (rCBF) and metabolism in prefrontal cortex, anterior cingulate gyrus and basal ganglia (for review, see Videbech, 2000). Consistent with EEG findings, positron emission tomography (PET) studies have reported a decrease in regional rCBF in the left dorsolateral prefrontal cortex in depression (Baxter *et al.*, 1989; Bench *et al.*, 1992, 1993; George *et al.*, 1993, 1994a; Martinot *et al.*, 1990).

In addition, specific changes in the medial prefrontal cortex have been associated with cognitive impairment in depression. The medial prefrontal region is characterized by reciprocal connections with other higher order association areas, mainly dorsolateral and caudal orbitomedial prefrontal cortex as well as anterior cingulate cortex (Kendell and Gourlay, 1970). In a study comparing patterns of rCBF in depressed patients with cognitive impairment to those of equally depressed patients without cognitive impairment, Dolan *et al.* (1992) reported decreased rCBF in the medial prefrontal brain region in the depressed cognitively impaired group. Similarly, significant decreases in rCBF in medial frontal gyrus were reported in patients with depression-related cognitive impairment (Bench *et al.*, 1992) and there was a significant correlation between a measure of global cognitive function and rCBF decreases in medial prefrontal cortex in patients meeting criteria for primary depression (Bench *et al.*, 1993).

Dolan *et al.* (1994) conducted a principal component analysis on two subtests discriminating most between depressed patients, non-patients and patients with cognitive impairment, which resulted in a two-factor solution (memory and attention) accounting for 50% of the variance in test scores. Increasing neuropsychological impairment on both factors was associated with decreased rCBF in the medial prefrontal and the frontal polar cortex. These findings were interpreted by Dolan and colleagues to indicate that the medial prefrontal cortex could mediate psychological components such as executive functions that are common to both attention and memory. In addition, each factor showed unique patterns of correlations with posterior brain regions. The memory factor was associated with rCBF in the retrosplenial precuneus and posterior cingulate cortices, regions implicated in auditory and verbal long-term memory (Grasby *et al.*, 1993). The attention factor was associated with rCBF in the posterior brain region extending from inferior post-central gyrus to the inferior parietal lobule.

Contrary to findings of decreased activity in particular brain regions, some studies have shown increased rCBF in areas of left prefrontal cortex, including ventrolateral prefrontal cortex, frontal polar cortex, medial orbital cortex, and the pregenual portion of the anterior cingulate gyrus as well as the left amygdala in individuals with familial pure depressive disease (FPDD), defined as primary major depression in an individual with a first-degree relative with primary depression. In light of these conflicting findings, Drevets and Raichle (1995) propose that the findings of decreased frontal flow and metabolism can be attributed to confounds introduced by medication effects and decreased frontal lobe size, as reported in post-mortem and MRI studies (Bowen *et al.*, 1989; Coffey *et al.*, 1993). They suggest using methods that correct PET measurements for volumetric differences. While these cautions are appropriate, a resolution of these inconsistencies awaits further research.

Drevets and Raichle (1998) also argue that different brain regions are involved in emotional versus cognitive conditions, and that there is a reciprocal relationship between them (Drevets and Raichle, 1998). Across a number of PET studies, they observed that regions implicated in emotional processing, such as the orbitofrontal cortex (OFC), ventral prefrontal cortex and amygdala show increased activity during emotional states but decreased activity during performance of cognitive tasks. Conversely, a complementary set of regions including dorsal anterior cingulate and dorsolateral prefrontal cortex show increased activity during attentionally demanding cognitive tasks, but decreased activity during experimentally induced and pathological emotional states such as depression. Drevets and Raichle (1998) hypothesize that suppression of neural activity in certain cognitive processing regions during intense emotional states could be a possible mechanism by which severe depression may interfere with cognitive performance.

An alternative to this cognition–emotion reciprocity position is the idea that tasks typically classified as 'cognitive' and 'emotional' may rely, at least in part, on common, overlapping neural systems due to their common, overlapping behavioural functions (Compton *et al.*, submitted; Heller and Nitschke, 1997; Miller, 1996; Nitschke *et al.*, 2000). We have argued that, because of the overlap in 'cognitive' and 'emotional' functions implemented by many brain regions, patterns of brain activity associated with changes in emotional state provide an important means for exploring the cognitive characteristics of emotional disorders (Heller and Nitschke, 1997; Miller, 1996; Nitschke *et al.*, 2000).

Earlier, we reviewed evidence examining the impact of abnormal response to feedback on cognitive functioning in depression. Some researchers have argued that cognitive deficits in depression are related to dysregulation of the neurobiological reinforcement and reward systems (Beats *et al.*, 1996; Elliott *et al.*, 1996, 1997). Using PET, Elliott *et al.* (1998) showed that behavioural response to feedback in depression is associated with attenuated activation in medial caudate and orbitofrontal cortex, regions that are implicated in reward mechanisms (Schultz *et al.*, 1992).

In summary, studies show that executive functioning deficits in depression are associated with abnormal patterns of brain activation in prefrontal brain regions. In future research, it will be important to examine more precisely how emotion modulates cognition and vice versa in depression. Further investigation into reciprocal brain mechanisms as well as integrated brain mechanisms for emotion and cognition could have important implications for cognitive deficits in depression. Another potentially important line of research appears to be that of the role of brain systems that regulate reinforcement and reward in depression.

Memory

Evidence from a large number of studies indicates that individuals with depression perform comparatively poorly on memory tasks. A meta-analytic study (Burt *et al.*, 1995) synthesizing 147 recall and recognition studies in clinically depressed and non-depressed samples revealed a significant, stable association between depression and memory impairment. Furthermore, the study showed that memory impairment in depression is linked to particular aspects of memory as well as particular subtypes of depression. Specifically, depression is associated with deficits on explicit, but not implicit, memory tasks. It is also associated with a tendency to remember negative material better than positive material.

Depression has been associated with impaired performance on tests of short-term memory, verbal and visual recognition memory, spatial working memory, and immediate or delayed recall (Cutting, 1979; Massman *et al.*, 1992; Richards and Ruff, 1989). Two main theoretical frameworks have been proposed to explain memory deficits in depression. According to one model, they can be attributed to impairment in effortful processing related to reduced attentional resources (Ellis and Ashbrook, 1988). Support for this model comes from studies that show that depressed individuals are more likely to show deficits on effortful or controlled tasks than on recognition tasks or tests of implicit memory (Denny and Hunt, 1992; Hertel and Hardin, 1990; Ilsley *et al.*, 1995; Hertel and Milan, 1994; Roy-Byrne *et al.*, 1986).

In contrast, Hertel (1994) proposed that attentional resources are intact in depression, but what is missing is the initiative to control

them. This model is strongly supported by studies that show that use of attentional control strategies such as focusing attention on the task and monitoring relevance of the past (Hertel, 1994) can result in improvement of memory deficits in depression. Similarly, a study conducted by Weingartner *et al.* (1981) reported that depressed individuals failed to use encoding operations that are useful in reorganizing input to facilitate later recall. This is consistent with findings that depressed patients perform more poorly than normal controls on memory tasks involving intentional coding strategies than on tasks in which memory is encoded incidentally (Hartlage *et al.*, 1993).

A phenomenon that is commonly demonstrated in memory studies in depression is that of mood congruency, that is, enhanced encoding and/or retrieval of negatively valenced material while the individual is in the corresponding mood state. The biasing effect of mood on recall of autobiographical memories has been demonstrated in studies of clinically depressed individuals (Clark and Teasdale, 1982, Gilligan and Bower, 1984; Williams and Scott, 1988). For example, Lloyd and Lishman (1975) reported that the speed of retrieval of unpleasant memories in depressed individuals correlated positively with severity of depression. Similarly, Williams (1992) showed that depressed individuals demonstrate a biased pattern of recall with fewer memories of positive experiences.

However, biased recall of negative memories could be attributed to depressed patients actually having more depressing experiences or, conversely, being more likely to interpret neutral experiences in a negative way. Some studies have tried to resolve this ambiguity by using mood induction techniques and randomly assigning normal individuals to 'happy' and 'sad' conditions with the aim of controlling the number of depressing experiences across groups. Results from these studies show that when mood is 'sad', people tend to recall more negative personal memories and fewer positive personal memories, whereas the trend is reversed when mood is more positive (Teasdale and Fogarty, 1979; Teasdale *et al.*, 1980). A similar experiment was conducted on individuals with clinical depression characterized by diurnal mood swings. Results showed that when patients were more depressed, negative memories were more probable and positive memories were less probable, whereas the pattern was reversed for patients in a more positive mood (Clark and Teasdale, 1982).

Similar to findings on autobiographical memory, the biased recall of mood congruent information in depression has also been demonstrated for explicit memory tasks, which are tasks that require conscious recollection of facts or episodes. Studies with normal individuals with varying levels of depression (Gotlib and McCann, 1984) as well as clinically depressed individuals (Bradley and Mathews, 1983; Dunbar and Lishman, 1984) provide evidence for increased recall of negative material and/or decreased recall of positive materials. Depressed mood has been shown to be associated with higher recall for negative content than positive content in incidental free-recall paradigms (Gotlib *et al.*, 2000). The memory bias for negative material appears to be specific to depression-relevant stimuli (Gotlib *et al.*, 2000). For example, Watkins *et al.* (1992) found that depressed individuals exhibited a bias for depression-relevant but not threat-related words. Although there is substantial evidence indicative of memory bias related to explicit memory in depression, there is little evidence supporting this bias on implicit memory tasks (Gotlib *et al.*, 2000; for review see Roediger and McDermott, 1992).

Some researchers hypothesize that the enhanced recall of negative memories and material in depressed mood may provide a cognitive mechanism for the maintenance of depression (Teasdale, 1983), and cognitive theories of depression (Beck, 1967) assert that negatively biased information processing may constitute a vulnerability factor for the onset and/or maintenance of depression. Gotlib and Krasnoperova (1998) reviewed research focusing on information-processing paradigms to examine depression-associated biases in attention to, and memory for, negative information. They concluded that there is strong evidence that depression is associated with concurrent information-processing biases and that some aspects of cognitive functioning, particularly those involving memory processes, may represent a vulnerability factor for depression. Thus, the research on memory biases in depression has potential clinical implications as these maladaptive biases may represent an enduring cognitive characteristic that can be measured and utilized as an indicator of vulnerability for depression (Dalgleish and Watts, 1990).

Neural Correlates of Memory

The cognitive findings described in the previous section are consistent with the neuropsychological data suggesting deficits in a variety of executive functions associated with prefrontal cortex, as reviewed above. Impairment in the strategic use of information, inappropriate deployment of attentional resources, and inadequate maintenance and updating of online material could account for many of the observed memory deficits (for review, see Heller and Nitschke, 1997). A recent study by Herrmann *et al.* (2001) also emphasized the importance of prefrontally mediated executive control strategies in efficient episodic memory retrieval. Additional support comes from an electrophysiological study conducted by Etienne *et al.* (1999), which reported that participants higher in depression did not initiate frontal lobe functions in anticipation of the task that would later improve their memory performance. However, when asked to attend to stimuli, high-depressed individuals showed an association between better memory performance and increased frontal activity. Thus, deficits could be attributed to a lack of initiative in the use of frontally-mediated strategies that are required for memory processing.

The extensive literature on cognitive biases in depression is also highly relevant to the relationship between anterior brain asymmetry and emotional valence (Heller and Nitschke, 1997). There is considerable evidence from a variety of research paradigms that anterior brain asymmetries are associated with valence (for review, see Heller, 1990) with relatively more right than left anterior activity associated with unpleasant affect and sad mood states. This model of asymmetric brain activity would lead us to predict an increased bias towards negatively valenced material and a bias in the opposite direction for positively valenced material, a pattern that is commonly seen in depression.

Besides the anterior brain regions, posterior brain regions have also been implicated in memory deficits in depression. Beats *et al.* (1996) argue that the profile of memory deficits seen in depression can best be characterized by temporal lobe dysfunction in addition to frontostriatal dysfunction. Austin *et al.* (1992) reported that depression-related clinical variables were significantly associated with tests of temporal lobe function such as pattern recognition, spatial span and delayed match to sample task (DMST). A study by Sweeney *et al.* (2000) showed that deficits in bipolar and nonbipolar depressed patients were restricted to episodic memory, suggestive of a more selective dysfunction in mesial temporal lobe function during episodes of depression. It is also possible that these memory deficits are a function of poor visuospatial spatial functioning rather than memory *per se*. Visuospatial functions, implemented in the posterior brain regions, are commonly impaired in depression and could lead to poor encoding resulting in impairment in memory retrieval.

Researchers have also hypothesized that hippocampal dysfunction may contribute to the verbal declarative memory deficits observed in depression. Depressed individuals have smaller left hippocampal volume (Bremner *et al.*, 2000; Mervaala *et al.*, 2000)

and exhibit reduced grey matter density in the left temporal cortex, including the hippocampus (Shah *et al.*, 1998). Additionally, neuropsychological impairment in depressed individuals has been shown to be associated with smaller left hippocampal volume (Shah *et al.*, 1998; von Gunten *et al.*, 2000). Individuals with a history of major depressive episodes but who are currently in remission show significantly smaller left and right hippocampal volumes with no difference in total cerebral volumes (Sheline *et al.*, 1996). It has been hypothesized that hippocampal volume loss in depression is due to neuronal cell death in the hippocampus resulting from adverse effects of stress hormones (Bemelmans *et al.*, 1996). Brown *et al.* (1999) reviewed studies to examine the hypothesis that hypercortisolaemia leads to hippocampal dysfunction and found cognitive impairment consistent with hippocampal dysfunction in depression, bipolar disorder, Cushing's disease, and in those individuals receiving exogenous corticosteroids. It will also be important in future research to take into account the comorbidity of anxiety with depression, because hippocampal involvement has been reported in post-traumatic stress disorder (see Chapter XIX-8).

Attention

Impaired attention has been considered one of the cardinal features of clinical depression. Decreased concentration is an important criterion for the diagnosis of depression in DSM-IV (American Psychiatric Association, 1994). In a thorough review of the attention literature in depression, Mialet *et al.* (1996) reported that impairment was consistently observed on tasks such as simple and choice reaction time tasks (Cornell *et al.*, 1984), the Digit Symbol Subtest (Austin *et al.*, 1992), the Continuous Performance Task (Cornblatt *et al.*, 1989) and sustained attention tasks (Mialet *et al.*, 1996).

Despite the claim that attentional biases are more common in anxious individuals and memory biases more common in depressed individuals (Dalgleish and Watts, 1990; Mathews and MacLeod, 1994), a number of studies have reported an attentional bias for negatively valenced stimuli in depression. Studies have shown that both depressed non-patients and clinically depressed patients took longer to name the colours of tachistoscopically presented depression-relevant words than neutral words (Gotlib and Cane, 1987; Gotlib and McCann, 1984). Furthermore, this bias was not evident after symptomatic recovery. Depressed individuals were also slower on a reaction time task when the distractor words were negative than when they were positive or neutral (Ingram *et al.*, 1994; McCabe and Gotlib, 1993). Similarly, studies have shown that on an emotional dot probe task depressed individuals were faster to detect a dot that replaced negative stimuli (e.g. words and emotion faces) than a dot that replaced positive stimuli (Krasnoperova *et al.*, 1998; Mathews *et al.*, 1996). However, a number of studies failed to find the association between depression and attentional bias to negative stimuli (Clark *et al.*, 1983; Mogg *et al.*, 1993, 2000). Due to substantial comorbidity between depression and anxiety, the possibility that attentional biases observed are not due to depression but to accompanying anxiety cannot be ruled out.

ERP studies have also demonstrated deficits in attentional processing in depression. For example, N2b, an ERP measure of selective attention, was enhanced in non-patients with dysthymia and anhedonia (Fernandes *et al.*, 1999; Giese-Davis *et al.*, 1993) and in patients with major depression (Keller *et al.*, 1999). Due to substantial comorbidity of anxiety and depression, Mohanty *et al.* (2000) used self-report scales that have been shown to distinguish between anxiety, depression and negative affect (Nitschke *et al.*, 2001) to determine which among these is related to the attentional deficit measured by N2b enhancement. The results indicate that it is negative affect, not something specific to depression or anxiety, that carries the N2b enhancement.

Two main hypotheses have been proposed to explain attention disturbances in depression; the resource allocation hypothesis, discussed in detail in earlier sections, and the distractor inhibition hypothesis (Ellis, 1991; Ellis and Ashbrook, 1988; Hertel and Rude, 1991). According to the distractor inhibition hypothesis, depressed individuals show an impaired ability to suppress external and internal distractors, resulting in inappropriate allocation of resources. The increased interference seen on attentional tasks requiring distractor inhibition such as the colour-word Stroop task (Raskin *et al.*, 1982; Trichard *et al.*, 1995) led Lemelin *et al.* (1997) to hypothesize that there is a difficulty in inhibiting distractors in depression. Support was provided for this hypothesis by a study using a negative priming paradigm which demonstrated that depressed patients had reduced ability to inhibit features of a distractor (MacQueen *et al.*, 2000).

Neural Correlates of Attention

An area that has been strongly implicated in both attentional and emotional processing is the anterior cingulate cortex. Bush *et al.* (2000) conducted a meta-analysis of activations and deactivations in the anterior cingulate during cognitive and emotional studies. They reported that the dorsal portion of the anterior cingulate was activated by Stroop and Stroop-like divided attention tasks as well as by complex response selection tasks. This area was deactivated (reduced blood flow or MR signal) by emotional tasks. The rostral ventral portion of the anterior cingulate was activated by tasks that relate to emotional content or symptom provocation (e.g. anxiety and induced sadness in depressed subjects) whereas this region was deactivated by cognitively challenging tasks. The reciprocal suppression of the so-called cognitive subdivision during intense emotional states such as severe depression (Bench *et al.*, 1992; Mayberg, 1997) or film-induced negative emotion could have important implications for attentional deficits in depression. Studies have shown that the cingulate gyrus is important in directed attention and in choosing the appropriate response in the presence of distractions or competing responses (Bench *et al.*, 1993; George *et al.*, 1994b). In a study designed to investigate the neural regions that could be involved in an attentional task in depression and normal controls, George *et al.* (1997) used a Stroop interference task and a modified Stroop interference task consisting of sad words. Results showed that control subjects activated the left anterior cingulate gyrus during a response interference task, whereas depressed individuals failed to show a corresponding increase in activity in this area. Instead, depressed individuals showed increased activation in the dorsolateral prefrontal cortex and visual cortex.

Mayberg (1997) postulated that the dorsal anterior cingulate (in conjunction with the dorsolateral prefrontal cortex) modulates the cognitive and psychomotor aspects of depression while the ventral anterior cingulate appears to modulate the vegetative, autonomic and affective aspects of depression, via the modulation of subgenual prefrontal cortex. Mayberg *et al.* (1999) reported increases in limbic–paralimbic blood flow (subgenual cingulate, anterior insula) and decreases in neocortical regions (right dorsolateral prefrontal, inferior parietal) during depression. After recovery, the reverse pattern, with limbic metabolic decreases and neocortical increases, was seen. A significant correlation between subgenus cingulate and right dorsolateral prefrontal activity was also demonstrated in both conditions. According to Mayberg and colleagues, the reciprocal regional relationship between ventral limbic and dorsal cortical compartments suggests that the negative influence of depressed mood on attention can be attributed to abnormal functional connections between these regions rather than concurrent changes in both sites independently.

Visuospatial Functioning

Depressed individuals have been shown to display deficits on tasks associated with cognitive functions implemented by right posterior regions of the brain (Heller and Nitschke, 1997). Individuals with depression show impaired performance on right hemisphere tasks such as judgement of line orientation, three-dimensional constructional praxis, spatial association learning, and subtests of the WAIS-R performance scale (Flor-Henry, 1976; Asthana *et al.*, 1998; Silberman and Weingarter, 1986). Furthermore, severity of depression has been found to correlate with performance on tests of visuospatial function such as spatial span, pattern recognition and delayed matching to sample (Elliott *et al.*, 1996).

NEUROPSYCHOLOGICAL FUNCTIONING IN MANIA

In contrast to the extensive literature describing the cognitive characteristics of depression, only a few studies have focused on the nature of cognitive impairment accompanying mania. According to Murphy *et al.* (2001) possible explanations for the scarcity of research in this area include practical problems in using standardized neuropsychological assessment procedures in mania, as well as the nature of a manic episode, which may prevent individuals from serving as reliable participants. In general, findings from a variety of studies show that mania is associated with impaired performance on neuropsychological tasks of attention (Bulbena and Berrios, 1993; Taylor and Abrams, 1986), memory, including pattern and spatial recognition memory as well as delayed visual recognition (Bulbena and Berrios, 1993; Bunney and Hartman, 1965; Henry *et al.*, 1971; Johnson and Magaro, 1987; Murphy *et al.*, 1999; Taylor and Abrams, 1986), and visuospatial functioning (Bulbena and Berrios, 1993; Taylor and Abrams, 1986). Some researchers attribute memory deficits in mania to altered patterns of verbal associations (Henry *et al.*, 1971), loosening of associations, and overinclusiveness, which results in an impaired ability to filter environmental stimuli (Andreason and Powers, 1974).

Another major area of functioning that is affected in mania is executive functioning. Individuals with mania exhibit disruptions in social behaviour and decision making similar to those observed in individuals with frontal cortical lesions (Bechara *et al.*, 1994). Mania is associated with deficits on executive functioning tasks such as attentional set-shifting tasks (Clark *et al.*, 2000; Morice, 1990), planning ability (Murphy *et al.*, 1999) and decision making (Clark *et al.*, 2000; Murphy *et al.*, 2001). Ferrier *et al.* (1999) also reported residual impairment in executive function in individuals with euthymic bipolar disorder after controlling for age, premorbid intelligence and depression symptoms.

Depression and mania are associated with distinct physiological, emotional and cognitive characteristics that seem to reflect the activity and function of different brain regions. Gainotti (1972) reported dramatic differences between patients with right brain damage, who exhibited cheerful and euphoric emotions (the 'indifference reaction'), and patients with left brain damage, who exhibited distressed and tearful emotions (the 'catastrophic reaction'). Similarly, another lesion study showed that mania was associated with damage to areas of the right hemisphere that are connected with the limbic system whereas depression was associated with damage to anterior cortical and subcortical regions of the left hemisphere (Robinson *et al.*, 1988). These findings suggest that brain mechanisms implemented in the right hemisphere are associated with negative affect while those associated with positive affect are implemented in the left hemisphere (Shackman, 2000). This is based on the assumption that focal brain lesions act as deactivating forces in the region in which they are located (Heller, 1990). Conversely, in normal individuals, one would expect that negative affect would be associated with increased activity in the right hemisphere while positive affect would be associated with increased activity in the left hemisphere.

Studies examining physiological activity during different emotions have shown that individuals who show greater left anterior activity report increased positive affect, engage in more approach behaviours, and respond more intensely to positive than negative stimuli, whereas individuals who show increased right hemisphere activity report more negative affect, engage in more avoidance behaviours, and respond more to negative than positive stimuli (for review, see Davidson, 1995). Consistent with these findings, the prefrontal cortex has been found to be differentially involved in negative and positive emotions, with increased left anterior activation during positive affect and increased right-sided activation during negative affect (Davidson, 1992, 1998; Davidson *et al.*, 1990).

Some studies comparing the performance of individuals on a range of neuropsychological tests report no differences between mania and depression (Bulbena and Berrios, 1993; Goldberg *et al.*, 1993). These findings have led to the hypothesis that similar neuropsychological processes are involved in mania and depression despite different clinical presentations (Murphy and Sahakian, 2001). However, differences in cognitive functioning between mania and depression emerged on a shifting task using affective stimuli (Murphy *et al.*, 1999). Individuals with mania were impaired in their ability to inhibit behavioural responses and focus attention, while depressed individuals were impaired in their ability to shift the focus of their attention. Furthermore, individuals with mania tended to show a bias towards positive stimuli while depressed individuals displayed a bias towards negative stimuli. These findings are in line with the prediction that different patterns of lateralized prefrontal activity would cause differences in cognitive performance, at least for affective stimuli, between mania and depression (Murphy *et al.*, 1999). Differences in performance were also seen on a recognition memory task, with a more conservative response bias associated with depression and a more liberal response bias associated with mania (Corwin *et al.*, 1990). Thus, cognitive performance in depression and mania seems to be influenced by the affective nature of the task as well as affective responses to task stimuli.

CONCLUSION

We have reviewed extensive evidence from a variety of sources suggesting that depression is associated with biases or deficits in cognitive functioning. Areas of cognitive functioning that are impaired in depression include executive functioning, attention, working memory, memory, particularly explicit memory, and visuospatial functioning. Further research aimed at the identification of moderator variables such as depression severity, psychosis, patient status and medication status is required to clarify the extent and nature of the relationship between depression and the aforementioned cognitive deficits.

Evidence from a variety of techniques such as neuropsychological testing, as well as lesion, electrophysiological and haemodynamic measurement, has implicated the role of different brain regions in cognitive functioning in depression. In general, cognitive deficits are associated with abnormal patterns of activity in anterior brain regions, particularly dorsolateral prefrontal, medial prefrontal, orbitofrontal and anterior cingulate cortex. Additionally, memory, attentional and visuospatial deficits appear to be related to abnormal activity in posterior brain regions, including the temporal lobe, and limbic structures such as the hippocampus.

Despite extensive evidence indicating of cognitive functioning deficits in depression, researchers examining the neuropsychology of depression have focused largely on the relationship of regional

brain activity to emotion and not cognition (Heller and Nitschke, 1997). For a better understanding of cognitive neuropsychological disturbances in depression it is important to develop a comprehensive theoretical formulation elucidating the relationship between various brain regions and cognition. Examples of theoretical formulations relating regional brain activity and cognition in emotional disorders include Heller (1990, 1993), Davidson (1998), Drevets and Raichle (1998) and Mayberg (1997). For better characterization of cognitive deficits in depression it is also important to examine how emotion modulates cognition and vice versa. More specifically, studies designed to investigate reciprocal brain mechanisms as well as integrated brain mechanisms for emotion and cognition have important implications for cognitive deficits in depression. Insights into these issues could also benefit research on the cognitive correlates of mania. For example, earlier studies using affectively neutral stimuli have yielded findings suggesting similarities in cognitive dysfunction for mania and depression. However, recent studies utilizing experimental designs that require both cognitive and emotional processing have indicated biases in information processing and abnormal responses to feedback that appear to be consistent with distinct patterns of regional brain activity in mania and depression.

The association between neuropsychological impairment and depression has a number of clinical and research implications. According to Austin *et al.* (2001), both affective disturbance and cognitive impairment need to be considered as equally important manifestations of depressive disorder. They propose that cognitive testing can be used as a helpful adjunct in clinical evaluation of patients both at initial episode and following recovery. Cognitive testing in depression has potential as an evaluative tool because performance on cognitive tests has been shown to be sensitive to a variety of depression-related clinical variables such as severity (Moffoot *et al.*, 1994), presence of psychosis (Basso and Bornstein, 1999) and depression subtype (Silberman *et al.*, 1985). Despite evidence supporting sensitivity of cognitive measures to depression, it is still debatable whether cognitive deficits such as memory impairment are specific to depression or a reflection of a more general form of psychopathology (Burt *et al.*, 1995). Thus, an important diagnostic issue for clinicians is to determine whether findings of impaired memory in depression are specific to depression and distinctive enough to be used for purposes such as differentiating between depression and dementia (Burt *et al.*, 1995).

An area of research that has immense clinical utility involves the role of cognitive biases and deficits in the onset, course and maintenance of depression. For example, cognitive precursors such as information-processing biases may taint perceptions of previously depressed individuals and predispose them to the mood disturbance (Mathews and MacLeod, 1994). According to another cognitive perspective, known as the diathesis-stress model of cognitive vulnerability (Beck *et al.*, 1979), depression-relevant experiences trigger latent negative thoughts, leading to an increased risk of depression. Mineka and Gilboa (1998) argue that cognitive biases may play a threefold role, in which they mediate the course of emotional disorders, predict vulnerability to relapse, and contribute to the onset of depression.

In conclusion, an understanding of the neuropsychological concomitants of depression has the potential not only to enhance basic scientific knowledge of the disorder but also has important implications for intervention. Since researchers have proposed that cognitive characteristics such as executive functioning deficits, memory biases and attentional deficits play an important role in increasing risk for, as well as the maintenance of, depression (Mineka and Gilboa, 1998), treatment strategies aimed at improving neurocognitive functioning in these domains may prove to be beneficial in depression. Support for such strategies comes from treatment of schizophrenia and organic brain syndromes where neurocognitive rehabilitation programmes focusing on cognitive flexibility, working memory and planning ability have been shown to be effective (Delahunty and Morice, 1996; Garety *et al.*, 2000; Moore *et al.*, 2001; Morice and Delahunty, 1993). Similarly, research on patterns of abnormal brain activity in depression may contribute to an understanding of the mechanisms underlying pharmacological and psychological treatment in depression. For example, Mayberg *et al.* (1999) has suggested that the neural pathology associated with depression can be addressed via a top-down (psychotherapeutic) or bottom-up (pharmacological) intervention strategy, based on a model of reciprocal relationships between limbic and prefrontal cortical regions. Bench *et al.* (1995) reported that recovery from depression was associated with increases in rCBF in the same areas in which focal decreases in rCBF were described in the depressed state. Another example comes from the field of transcranial magnetic stimulation (TMS), a method that allows non-invasive stimulation of regions of the cerebral cortex. Using this method, researchers have demonstrated that left dorsolateral prefrontal cortex TMS resulted in significant decreases on measures of depression (Pascual-Leone *et al.*, 1996). Future research might capitalize on these findings by using cognitive and emotional activation paradigms to activate regions of the brain such as the left dorsolateral prefrontal cortex, and examining the effect of these manipulations on both depressed mood and cognitive deficits in depression.

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XVIII-8

Functional Neuroscience of Mood Disorders

Michel Le Moal and Willy Mayo

INTRODUCTION

The evolution of criteria, symptoms and models for diagnosing mood disorders, as reflected in classical twentieth-century textbooks, has been a long process, if we consider Kraepelin's (1921) phenomenological characterizations to constitute a historical landmark. This is especially true for classification and the progressive importance of biological theories. A depressive state is considered to result from either endogenous determinants or a reaction to environmental or life events and a failure to adapt. The evolution from the first edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-I) (American Psychiatric Association, 1952) to the fourth edition (DSM-IV) (American Psychiatric Association, 1994) illustrates, through the push for more reliable and valid diagnoses and for a change to a categorical system (notably for research), profound changes due largely to the new pharmacological thought and its aminergic theories and derivatives. However, it remains clear that the parallel neurobiological investigation on mood has not played a pivotal role, and that pathophysiological mechanisms have still not been identified, at least in a specific manner, in relation to the categories proposed by the last DSM (Boland and Keller, 1999). Moreover, the relation between the genetic aspects of susceptibility to mood disorders (mainly bipolar) and possible defects at the neuronal levels have not yet been demonstrated. These problems are now at the forefront of psychiatric research. Thanks to advances in statistical analysis combined with powerful new biotechnology methods, hypotheses can now be explored more rapidly. Replicated linkage has, at present, proposed susceptibility loci on chromosomes X, 18 and 21, but a single locus genetic model is in no way responsible for unipolar, bipolar and recurrent disorders (for review, see Sanders *et al.*, 1999), leading to multifactorial vulnerabilities, including environmental factors as well as many genes. Finally, promising data from brain-imaging techniques have opened a new era to help identify circuitries involved in the physiopathology and proposed new neuroanatomies of mood disorders (Manji *et al.*, 2001). However, these investigations raise methodological problems.

NEUROSCIENCE OF MOOD DISORDERS: THEORETICAL AND METHODOLOGICAL CONSIDERATIONS

Difficulties for an Experimental Psychopathology of Mood Disorders

Basic and experimental neurosciences and clinical neuropsychiatry have joined forces over the past decade to promote the biological foundations of mental illness. The task is so difficult that it can be considered, together with the mind–consciousness problem, as the last frontier in biomedical research and the definitive inclusion of psychiatry in medical sciences. In spite of significant advances that have transformed the clinical perspectives in neurology and related disorders (obsessive–compulsive disorders and Tourette's syndrome are good examples), the growing tendency to 'neurologize' classic psychiatric syndromes, such as the various forms of schizophrenia, has not yet led to universal consensus regarding the aetiology and pathophysiology of such major mental illnesses.

From a neurobiological standpoint, and in the perspective of the neuroanatomical bases of the syndromes, three main general questions remain to be solved. First, when considering a given syndrome or disorder, or a specific mental disease, the symptoms that define the disease still have to be related to a given (or a set of related) 'normal' integrated functions. In other words, a continuum from physiology to pathology and vice versa is expected and, if so, then these functional dimensions should correlate with anatomical loci. The neurobiological interpretations of the symptoms have to be placed within the framework of the structure–function paradigm (Pearlson, 1999), in which modern neurosciences and neuropsychology have made enormous progress, even regarding mental functions and higher cognitive processes. The neuropsychological paradigm first applied to the neurology of cortical functions is now open to psychiatric symptoms. For instance, the disinhibition syndrome characteristic of acute mania will be referred to a similar syndrome sometimes seen after frontal brain injury, and mania will be attributed to a dysfunction of this region.

Second, by the same token, using working hypotheses inspired by experimental medicine, and considering that the concept of experimental psychopathology results directly from the structure–function paradigm, the neurobiologist could create appropriate animal models of the given illness by manipulating either the function or the underlying structure. In this context, the interpretation of experimental conditions is limited by the fact that most of the major human mental disorders do not exist *per se* in animals. Moreover, two other problems render experimental psychopathology difficult: on the one hand, manipulation of the model to obtain all the basic symptoms or dimensions that could characterize the illness or syndrome is theoretically impossible because they are known mainly by subjective report, and on the other hand, these basic symptoms may also be found in other heterogeneous disorders, because of the difficulty of defining mental illness, as reflected by ever changing concepts in psychiatry, or due to the clinical reality of considerable comorbidities. For instance, many depressed patients display anxiety symptoms, while anxiety and depression are also considered separately in DSM-IV.

Third, the pharmacological paradigm is not totally satisfying. It is based on a well-known working hypothesis: (1) the active drug acts on neuronal systems that are defective and part of the pathophysiology of the disease; (2) it is of major interest to discover the site of action of therapeutic drugs, and these sites or structures have generally been characterized as being the specific

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neurotransmitter systems; and (3) the more a given drug acts on many transmitter systems, the better is its therapeutic efficacy. However, the transmitter systems cannot be considered as the anatomical locus of the mental functions whose defects are reflected in the symptoms; in other words, it is difficult to imagine that the serotoninergic neurons are the anatomical substrates of symptoms characteristic of mood disorders. Of course, it is possible that the primary defect has taken place at this level, but that the symptoms result from the dysfunction of the region modulated by these neurons whose cell bodies are located in the reticular formation and related structures (Le Moal, 1995; Le Moal and Simon, 1991).

From the Physiology of Emotion to the Pathophysiology of Mood

Mood disorders have — as a trivial definition — a disturbance of mood as the predominant feature (DSM-IV). The term 'mood' refers to something based on an empirical consensus but nevertheless represents a construct with multiple facets that needs to be clarified according to its roots (old English *mod*, meaning mind, soul, courage, or German *mut*, meaning mental disposition, spirit, courage, or an Indo-European prefix, *me-*, adding the sense of strong activity, energy), leading to the general definition of a particular state of mind or feeling, humour, temper or spirit. According to common sense, mood is a mixture of feeling, humour and state of mind, i.e. a state of emotional tone and cognition. The construct emotion is not considered in the DSM-IV or in classical psychiatric dictionaries as concerns its generally understood meaning (strong feelings aroused to the point of awareness, complex reactions with both physical and mental manifestations, whatever the specific labels used, e.g. fear, anger, love, hate). Conversely, the neurobiologist deals with emotion and the construct affect but not mood. After two decades in which studies on emotion were not a central theme in neurosciences — a topic blunted largely by a hard-line, reductionistic, new-wave computational cognition that neglected feelings and emotions as important aspects of everyday life — a huge amount of knowledge has now been accumulated (LeDoux, 2000), and publications have acknowledged the neurobiology of emotion, or affective neuroscience, as a major discipline (Damasio, 1994; Damasio, 2000; Lane and Nadel, 2000; LeDoux, 1996; Pankseep, 1998; Rolls, 2001). Classically, specialists attempt to identify genetically dictated emotional operating systems considered to exist in both animal and human brains, and to evaluate how emotional feelings are generated. The vast difference in cognitive abilities among species contrasts with the homology in mammals concerning ancient subcortical evolutionarily derived systems. The problem is to appraise whether these data have something to do with mood, and whether they represent the neurophysiological foundations of mood disorders. As stated by Pankseep (1998; p. 5), 'Clinical psychology and psychiatry attempt to deal at a practical level with the underlying disturbances in brain mechanisms, but neither possesses an adequate neuroconceptual foundation for the sources of emotionality upon which systematic understanding can be constructed.' It is a challenge for this decade to relate modern cognitive neuropsychology to a new 'neuropsychology of emotion' (Lane and Nadel, 2000) and a neuropsychology of mood disorders.

NEUROSCIENCE AND NEUROENDOCRINOLOGY OF EMOTIONS

Anatomical Networks

Early in the twentieth century, neurophysiologists and clinicists suggested that emotions originated from (or were processed) at a subcortical level (Dupré-Devaux, 1901; Nothnagel, 1889) and that this subcortical 'emotional' level would be under the control of the cortex (Cannon, 1929). During the same period, Bard (1928) demonstrated in animals the critical role of a subcortical structure, namely the hypothalamus. These data were confirmed in humans through the observations of Foerster and Gagel (1933), indicating that lesions located in the hypothalamus could induce profound emotional disturbances. However, the first description of a network devoted to the processing of emotions was due to Papez (1937). For Papez, the 'stream of feeling' flows through interconnected medial structures. External inputs through primary sensory cortices reach the hippocampus, and via the mammillary bodies this information arrives at the hypothalamus. For Papez, the hypothalamus still represents the core structure of the emotional circuitry; its role is to associate an emotional value with the sensory afferents and to induce the associated motor response. From mammillary bodies, information can also reach the cingulate gyrus, and through the retrosplenial cortex it induces a feedback on the hippocampal formation. Interestingly, a direct route has also been described from the thalamic nuclei to the hypothalamus, possibly explaining the relatively quick and direct processing of some emotional information (Figure XVIII-8.1).

A few years later, Yakovlev (1948) suggested that emotional and motivational expressions are controlled by a 'middle layer' of the brain composed of partially myelinated long neurons located in the limbic thalamus, basal ganglia and hippocampal and olfactory structures. Yakovlev also dissociated a mediodorsal pathway from a basolateral pathway. The mediodorsal pathway centred on the hippocampus is similar to the circuit of Papez and seems to be involved mainly in memory functions (Livingston and Escobar, 1971). The basolateral pathway, more involved in mood regulation, involves the amygdala and its connections with the hypothalamus, dorsomedial thalamus and orbitofrontal cortex. The functional anatomy of emotional processes proposed by Papez and Yakovlev was reanalysed by McLean (1949) as the concept of the limbic system (referring to the *grand lobe limbique* initially identified by Broca in 1878). This limbic system concept is still in debate (Kötter and Meyer, 1992; LeDoux, 1991), but the recurrent problem in the search for neural substrates of emotions again lies in the definition of the word and the construct of emotion itself (Lewis and Haviland, 1992). Currently, only the circuits involved in the detection and response to danger seem to be well defined. The core structure is the amygdala: the lateral part receives cortical sensory or hippocampal inputs, and the central part controls fear responses by way of projections to the brainstem areas. The crucial role of the amygdala was evidenced by numerous studies in animals (Gloor, 1960; Kaada, 1951) and, more recently, by the experiments of LeDoux (for review, see LeDoux, 2000). These experiments also emphasize the previously suggested dual networks of Papez, i.e. a direct thalamolimbic pathway and a classical thalamocorticolimbic pathway (Figure XVIII-8.1b).

The Monoaminergic Hypothesis

Among the neurotransmitter circuits of the brain studied for their possible roles in mood disorders, monoaminergic systems have been implicated repeatedly since the 1960s. In 1965, the hypothesis of a deficit in monoamines — mainly noradrenaline — was proposed for the first time (Bunney and Davis, 1965; Schildkraut, 1965). This hypothesis was supported by evidence of low urinary levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the principal metabolite of noradrenaline, in depressed patients (mainly in bipolar depression) compared with control subjects (Maas *et al.*, 1972), and by the fact that antidepressant drugs (tricyclics and monoamine oxidase inhibitors) are known to increase noradrenergic transmission. As well as the depressive phase of bipolar disorders,

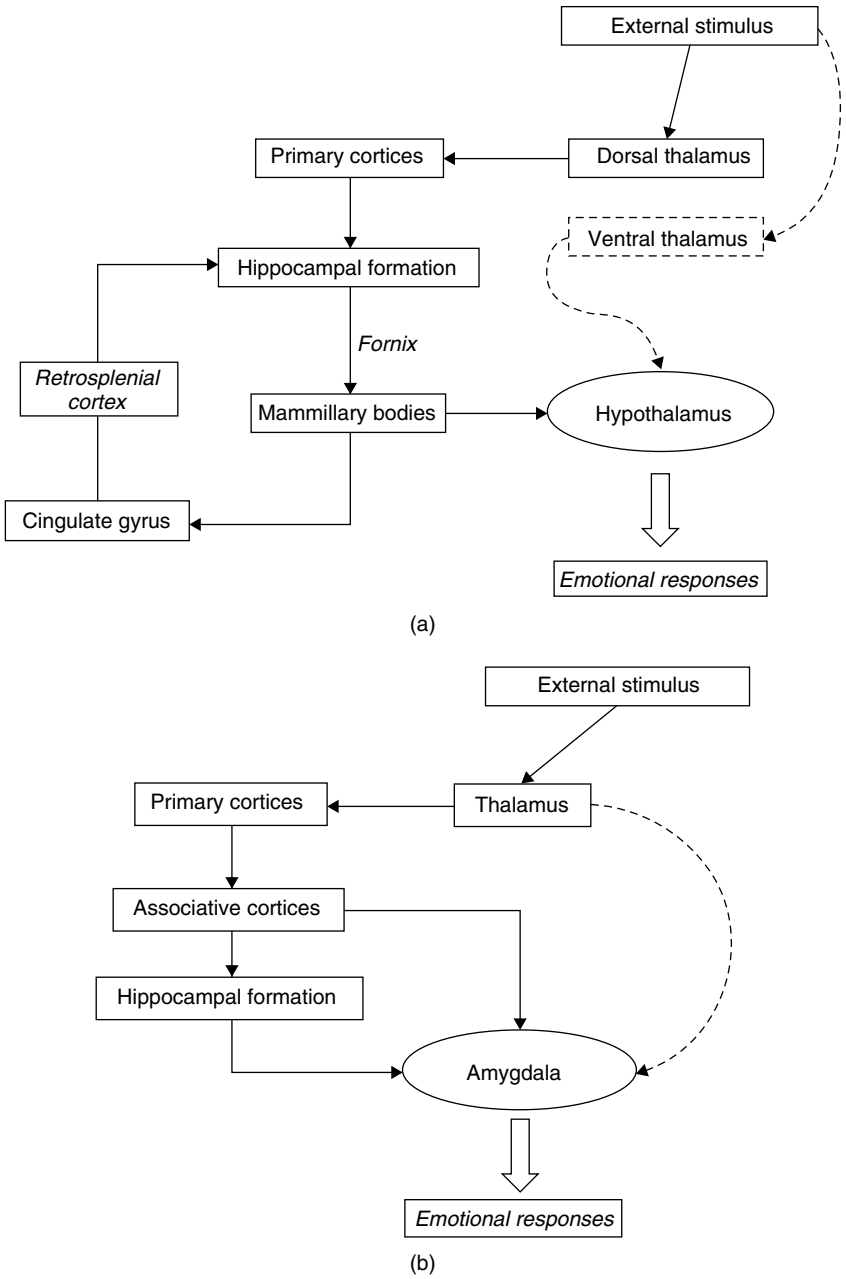

Figure XVIII-8.1 Models of anatomical networks integrating cortical and subcortical structures that could associate emotional values with sensorial afferents. (a) the model of Papez; (b) the model of LeDoux

the noradrenaline hypothesis was proposed to explain the manic phase of the illness in terms of increased noradrenaline activity (Schildkraut, 1965), and was supported more recently by Maj *et al.* (1984) and Swann *et al.* (1987). Further evidence came from the use of α-methylparatyrosine (AMPT), a competitive inhibitor of the rate-limiting enzyme for the synthesis of noradrenaline that reinstalls depressive symptoms in patients previously treated with noradrenaline uptake inhibitors (Miller *et al.*, 1996). Interestingly, AMPT has a far less significant effect in normal subjects, and is ineffective in patients treated with selective serotonin reuptake inhibitors (SSRIs) (Delgado *et al.*, 1993), indicating that noradrenaline alterations alone are not sufficient to induce depression. α2-Adrenergic and β-adrenergic receptors seem to be implicated in the pathophysiology of depression. Indeed, an increase of α2 receptors has been described both peripherally (platelets) and centrally (Garcia-Sevilla *et al.*, 1987; Meana *et al.*, 1992), indicating increased sensitivity of these receptors, inducing a decrease of the activity of noradrenergic neurons. β-Adrenergic receptors have also been implicated in depression, but there are many discrepancies in the reports available.

A serotonin (5-hydroxytryptamine, 5-HT) hypothesis for depression was also proposed to explain the pathophysiology of the disease as well as the mechanisms of antidepressants. This hypothesis is supported by various observations. Concerning precursor availability, there are data indicating that plasma concentrations of L-tryptophan are lower in some depressive subjects compared with

Table XVIII-8.1 Relationships between the HPA axis, neurotransmitter systems and some trophic factors on the hippocampal formation integrity in depression, antidepressant treatments and stress. The data refer to a cellular hypothesis of depression in which neural plasticity plays a crucial role. Neuronal atrophy or death in the hippocampal formation and prefrontal cortex contribute to the pathophysiology of mood disorders. The stress factor and life events may contribute to differential individual vulnerabilities to subsequent stressors and to depressive trends. It is possible that antidepressant treatments could oppose these adverse cellular effects, resulting in loss of neuronal plasticity by increasing either neurogenesis or cell survival. The molecular mechanisms underlying these effects include the role of cAMP signal transduction cascade and neurotrophic factors (after Duman and Charney, 1999)

				Hippocampal plasticity	
	Hormones (glucocorticoids, CRF)	Neurotransmitters (5-HT, noradrenaline)	Trophic factors (BDNF)	Neurogenesis (DG)	Cell number (CA3) or volume
Depression	↗	↘	?	?	↘
Antidepressant treatment (SSRI, lithium, ECS)	↘	↗	↗	↗	↗
Stress	↗	↘ ?	↘	↘	↘

DG, dentate gyrus; ECS, electroconvulsive shock.

normal subjects (Meltzer and Lowy, 1987). These lower levels of the precursor could lead to decreased serotonin synthesis in the central nervous system (CNS), and experimental reductions of plasma L-tryptophan can induce some symptoms of depression (Delgado *et al.*, 1989) or reverse the action of SSRIs (but not noradrenergic antidepressants) (Delgado *et al.*, 1999). Concerning the evaluation of metabolites, there are no clear data linking the cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and the depressive symptoms, except for impulsive suicidal behaviour (Asberg *et al.*, 1976; Roy *et al.*, 1989). A decrease in the binding of the serotonin transporter (SERT) in platelets of depressed subjects compared with normal subjects was reported frequently (Ellis and Salmond, 1994), and this decrease was also observed in the occipital cortex of depressed subjects (Perry *et al.*, 1983). A decrease in SERT binding has been described in suicide victims in the frontal cortex (Stanley *et al.*, 1982) and in the ventrolateral prefrontal cortex (Arango *et al.*, 1995). Concerning the possible alteration of serotonin receptors in depression, the complexity of these receptors must be taken into account (there are at least seven receptor families with multiple members in each family) (Saxena, 1995). Nevertheless, most research has focused on 5-HT_{2A} and more recently on 5-HT_{1A} receptors. 5-HT_{1A} receptors are found mainly in the CNS, whereas 5-HT_{2A} receptors are found in various central and peripheral tissues. Many studies report an increase in the binding of 5-HT_{2A} receptors in platelets of depressed subjects compared with normal subjects, and treatment of depressed patients with antidepressants downregulates 5-HT_{2A} receptors in platelets (Biegon *et al.*, 1990; Cowen *et al.*, 1987). The 5-HT_{1A} receptor, which controls the rate of firing of 5-HT neurons, exhibits a reduced binding in the frontal cortex of depressed medicated subjects compared with controls, suggesting a downregulation effect of antidepressants (Yates *et al.*, 1990). Altogether, these studies evidence the implication of noradrenaline and 5-HT systems in the pathophysiology of depression and an alteration of their functioning with compounds having antidepressant properties.

The pathogenesis of mood disorders cannot be explained only in terms of altered noradrenaline and/or 5-HT neurotransmission. Indeed, an involvement of dopamine was suggested several years ago (Serra *et al.*, 1979). Subsequent animal and clinical studies have suggested that drugs that increase dopamine levels (amphetamine, cocaine) induce mood elation, while drugs that decrease dopamine levels (reserpine) and dopamine receptor blockers (neuroleptics) can depress mood and induce dysphoria, respectively (for review, see Willner, 1995). Chronic antidepressant treatments increase the sensitivity to dopamine receptor stimulations, possibly trough an increase of D2/D3 receptor function and a decrease in D1 receptor number and activity (D'Aquila *et al.*, 2000). Interestingly, these changes occur mainly in the mesolimbic dopaminergic system. This system is crucial for reward-related behaviour and incentive motivation (Le Moal, 1995), which are altered in depression. In this circuit, the modulation of the dopaminergic transmission in the nucleus accumbens may represent 'a common final pathway responsible for at least part of the spectrum of behavioural actions of antidepressant drugs' (Willner, 1995); interestingly, recent imaging studies report significant modifications of the volume of this nucleus in mood disorders (see Anatomical considerations following postmortem studies below).

It remains to be determined whether the aminergic changes are a primary cause, a consequence, or only a part of a complex chain of pathophysiological events. Answering this question would help us to understand the real place of antidepressants as therapeutic agents. These agents will be taken into account when neuroplastic changes due to therapeutics are considered (see Table XVIII-8.1).

The Hypothalamic-pituitary-adrenal Axis, Stress, and the Neurotrophic Hypothesis

Patients suffering from depression exhibit some symptoms that indicate a deregulation of the main neuroendocrinological system implicated in the stress response, the hypothalamic-pituitary-adrenal (HPA) axis, notably hypercortisolaemia and the absence of suppression in the dexamethasone suppression test. Corticotropin-releasing factor (CRF) is a peptide involved in the regulation of the HPA axis, and its physiology is affected in depression. In depressed patients, there is an increase of CRF concentrations in the CSF (Nemeroff *et al.*, 1984), and these concentrations can be normalized by various antidepressant treatments (De Bellis *et al.*, 1993; Nemeroff *et al.*, 1991). Thus, an alteration of HPA axis regulation in depression could magnify some deleterious effects of stress (Figure XVIII-8.2).

It is well known that stress can induce neuronal atrophy in some limbic structures, particularly the hippocampus (Sapolsky *et al.*, 1990), and a reduced hippocampal volume has been described in depressed patients (Sheline *et al.*, 1996). Furthermore, it is now established that stress and the associated glucocorticoid release decreases neurogenesis in the hippocampus (Gould *et al.*, 1997; Tanapat *et al.*, 2001). Conversely, antidepressant treatments increase hippocampal neurogenesis (cell proliferation

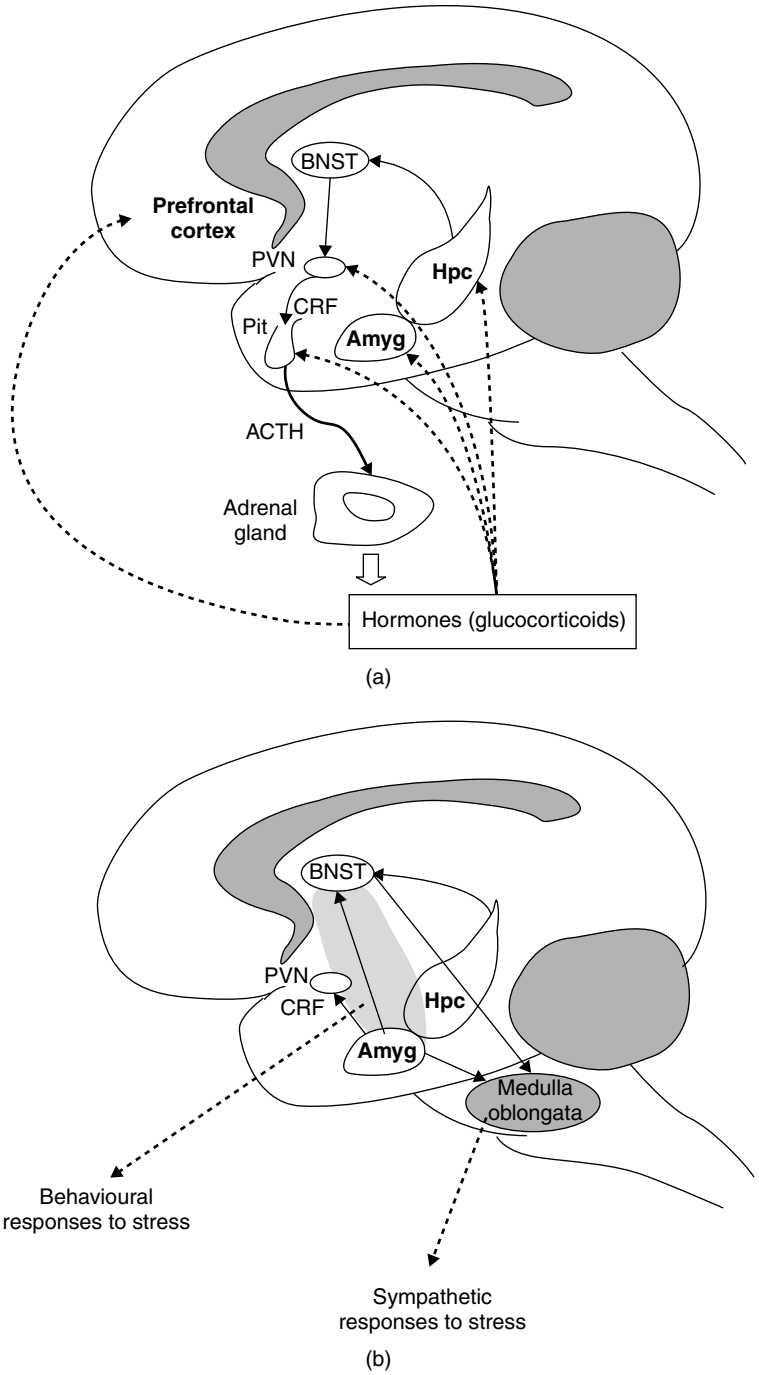

Figure XIII-8.2 The brain stress systems. (a) HPA axis stress system: Stressful stimuli increase CRF, which in turn stimulates adrenocorticotropic hormone (ACTH) release from the pituitary, which results in enhanced release of glucocorticoids from the adrenal gland. High levels of glucocorticoids, through negative feedback, decrease CRF synthesis at the level of the paraventricular nucleus (PVN) but activate CRF activity at the level of the central nucleus of the amygdala. (b) Extrahypothalamic stress system: Stressful stimuli also activate CRF systems in the basal forebrain, notably the bed nucleus of the stria terminalis and the central nucleus of the amygdala, to help mediate behavioural responses to stressors and to mediate sympathetic activation associated with stressors Amyg, amygdala; BNST, bed nucleus of the stria terminalis; Hpc, hippocampus; Pit, pituitary gland Redrawn from Koob and Le Moal (2001)

and cell survival) (Malberg *et al.*, 2000). The mechanisms underlying such actions of antidepressants are still unknown. Antidepressants may normalize the HPA axis activity, which may lead to an upregulation of brain-derived neurotrophic factor (BDNF), reported to be increased in the hippocampus of subjects treated with antidepressants (Nibuya *et al.*, 1995), possibly by an activation of the cyclic adenosine monophosphate (cAMP)–cyclic adenosine monophosphate response element binding protein (CREB) cascade. BDNF is known to enhance the growth and survival of 5-HT cells (Mamounas *et al.*, 1995), and to increase synaptic strength in the hippocampus (Kang and Schuman, 1995), and it is downregulated in the latter by stress (Smith *et al.*, 1995). In some animal models of

depression, BDNF seems to have antidepressant-like effects (Siuciak *et al.*, 1996). Imaging methods have shown smaller pituitary volumes in bipolar but not unipolar disorders, suggesting a dysfunction in the HPA axis (Sassi *et al.*, 2001).

According to the neurotrophic hypothesis, depression may result from damage of hippocampal neurons, possibly stress-induced and amplified by the hypercorticosterolaemia of depressed patients (Table XVIII-8.1). Some antidepressant treatments could enhance the expression of neurotrophic factors (BDNF), thus stimulating hippocampal neurons. Nevertheless, this hypothesis only seems to be related to depression associated with stress, and additional studies are required to understand better the link between the neuroprotective role of antidepressants and the compensation of hippocampal atrophy.

MORPHOLOGICAL STUDIES IN A NEUROPSYCHOLOGICAL CONTEXT

Anatomical Considerations Following Post-Mortem Studies

Post-mortem studies may provide evidence for the hypothesis that structural-morphological changes are responsible for the chronic functional disorders (for review, see Baumann and Bogerts, 2001). In contrast to schizophrenia and other major psychoses, little has been done to investigate the neurohistological correlates of mood disorders. Studies resulting from subjects who committed suicide frequently suffer from the lack of robust amnestic and retrospective diagnosis that establishes firmly the existence of a chronic monopolar or bipolar disorder. Future studies should use well-characterized brain specimens, well-constructed experimental designs, and well-controlled confounds (for review, see Lewis, 2002). However, investigations suggest structural disturbances in the parahippocampal cortical regions with malformations in the entorhinal lamination (Bernstein *et al.*, 1998), reduction in size (Altshuler *et al.*, 1990), and alterations in the temporal lobe. The prefrontal regions have also been investigated, and a decrease in laminar thickness was found without a change in laminar density (Goodwin, 1997; Rajkowska, 1997), while layer-specific reduction of interneurons was noticed in the anterior cingular cortex (Vincent *et al.*, 1997). No firm data or significant investigations can be reported concerning the cytoarchitecture of monoaminergic neurons, although some studies favour a role for indolaminergic neurons with a reduced neuron number in the ventral subnuclei of the dorsal raphe nucleus in major depression without a clear generalization for bipolar disorder (Baumann and Bogerts, 2001).

Volume measures have been undertaken for large regions, such as the telencephalic basal ganglia and limbic regions (Baumann *et al.*, 1999b; Baumann *et al.*, 1999c), but possible post-mortem artefacts (autolysis, medication, age, gender) have to be excluded. Results suggest that predominantly limbic-affiliated basal ganglia are involved in the pathology of mood disorders, irrespective of the diagnosis of polarity: there are reduced volumes in the right putamen, right and left pallidum (external part), and, in the most affected patients, the left nucleus accumbens. Moreover, the data suggest a trend towards reduction of the left amygdala in current major depression but not in bipolar disorder. These studies do not evidence changes in the volume of the hippocampus, stria terminalis, temporal horn or thalamus. In major depression, the right hypothalamus was smaller.

In parallel, brainstem structures have been explored, in particular for a possible pathomorphology of the aminergic nuclei. In the locus coeruleus, the main source of forebrain noradrenergic innervation, the total number of neurons was higher bilaterally in bipolar disorder (Baumann *et al.*, 1999c), in particular in the rostral two-thirds and in the dorsal part. This finding is considered to be consistent with others (for review, see Baumann and Bogerts, 2001). Fewer tyrosine hydroxylase-immunoreactive neurons were found in a depressed non-suicide group compared with depressed suicide and control groups (Baumann *et al.*, 1999a). The cytoarchitecture of the dorsal raphe has also been investigated, and the ventral part shows fewer neurons, especially of the ovoid and round types, in patients with unipolar or bipolar mood disorders; a compensatory adaptive process is noticed in the form of increased nucleolar size. Such structural changes may contribute to an impaired serotoninergic innervation of brain regions supposed to be involved in the pathophysiology of mood disorders (Arango *et al.*, 1990; Baumann and Bogerts, 2001).

Structural Imaging

Structural imaging for evaluating morphology changes includes computed tomography (CT) and magnetic resonance imaging (MRI). These studies are just beginning to guide proposals for functional and structural models. Most of the studies reveal significant prevalence of hyperdensities in deep and periventricular white matter, which have also been found in the thalamus, basal ganglia, putamen and globus pallidus, especially in elderly subjects during their first experience of unipolar depression (Chimowitz *et al.*, 1992; Drevets *et al.*, 1999; Krishnan *et al.*, 1988; Soares and Mann, 1997), in the course of late-life unipolar depression (Lidaka *et al.*, 1996). One group of subjects, who experienced their first major depressive episode later in life and exhibited cerebrovascular defects as an aetiological factor, had left frontal lobe and striatum hyperdensities (Greenwald *et al.*, 1996; Greenwald *et al.*, 1998). These anatomical characteristics do not seem to be found in bipolar disorder. In parallel, neuromorphometric explorations report regional volumetric reductions in the temporal and frontal cortices. There are some data on volumetric reduction of the hippocampus and amygdala in unipolar depression (Botteron and Figiel, 1997; Duman and Charney, 1999; Pearlson *et al.*, 1997; Sheline *et al.*, 1996), and the same type of information was found for the prefrontal cortex (Drevets *et al.*, 1997a). Observations are contradictory for the basal ganglia.

The safer conclusion deduced from structural neuroimaging is that some structural changes can be observed in unipolar depression, essentially in late-onset cases, while in other pathological situations, and in young or adult subjects or bipolar episodes, brain morphology seems to be relatively preserved (Soares and Mann, 1997). The regions targetted were those shown to participate in emotional processing (thalamus, basal ganglia, frontal lobe, hippocampus, amygdala), with the exception of the hypothalamus, brainstem nuclei and regions related to neuroendocrine-autonomic systems less tractable to neuroimaging approaches.

Functional Imaging

Functional imaging is open to new concepts closer to behavioural physiology, revealing physiological correlates of depressive states but also of asymptomatic situations. As discussed previously, the theoretical assumption is that mood disorders correspond in terms of symptoms to the disruption of emotional processes normally depressed or regulated in normal subjects, and reflect the passage from homeostatic regulation to allostasis and pathology (Koob and Le Moal, 2001). Again, such a hypothesis includes the fact that hyperemotional situations experienced by normal subjects (fear, anxiety, sadness) are correlated with metabolic and blood flow–haemodynamic changes that might be related functionally to pathophysiological changes thought to take place in mood disorders (Banki *et al.*, 1992). It is becoming possible to quantify neuroreceptor binding and neurochemical abnormalities, and such an approach aims to provide neuroimaging bases for research concerning vulnerability. In practice, as regards mood disorders, the

literature is composed essentially of glucose metabolism and blood-flow studies. The elevation or decrease of the latter may reflect an increase or inhibition of neurotransmitter functioning, regardless of the cause, and comparison with normal-state or healthy subjects aims to provide evidence on correlative changes due to depressive symptomatology (affective, cognitive, behavioural).

Reviews of more than 30 studies confirm that deficits are present when these parameters are examined regionally (Soares and Mann, 1997). A decrease in the cerebral frontal cortex was observed and confirmed with a variety of methodologies, as well as abnormalities in the basal ganglia and thalamic and cerebellar areas, in both unipolar and bipolar patients. This seems to correlate with the severity of the illness, especially for the frontal cortex. Data concerning the temporal lobe and other limbic regions, such as the hippocampus and amygdala, are more conflicting, at least for bipolar patients.

Integration of Different Approaches

Integration of data from functional and structural imaging is not easy. With respect to the main structural characteristics, the following points can be observed: (1) increased incidence of white-matter lesions in bipolar subjects and hyperdensities in unipolar major depressions; (2) smaller basal ganglia in unipolar patients and smaller cerebellum in unipolar and bipolar patients; and (3) conflicting findings for smaller frontal lobe in unipolar patients. The complex symptomatology and the various syndromes that characterize mood disorders suggest the existence of an extensive network and subtle imbalances and dynamics between regions as well as between neurotransmitters due to various causes, such as genetic-developmental, environmental and vascular ageing, and these aetiologies penetrate the circuits in different loci (Figure XVIII-8.3). Moreover, a dysfunction at one level of the circuit results in dysfunction in other areas. These modular systems could also be involved in other pathologies or comorbidities, such as Tourette's syndrome or obsessive–compulsive disorders.

The prefrontal cortex, the most consistently identified functional neuroimaging finding, represents a set of regions and plays a role in cognitive processes, working memory, planning, control and inhibition, attentional set shifting, motivation and mood regulation (George, 1994; Weinberger, 1993). Its dysfunction, lesion or atrophy seems consistent with a role in the pathophysiology of mood disorders. It is proposed that depressed mood and psychomotor retardation are related to changes in the dorsolateral part but also in the anterior cingulate due to its extensive connections with the limbic regions: suicide is related to the ventral prefrontal and anxiety to the posterior cingulate. Such phrenological approaches are still working hypotheses.

However, contradictions do exist, especially when neuropsychological data are confronted with possible underlying neurostructural abnormalities. Rubinsztein *et al.* (2001), departing from the observations that decision-making and task-related activation are associated with activation in the ventral prefrontal cortex and the anterior cingulated gyrus, investigated these regions in manic, unipolar depressive, and control subjects. They showed that during appropriate tasks, activation was increased in the left dorsal anterior cingulate, but decreased in the right frontal polar region, in the manic patients compared with the control patients. Moreover, less activation was noticed in the inferior frontal gyrus. Depressed patients did not show significant task-related differences in activation compared with control subjects. These patterns point to abnormal responses in specific frontal regions in manic patients and are consistent with neuropsychological observations in patients with lesions in the ventromedial prefrontal cortex. However, in a parallel study, another group did not reach the same conclusions (Clark *et al.*, 2001). In this context, the authors wanted to describe the neuropsychological profile of severe acute mania using a range of tasks selected primarily for the detection of localized neural disruption within the prefrontal cortex. They showed that manic patients did not resemble patients with ventromedial prefrontal cortex damage, and concluded that this region is not implicated as a locus of pathology in mania.

Coupled with the cortical regions, functional abnormalities are noticed in the basal ganglia in unipolar and even bipolar depression.

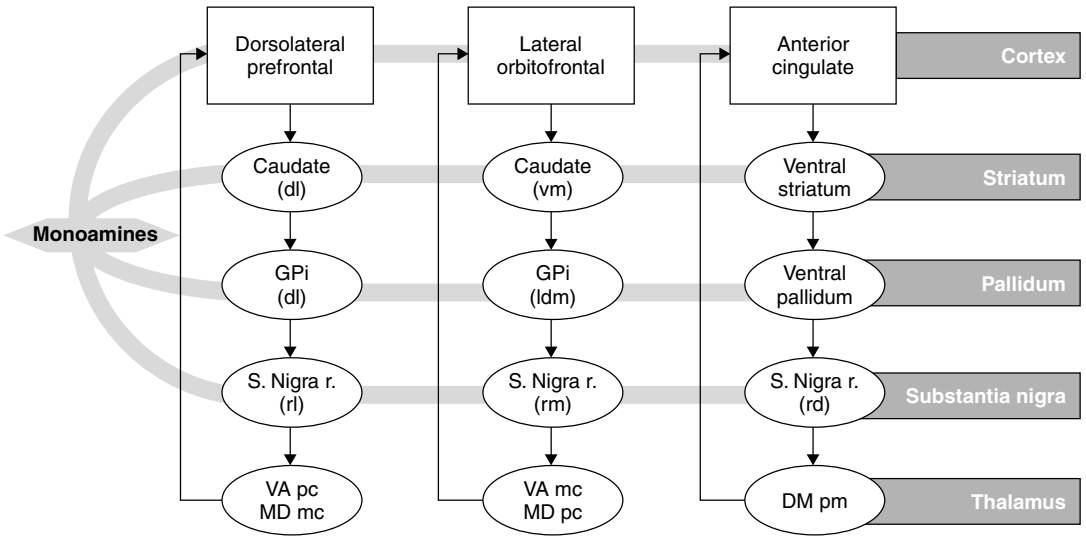

Figure XVIII-8.3 Modular organization of thalamocorticostriatal circuits. Note that each circuit engages specific regions at five levels of integration of the forebrain, and that the monoamines regulate the functioning of each circuit and allow each element to function in relation to the others. Interestingly, monoamines modulate numerous structures included in classical emotional anatomical networks. dl, dorsolateral; DM, dorsomedial; GPi, internal segment of globus pallidus; ldm, lateral dorsomedial; MD mc, medialis dorsalis pars magnocellularis; MD pc, medialis dorsalis pars parvocellularis; pm, posteromedial; rd, rostrodorsal; rl, rostrolateral; rm, rostromedial; S. Nigra r, substantia nigra pars reticulata; VA mc, ventralis anterior pars magnocellularis; VA pc, ventral anterior pars compacta; vl, ventrolateral; vm, ventromedial Redrawn from Alexander *et al.* (1986) and Le Moal (1995)

Atrophies have been noted in the caudate and putamen. Specific neurological diseases of these areas are associated with prefrontal mood changes. Moreover, the caudate and putamen have major connections with the medial temporal structures, hippocampus and amygdala, which in turn have relations with the frontal cortex. The metabolic rate in the amygdala region predicts severity of depression in depressed patients (Abercrombie *et al.*, 1996); in this circuit, potential white-matter abnormalities may contribute to the pathogenesis. An increasingly well-documented role of the amygdala-hippocampal complex has been described in dementia subsequent to recurrent stress, HPA feedback inhibition and neuronal destructions (McEwen and Sapolsky, 1995) (see Figure XVIII-8.2). This process may occur in depression characterized by HPA axis overactivity, and indeed hippocampal atrophy seems to exist in depression, although this is disputed in the literature (Pearlson *et al.*, 1997; Sheline *et al.*, 1996). The cerebellum and brainstem structures are connected with the limbic regions, and structural abnormalities have been described. In total, many studies are in agreement regarding a frontotemporal-basal ganglia circuitry in primary depression. In general, data also imply connected circuitries in major unipolar depression: a limbic (amygdala)-thalamo-(mediodorsal)-corticofrontal circuit and a limbic-striatal-pallidal-thalamic circuit (Cummings, 1993; George *et al.*, 1993a; Grodd *et al.*, 1995; Guze and Gitlin, 1994; Gyulai *et al.*, 1997; Schneider *et al.*, 1995). Cellular physiologists propose the existence of excitatory projections and subsequent increase of synaptic transmission from the limbic and frontal regions. These converging data, mainly from functional imaging techniques, remain to be interpreted in the course of major depression. Indeed, these imaging abnormalities may reflect neuromodulator dysfunction, and/or real concomitants of one or some of the symptoms of a general emotional state and/or a general pathophysiological trait and a vulnerability to depression, and/or a neuroplasticity and circuit reorganization (see Table XVIII-8.1).

Again, the main problem that confronts these neuroanatomical findings is the correspondence between what is known from behavioural physiology and neuropsychology on the one hand and symptoms on the other hand, i.e. neural and biological substrates or correlates of the various emotions and mood disorders expressed by the symptom that characterizes the different forms of depression.

Regarding the prefrontal cortex, interesting data are provided by imaging changes (increase or decrease in the imaging aspect) according to the mental state, cognitive operations, positive or negative emotions and, even more interestingly, when cognition and emotion interact in the same region. These functional correlations facilitate interpretations of the pathological changes from the symptom point of view. In view of these neuropsychological dysfunctions, reduced blood flow in the dorsomedial frontal cortex may be related to the classic impaired attentional and memory processing observed in major depression, while slowing of cognitive processes is confined to reduced dorsolateral functioning (Dolan *et al.*, 1994), regions where increased blood flow was observed in normal subjects who increased their mental activity. Moreover, the orbital subregion is assumed to play a role in modulating psychological perseveration and emotional states, and increased blood flow is observed in ventrolateral, pregenual anterior cingulate and orbital subregions during emotional processing and obsessive–compulsive states and during experimentally induced anxiety. These regions also constitute part of the circuitries described for obsessive–compulsive disorders, due to their connections with the amygdala, accumbens and hypothalamus, and decreased blood flow appears in the course of active pharmacological treatments. It has been shown that these orbital cortical regions are activated during depression in order to modulate — or inhibit — emotional responses, or to break perseverative problems and negative thoughts (Drevets and Botteron, 1997). Finally, some authors described increased functional imaging correlates with severity of symptoms (Abercrombie *et al.*, 1996; Drevets *et al.*, 1997b; Drevets and Raichle, 1992). Regional glucose metabolism in the right amygdala was shown to predict the severity of predispositional negative affect in depressed patients (Abercrombie *et al.*, 1996).

HYPOTHESIS AND CONCLUSION

Research into the pathophysiological bases of mood disorders is progressing at each level of organization under investigation (Manji *et al.*, 2001): (1) the genetic-molecular level (transcription factors, mRNA stabilization, nuclear import/export) and the subsequent gene expression-transcriptome processes; (2) the cellular level with signal transduction and modulation processes (cytoskeletal events, G-proteins, protein kinase C (PKC) and other substrates); (3) the system levels, neuronal circuits, and circadian and neurovegetative regulations (neuroplasticity processes); and (4) the cognitive-affective interactions. However, from molecular genetics (Craddock and Jones, 2001) to structural and functional defects, very little is known that might suggest a possible lead or causality between these levels.

A functional neuroanatomy of mood disorders (Soares and Mann, 1997) has emerged recently, and data obtained from morphological and imaging techniques are confronted and interpreted on the grounds of affective neuroscience. A frontolimbic-basal ganglia circuitry has been proposed, but such a structural approach needs to be refined. The regions involved account for two of the main groups of symptoms on the cognitive side (Austin *et al.*, 2001) and on the affective side, both interacting in everyday life (Buck, 1999; Cacioppo and Gardner, 1999; van Weelden, 1997). In summary, the prefrontal cortex is believed to play a role in volition, control and inhibition, working memory, and perhaps motivation and mood regulation. Dysfunction of the dorsolateral part orients the pathology to psychomotor slowing and depressed mood. Anxiety seems to be related to the posterior cingulate and also the amygdala. It is clear that the hierarchical position of these cortical regions results in dysfunction in the other areas. Defects in another group of regions seem to be present in unipolar and — in some studies — bipolar mood disorders, and are translated into psychomotor and cognitive symptoms. Both the putamen and caudate have many relations with the amygdala and hippocampus, suggesting possible trouble in affective, cognitive and inhibition abilities, the appearance of anxiety thus rendering comorbidities plausible. The amygdala-hippocampal complex may be subjected to structural abnormalities, in which overactivity of the HPA axis might have a role (McEwen and Sapolsky, 1995). Many studies have noted lateralization of functional or structural abnormalities; these data are very important (the classic interhemispheric differentiation of mood disorders after stroke) and need more clarification. Needless to say, autonomic, motivational and hormonal symptoms might be caused by sympathetic hormonal peripheral systems.

Neuropathological and imaging techniques have provided data that strengthen working hypotheses for future investigations. Again, as in pathologies such as anxiety, obsessive–compulsive disorders (Baxter *et al.*, 1992), Tourette's syndrome (George *et al.*, 1993b; Lombroso and Leckman, 1999), and drug dependence (Koob and Le Moal, 1997; Koob and Le Moal, 2001), a network including the prefrontal cortex, basal ganglia and limbic regions, including the anterior cingulate cortex (Bush *et al.*, 2000), is undoubtedly implicated (Figure XVIII-8.4).

Neuroimaging (structural and functional) abnormalities observed *in vivo* suggest neurohistological alterations linking macro- and micropathology: the basal ganglia are smaller, and structural changes are observed in noradrenergic and serotonergic nuclei (Baumann and Bogerts, 2001). However, clear distinctions between

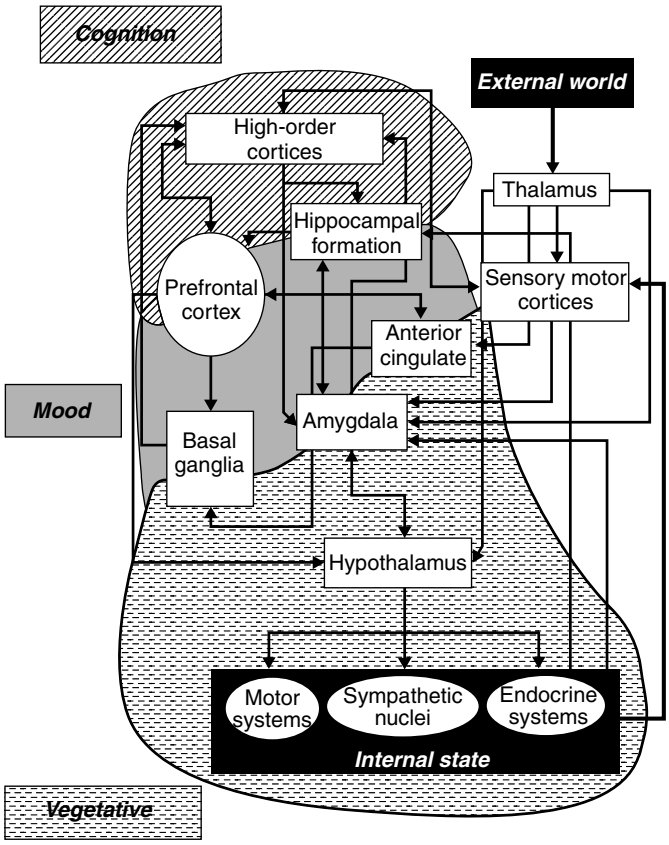

Figure XVIII-8.4 Neuroanatomical model of the central processing of emotions. Parts of these circuits are related mainly (but not exclusively) to cognitive, mood or vegetative processing and are modulated by monoamines (cf. Figure XVIII-8.3). The different circuits presented here are involved in the dialogue between the external and the internal world. External input, such as sensory information, via a classical thalamic relay, could be processed by the cortex or directly by the amygdala. The amygdala can also be influenced by the internal state (mainly by way of hormonal signalling) and, in turn, through the hypothalamus, which modulates this internal state. The evaluation of the nature of information is mainly via the prefrontal cortex, while comparison between this information and previous personal experiences involves the hippocampal formation. The prefrontal cortex and hippocampus are interconnected closely with the amygdala, and these three structures emerge as a possible mood-processing network integrating cognitive and vegetative inputs in order to make sense of the dialogue between the external and internal worlds

unipolar and bipolar syndromes, between the variety of syndromes and collateral symptoms, between acute and chronic defects, between early and late onset, and between the various aetiologies are needed for future investigations. Hemispheric lateralization dysfunction in the limbic system has been observed and merits confirmation. Besides the variety of technical manifestations and the problem of comorbidity (Nathan and Langenbucher, 1999), refinements of the techniques and methodologies will allow a more precise dissection of the circuitries, substructures or functional modules involved, such as the frontal cortex (Schoenbaum and Setlow, 2001), amygdala (Davis, 1998) or basal ganglia (Le Moal, 1995) in both acute and chronic depression.

There is still a gap between the enormous advances that have been made in past decades in affective neurosciences and a neuroscientifically based physiopathology of mood disorders, in particular in terms of structure–function relationships. It is possible that different circuitries are recruited for different emotional expression of feelings in normal subjects. For instance, experiments in humans support the hypothesis of a left hemisphere specialization for positive emotional expression and a right hemisphere specialization for expression of a negative emotion (Lee *et al.*, 1993). Moreover, there may be relationships between emotional and pre-existing personality traits, especially the harm avoidance dimension, which covaries with mood and anxiety (Svrakic *et al.*, 1992). During the past few years, progress has been made concerning (1) the neurofunctional and neuroadaptive changes, considered longitudinally, in the course of the illness, before and during mood disorders, and also in the recovery phases, in parallel with symptom evaluation; and (2) the neurofunctional changes due to pharmacotherapeutic agents or to behavioural-cognitive therapies, from disorder to recovery. Although differences in overall efficacy among groups receiving lithium, imipramine or paroxetin were not statistically significant, these drugs may affect the brain in different ways, the same being true for the mechanisms of mood stabilizers, which also have underappreciated neuroprotective effects (Manji *et al.*, 2001).

Further knowledge concerning the neurobiology and neuroanatomy of mood disorders will benefit from intense collaborative efforts in imaging techniques, neuropharmacology, physiological approaches and behavioural neurosciences.

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XVIII-9

Brain Imaging in Mood Disorders

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INTRODUCTION

Depression, as the reversible psychiatric condition par excellence, is clearly an ideal object for functional neuroimaging studies. In theory, patients return to their initial (healthy) brain state so that any image changes observed during an affective episode should mark the brain structures and circuits involved in the expression of symptoms and signs. Authors have imaged patients when ill and after recovery and used a number of strategies to exploit short-term fluctuations of symptoms. Such fluctuations occur naturally, as in the typical diurnal variations of mood (Moffoot *et al.*, 1994b), or they can be provoked by interventions, such as mood induction (Baker *et al.*, 1997), sleep deprivation (Ebert *et al.*, 1994a) or tryptophan depletion (Smith *et al.*, 1999b). If, on the other hand, anatomical changes did exist in depression, they would be predicted in cases of treatment resistance (Shah *et al.*, 1998), in secondary or late-onset depression (Ebmeier *et al.*, 1998), or possibly a priori in certain patients with a genetic predisposition for the illness (Drevets *et al.*, 1998).

Anatomical systems involved are likely to be medial limbic, with the anterior cingulate cortex and orbitofrontal cortex playing a prominent role (Ebert and Ebmeier, 1996). There is also the well-rehearsed hypothesis of hypercortisolaemia, which occurs frequently in depression and, at least in animal models, leads to hippocampal damage. Hippocampal damage, in turn, would release the pituitary secretion of adrenocorticotropic hormone (ACTH) from hippocampal suppression and result in a positive feedback loop (Sapolsky *et al.*, 1986). This mechanism may not be specific to depression (Welberg *et al.*, 2001), as some authors have also used it to explain cognitive impairment or dementia (Hibberd *et al.*, 2000) and the sequelae of severe psychological trauma (Bremner *et al.*, 1995).

Neuropsychological tasks have been employed in imaging studies to activate brain systems thought to be implicated in depression, in particular using 'frontal' (e.g. word-generation) or 'temporal' (memory) tasks. In such experiments, limited task performance may be responsible for group differences. Attempts to control for such performance differences include pacing tasks at a speed that all patients can manage, and post-hoc correlation of brain activity with task performance, e.g. by using analysis of covariance. A further complication of functional imaging protocols is that it is now very difficult to recruit untreated patients in a psychiatric setting. Primary-care physicians have usually already treated their patients with a standard antidepressant (e.g. a selective serotonin reuptake inhibitor, SSRI) by the time of referral. The cost and effort required to recruit patients at the primary-care level is usually seen as prohibitive. For this reason, many studies contain samples of medicated patients and have to be interpreted with caution. It also cannot be excluded that changes in brain activity or even brain anatomy may be caused by medication (DelBello *et al.*, 1999).

Medication is, of course, a particular problem for receptor ligand studies. Based on effective pharmacological treatment, there are a variety of hypotheses, particularly involving the serotonergic and noradrenergic transmitter systems, which are theoretically amenable to *in vivo* testing with neuroimaging (Delgado *et al.*, 1990). Not only the availability of untreated patients but also the availability of receptor ligands has limited such research. The latter may be partially responsible for the dearth of noradrenaline ligand studies. Not all ligands are suitable; their use may be limited by their specificity for the receptor concerned, their affinity (i.e. the likelihood to be displaced by endogenous ligand) and their nondisplaceable (non-specific) binding fraction. In spite of these limitations, first results are now emerging that test some of the extant pharmacological hypotheses in depression.

Rather than giving a balanced review of all studies carried out in the field, we will focus on certain themes and future prospects that appear to be emerging. Our selection will no doubt be idiosyncratic, but we hope that we have captured the important paradigms and paths of current research. In order to limit the size of the chapter, we will focus on key publications of the last 5 years (at the time of writing), as earlier literature has been summarized well in a number of other reviews (Davidson *et al.*, 1999; Drevets, 1998; Kennedy *et al.*, 1997; Norris *et al.*, 1997; Stoll *et al.*, 2000; Videbech, 1997; Videbech, 2000). Rather than systematically dividing the imaging literature by image modality or diagnosis, we will attempt to present a logical narrative, proceeding from simple (e.g. neurochemical) hypotheses, such as the dopamine theory of psychomotor retardation in depression, to more complex models. Hypotheses that are, in a sense, post-hoc, i.e. exploit the natural history of depressive symptoms and their treatment, will be followed by experimental approaches, which imply complex neuronal systems and attempt to activate selectively such systems that are thought to be implicated in the expression of depressive symptoms. Mania is a rare condition that is very difficult to study with neuroimaging techniques, and reports are rare (Al-Moussawi *et al.*, 1996). This illness will, therefore, not be discussed, except when included in studies of bipolar depressed patients.

PHARMACOLOGY

Dopamine and Motor Function

Although dopamine is not thought to be involved primarily in the treatment and the experience of symptoms of depression, it may play an important role in the brain reward systems and in movement control. It has been implicated in retarded depression, both by the reduction of the dopamine metabolite homovanillic acid in cerebrospinal fluid (Jimerson, 1987) and by increased D2 receptor binding, particularly in psychomotor retarded patients (Ebmeier and

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Ebert, 1997). Increased postsynaptic receptor binding is interpreted mainly as evidence of reduced dopaminergic activity with resulting receptor supersensitivity or, alternatively, reduced displacement of the radioligand by endogenous dopamine (Shah *et al.*, 1997). Sleep deprivation, which may have an amphetamine-like effect, is associated with displacement of such ligands from their binding sites in the neostriatum (Ebert and Berger, 1998). Whether reduced availability of dopamine is ubiquitous in depression is, however, doubtful. At least one single photon emission computed tomography (SPECT) study (Klimke *et al.*, 1999) ($n = 15$) found a reduction in ligand binding to D2 receptors that normalized on clinical recovery and predicted response to SSRIs. Paillere-Martinot *et al.* (2001) reported a reduction in left caudate 18F-dihydroxyphenylalanine (DOPA) positron emission tomography (PET) only in blunted and retarded, but not in impulsive and anxious, depression. Examining presynaptic loci, 15 drug-naive patients with major depression showed significant increases in SPECT striatal dopamine transporter (DAT) binding capacity (Laasonen-Balk *et al.*, 1999). This may be interpreted similarly to the findings in D2 receptors, as a correlate of reduced availability of dopamine (see above). This effect, however, was not reported in 31 drug-naive children. Serotonin transporter (SERT) binding (see below) but not DAT binding in the hypothalamus and midbrain was increased (Dahlstrom *et al.*, 2000). Interestingly, during acute cocaine abstinence ($n = 28$), associated with depressive symptoms and a 20% increase in striatal DAT binding capacity measured by SPECT, there was a negative correlation of tracer binding with Hamilton depression scores (Malison *et al.*, 1998a), suggesting an adaptive response. Basal ganglia involvement in depression is demonstrated not only by abnormal dopamine ligand studies but also by imaging studies examining brain activation and blood flow. Hickie *et al.* (1999) reported a correlation between (delayed) reaction time and (reduced) left neostriatal activation from simple to choice reaction time tasks in psychomotor retarded patients. Finally, basal ganglia pathology may predispose to late-onset or secondary depression, as suggested by magnetic resonance imaging (MRI) lesion studies (Lauterbach *et al.*, 1997) and MRI studies of iron deposition (Steffens *et al.*, 1998).

Noradrenaline

Although noradrenaline has been implicated in depression for many years (Zis and Goodwin, 1982), there have been relatively few neuroimaging studies. After driving the noradrenergic system with clonidine, an $\alpha 2$ agonist, a study in six depressed and six healthy women found an increase in right prefrontal perfusion only in the depressed group, suggesting either presynaptic subsensitivity or a local supersensitivity of postsynaptic alpha2 receptors (Fu *et al.*, 2001). This may not be a specific finding, as Moffoot reported similar results for patients with Korsakoff psychosis (Moffoot *et al.*, 1994a).

Serotonin

Serotonergic Activation, Treatment Effects and Response

In accordance with the theoretical and practical importance of SSRIs in the treatment of depression, a number of studies have examined the effects of SSRIs on brain function, both explicitly and by comparing patients when they are ill and recovered. Mayberg *et al.* (1997) examined 18 hospitalized patients with unipolar depression and reported that rostral (anterior) cingulate (Brodman's area 24a,b) metabolism predicted response (hypermetabolism) and lack of response (hypometabolism) to fluoxetine. In a repeat measures study (Brody *et al.*, 1999), 16 depressed outpatients were imaged with 18F-fluorodeoxyglucose (FDG)–PET before and after treatment with paroxetine. SSRI responders showed a reduction in ventral prefrontal perfusion, but (left) ventral anterior cingulate reduction before treatment predicted a better response. These findings could be in contradiction to the data of Mayberg *et al.* (1997). However, patients in that study were more clinically unwell, different SSRIs were used, and the target areas do not seem to be congruent.

In reversal of SSRI treatment, depletion of serotonin by a low-tryptophan amino acid drink can lead to a temporary lowering of mood in recovered depressed patients. Smith *et al.* (1999b) found that 'increasing levels of depression after tryptophan depletion were associated with diminished neural activity in ventral anterior cingulate, orbitofrontal cortex and caudate nucleus regions' during paced verbal fluency performance. In addition, depressive relapse attenuated cognitive task-related activation in the anterior cingulate. This study illustrates potential complications in design arising when behavioural conditions are mixed with pharmacological interventions in neuroimaging: although not obvious in this study, the pharmacological interventions may affect task performance as well as mood. The separation of drug and clinical effects, and the statistical definition of the interaction between the two, are, up to a point, arbitrary. The same group also confirmed the specific hypothesis that mood deterioration induced by low-tryptophan drink would increase activity in the projection from the habenula to the raphe, structures that are part of the feedback loop controlling the release of 5-hydroxytryptamine (5-HT) throughout the brain (Morris *et al.*, 1999). A reliable differential effect of the indirect serotonin agonist fenfluramine on brain perfusion in healthy volunteers and depressed patients has not been established. A study of 13 depressed and 18 healthy women showed identical effects in both diagnostic groups after intravenous infusion (Meyer *et al.*, 1998).

Serotonin Transporter

After an initial rush of interest in the association of certain SERT alleles with depression, a number of imaging studies have used SERT ligands with patients (Battersby *et al.*, 2001). Such studies are understandably difficult to conduct, as medicated patients cannot be used. Unipolar depressed patients exhibited a characteristic reduction of 18% in SERT in the brainstem, where the highest concentrations of receptors can be found, compared with healthy volunteers (Malison *et al.*, 1998b). Patients with seasonal affective disorder showed reduced SERT binding capacity in the thalamus and hypothalamus but not in the midbrain or pons (Willeit *et al.*, 2000). This effect, however, was reversed in the hypothalamus and midbrain, where increased SERT binding correlated with concurrent depression in a group of drug-naive children ($n = 41$). Increased binding could be attributed to a reduction of serotonin within the synaptic cleft (thus allowing increased tracer binding), with developmental differences accounting for contradictory results for transporter binding in adults and children (Dahlstrom *et al.*, 2000).

5-HT2 Receptor

Post-mortem studies have suggested that a history of depression and, in particular, suicidal behaviour is associated with an increase in 5-HT2 receptors (Mann *et al.*, 1999). In contrast, a study of six drug-free, depressed patients examined with 18F-altanserin PET reported reduced binding in the right posterolateral orbitofrontal cortex and anterior insula with trends on the left side (Biver *et al.*, 1997). This apparent contradiction may be explained by receptor downregulation due to medication: eight of ten depressed patients who improved with desipramine showed a decrease in 18F-setoperone binding to the 5-HT2 receptor in many cortical areas

(Yatham *et al.*, 1999). Further, an 18F-setoperone study before and after 6 weeks of paroxetine medication in 19 depressed patients suggested a reduced binding capacity after treatment, which was found mainly in patients younger than 30 years of age. A study of 14, better controlled, drug- and self-harm-free (>6 months) depressed patients showed no abnormalities in prefrontal 18F-setoperone binding (Meyer *et al.*, 1999). Similarly, 11 elderly depressed patients did not show an *in vivo* reduction of 18F-altanserin binding capacity (Meltzer *et al.*, 1999).

5-HT1a Receptor

Rueter *et al.* (1998) have argued that antidepressant effects are due to a desensitization of 5-HT1a somatodendritic autoreceptors in the rat. This is responsible for the return to normal firing rate levels in the dorsal raphe nucleus. A single study has confirmed the hypothesized reduction in 5-HT1a binding capacity in depressed patients (Lesch *et al.*, 1990) using the PET ligand 11C-WAY-100635. Twelve primarily depressed patients with a family history of the illness showed reductions in binding capacity in the brainstem raphe, medial temporal cortex and possibly other cortical areas. This effect was greatest in bipolar patients and patients with a family history of bipolar illness (Drevets *et al.*, 1999).

'NATURAL EXPERIMENTS': CLINICAL PROFILES, TREATMENT RESPONSE AND TREATMENT RESISTANCE

What can neuroimaging studies tell us about depression that clinical description could not? Comparison of pretreatment and post-treatment neural activity is the most obvious way to associate *in vivo* neurobiological markers with depressive symptoms, cognitive ability or deficits. There is also mounting evidence for a correspondence between pretreatment metabolism in specific areas and treatment outcome. Characteristic changes in perfusion may help to classify patients with similar neuropathology. Changes in functional circuitry may emerge as predictors of treatment response and residual dysfunction whenever remission is not synonymous with recovery. Because substantial placebo or spontaneous remission effects contribute to the drug treatment of depression, it is unclear whether changes in cortical dynamics upon recovery are associated with the nature or the extent of drug-induced changes (Andrews, 2001).

Neuroanatomical and functional deficits may already be identifiable as vulnerability factors in patients' families. They may, at the other extreme, be the correlate of lingering abnormalities, such as perturbed cortical dynamics and impaired cognitive performance that are present beyond remission (Abas *et al.*, 1990). Persistent abnormalities can be a consequence of several factors. First, chronic administration of antidepressant, anticonvulsant or antipsychotic medication may contribute to the enduring brain changes (DelBello *et al.*, 1999). Second, impaired function during (or between) clinical episodes may compromise a system that is already fragile or affected in some way, and thus cause enduring damage (Sapolsky *et al.*, 1986). Finally, since affective disorders are often characterized by recurrent episodes over time, the normal process of ageing may be a confounder or may interact with the illness process (Kapur *et al.*, 1994). The interpretation of treatment progress in depression is also not straightforward. Change in behaviour, cognition or motor activity may not necessarily imply an associated modification in functional circuitry with return to normal activity (Goodwin *et al.*, 1993). Psychopharmacological agents target one or more neurotransmitter systems. Nonetheless, if the activity of one of the widely projecting neurotransmitter systems (dopamine, noradrenaline, 5-HT) increases, then global cortical dynamics will also be affected. Functional connectivity changes in mood disorders will be associated with abnormalities at rest (baseline) or, more likely, an abnormal pattern of recruitment during cognitive or motor activity. Where structural abnormalities have been reported in unipolar and bipolar depression, it is not unreasonable to assume that baseline and task-associated functional connectivity changes may occur. Brain imaging provides an assessment tool for the course of both illness-induced changes and active mechanism of recovery or compensation. In accordance with Alexander's (Alexander *et al.*, 1986) and Swerdlow and Koob's (1987) theories of functional cortico-subcortical loops, cortical areas (such as the frontal, temporal and parietal cortices), subcortical areas (such as the basal ganglia and thalamus), and most of all areas related to the limbic system (such as the anterior cingulate, hippocampus and amygdala) will be affected.

In order to better understand blood-flow abnormalities in affective disorders and consequent remission effected by different forms of treatment, findings can be separated into a number of distinct categories: pretreatment perfusion, post-treatment perfusion, activity in responders and non-responders, and the comparison of either or both with control subjects. Some studies do not include healthy volunteers; therefore, it is impossible to tell whether remission is associated with normalization of perfusion. The use of diverse methodologies in clinical studies makes the comparison between their findings difficult. The neuroanatomical maps or delineation of specific brain regions (Brodman's areas) used to localize activation may not be identical throughout the literature. Despite anatomical proximity, these areas may have very different connectivity patterns. For example, the medial or orbital frontal networks receive input from very different areas of the cortex (Barbas *et al.*, 1999; Bhashghaei and Barbas, 2001). Methodological constraints on studies in a clinical setting include great variations in the size of samples, which are often small and occasionally non-uniform in terms of age, medication and clinical history. Medication can influence outcomes in both neuropsychological and imaging studies, although the duration of medication effects is not known (Elliott *et al.*, 1998). An agreed standard for response to medication lies around 6 weeks, but it may take longer to establish remission. Therefore, brain imaging carried out at a later stage (Pizzagalli *et al.*, 2001) may be recording the long-term behavioural gain of therapeutic intervention, rather than incidental drug effects.

An alternative to measuring regional metabolic changes is to examine functional connectivity. Mallet *et al.* (1998) found decreased interhemispheric connectivity along with reduced connectivity within the right hemisphere (in a cortical–subcortical as well as anterior–posterior orientation) in schizophrenia, obsessive–compulsive disorder and unipolar depression. In depressed subjects, these deficits were mostly resolved on remission. A distinct 'melancholic pattern' was noted, with decreased correlation between the orbitofrontal cortex and the dorsolateral prefrontal cortex compared with controls and non-melancholic depressed subjects (Mallet *et al.*, 1998).

TREATMENT METHODS

Sleep Deprivation

Up to 60% of patients can improve after a night of total sleep deprivation (TSD) (Ebert and Berger, 1998). In contrast to the delayed cumulative effects of antidepressant medication, TSD has an immediate effect that does not appear to extend beyond the next full night's sleep (Ebert and Berger, 1998). The experimental advantage of TSD is that patients do not have to be medicated to show a short-term clinical improvement. The effects of TSD have been explained by increased dopamine release in the basal ganglia, which results in increased displacement of the D2/D4 receptor radio ligand 123I-iodobenzamide (IBZM) by endogenous dopamine, i.e. reduced

ligand binding (Ebert *et al.*, 1994b). However, an allele of the D4 dopamine receptor, which has been suggested previously to increase susceptibility to this treatment, was not linked to treatment response in 124 bipolar patients (Serretti *et al.*, 1999). A growing body of evidence is documenting hyperperfusion in the medial prefrontal cortex as a state or trait related change (Ebert and Berger, 1998; Ebert *et al.*, 1996). Allied with limbic hyperperfusion, a number of studies describe dorsolateral prefrontal cortex hypoperfusion in depression and during mood induction in normal volunteers (Mayberg *et al.*, 1999). The early sleep-deprivation literature reported increased perfusion in the orbitofrontal cortex that was found in the right anterior cingulate and bilateral orbitofrontal cortex and basal cingulate. Increased right hippocampal pretreatment flow was associated with greater treatment response (Ebert *et al.*, 1994a). Similar findings, indicating the association between successful drug treatment and pretreatment limbic hyperperfusion, have since been reported by a number of groups. Comparing glucose metabolism in hospitalized unipolar patients, Mayberg *et al.* (1997) reported hyperperfusion in the rostral anterior cingulate (Brodman's area 24a/b) to be indicative of a favourable treatment outcome. Although subsequent non-responders were reported to be marginally more impaired than responders on neuropsychological performance, no other correlation between perfusion and clinical ratings was found. Hypoperfusion in responders was greater than in non-responders in the dorsolateral prefrontal cortex (Brodman's area 45/46), anterior insula and inferior parietal cortex (Brodman's area 40). Pretreatment premotor cortex activation, on the other hand, was greater in responders.

The prognostic capacity of anterior cingulate activity with reference to the extent of treatment response can also be measured with electroencephalography (EEG) (Smith *et al.*, 1999a). Higher θ (6.5–8 Hz) activity in the rostral cingulate was associated with a greater response after 4–6 months of treatment with nortriptyline. A greater degree of response was associated with increased pretreatment θ activity in the medial frontal cortex (Brodman's area 24, 32), consistent with previous functional imaging reports (Mayberg *et al.*, 1997).

Pretreatment metabolism in the medial prefrontal cortex (Brodman's area 32), ventral anterior cingulate (Brodman's area 24) and posterior subcallosal gyrus (Brodman's area 25) was found to be higher in responders to TSD than in non-responders and healthy volunteers (Wu *et al.*, 1999). Normalization upon recovery was noted with decreased flow in the medial prefrontal cortex (Brodman's area 32) and the frontal pole (Brodman's area 10). All depressed subjects had a lower striatal (putamen) metabolic rate than controls, which persisted after treatment and, by contrast, decreased in normal volunteers. Frontal and occipital cortex metabolism was also higher in both groups before treatment. The activity in the right lateral prefrontal cortex (Brodman's area 46) and higher superior temporal cortex and right insula increased in responders. Perfusion was decreased in the lateral prefrontal cortex of controls. Moreover, in comparison with control subjects, the neuropsychological performance of all depressed patients deteriorated markedly after a night of TSD. Normal subjects performing a verbal learning task following 35 hours of sleep deprivation exhibited a pattern of increased activation in the prefrontal and parietal cortices, whereas activity in their temporal lobe was decreased (Drummond *et al.*, 2000). Decline in subjects' performance of a free recall task was correlated positively with activation of their parietal lobe. Bilateral prefrontal cortex activation, which was related closely to personal perception of fatigue, was interpreted as a competitive mechanism associated with the homeostatic urge for sleep. Compensation for decreased temporal lobe activity appears to be achieved by increased perfusion in the bilateral parietal lobes in verbal tasks, which are not associated with this area in the control condition. Just as TSD created characteristic changes in blood flow during the verbal learning challenge, elevated pretreatment regional cerebral blood flow (rCBF) in the right orbitofrontal cortex and basal cingulate of patients was normalized in responders to partial sleep deprivation (in the latter part of the night). Post-treatment left inferior temporal flow was correlated with treatment response (Volk *et al.*, 1997).

In summary, these studies appear to suggest that elevated metabolism and blood flow in the prefrontal or medial prefrontal cortex areas is an adaptive marker aiding response to different forms of therapeutic intervention, especially sleep deprivation. Depressed patients may share an abnormal functional network (decreased flow in subcortical structures) (Wu *et al.*, 1999) or persistent abnormalities throughout the temporal cortex (where structural changes have also been reported) (Shah *et al.*, 1998; Sheline *et al.*, 1998; Sheline *et al.*, 1999) that may accommodate behavioural changes by compensating for hypoperfusion elsewhere. In certain patients, this mechanism may later cease to be effective.

Sleep disturbance during rapid eye movement (REM) and non-rapid eye movement (NREM) sleep are well documented in patients suffering from affective disorders (Kupfer and Reynolds, 1992). Ho *et al.* (1996) studied the δ stage of slow-wave (high-amplitude) sleep of NREM sleep with FDG–PET in depression and found that metabolism was elevated in the occipital and parietal cortices to a greater degree than elsewhere. Limbic structures, such as the posterior cingulate, amygdala and hippocampus, were more active in depressed patients; however, metabolism in midline structures and the neostriatum (including the medial prefrontal cortex, medial thalamus, anterior cingulate, bilateral caudate, putamen and the head of the caudate) was reduced compared with controls. In conjunction with an EEG investigation, Nofzinger *et al.* (2000) studied the link between β EEG frequency (characterized by higher frequency and lower amplitude) and glucose metabolism in different areas of the cortex, defined on the basis of findings from control data. β-Wave frequency had been coupled previously with secretion of cortisol (Chapotot *et al.*, 1998) and, in this study, was present for longer in depressed subjects and was associated negatively with subjective sleep quality. No hypofrontality was found in this study; however, orbitofrontal cortex (including Brodman's areas 11, 25, 32) metabolism was higher in depressed patients than in controls, which may be indicative of dysfunctional arousal. Finally, the contrast between waking and REM sleep revealed that, unlike controls, depressed subjects failed to recruit anterior paralimbic areas (right parahippocampal gyrus, right insula and anterior cingulate). Instead, temporal-limbic areas (amygdala, subiculum, inferior temporal cortex, sensorimotor cortex) were activated (Nofzinger *et al.*, 1999).

Light Therapy

In patients suffering from seasonal affective disorder, light therapy produced dissimilar blood-flow changes in responders and non-responders. Responders expressed a globally increased activity, measured with hexamethyl-propyleneamine-oxim (HMPAO)–SPECT, relative to the cerebellum mainly in the frontal and cingulate cortices along with the thalamus (Vasile *et al.*, 1997).

Pharmacotherapy

Ogura *et al.* (1998) found in an HMPAO–SPECT study of patients with major depression that the severity of depression was correlated negatively with perfusion in the left superior frontal, right lateral temporal and right parietal cortex. After treatment with tricyclic antidepressants (clomipramine and amoxapine), remitted patients' perfusion did not differ significantly from that of controls.

Brody *et al.* (1999) reported that a better response to the serotonin reuptake inhibitor paroxetine was associated with lower pretreatment glucose uptake in the left ventral anterior cingulate. In

responders, values returned to normal in the ventrolateral prefrontal cortex and orbitofrontal cortex, but not in the dorsolateral prefrontal cortex or inferior frontal gyrus. Hamilton depression score changes were correlated with changes in glucose metabolism in some areas (ventrolateral prefrontal cortex and inferior frontal gyrus). Remission was associated with increased baseline perfusion in the left premotor and supplementary motor areas, along with decreased left ventral anterior cingulate metabolism (Brody *et al.*, 1999). These findings do not fit the Mayberg *et al.* (1997) study, but patients in Brody's study were more depressed and the researchers identified an area that lies ventral to Mayberg's 'rostral cingulate'.

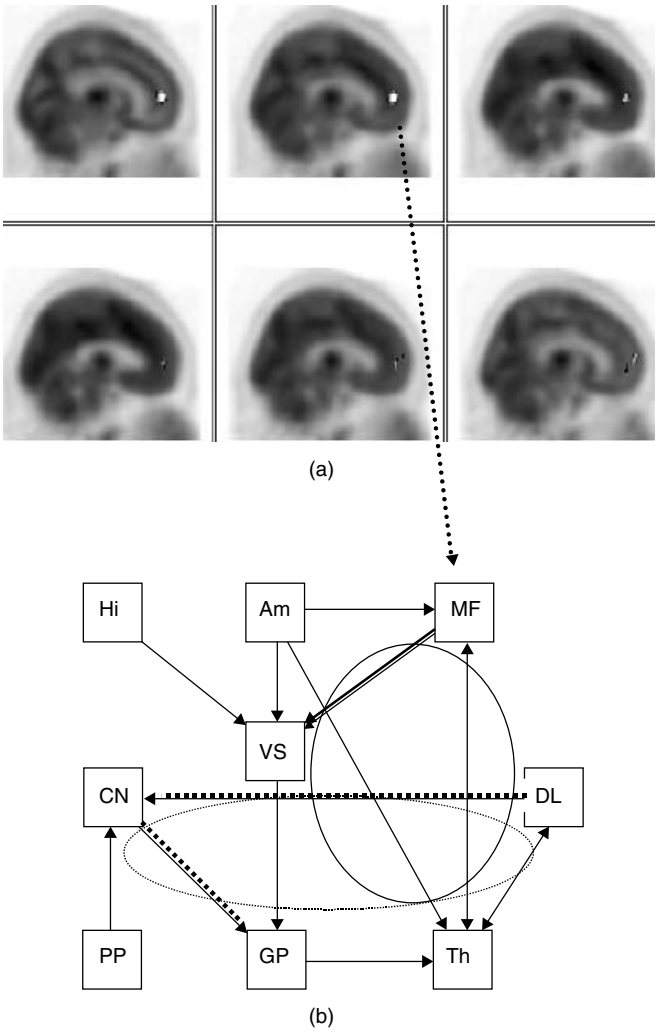

Figure XVIII-9.1 Effect of one session of TMS (5–20 Hz at 80% motor threshold over the left dorsolateral prefrontal cortex) on rCBF during a word-generation task. (a) Statistical parametric map of areas with $P < 0.01$ for effect size (z) and contiguous area (k), using Statistical Parametric Mapping, version 1996. (b) Neuroanatomical projections. Am, amygdala; CN, caudate nucleus; DL, dorsolateral prefrontal cortex; GP, globus pallidus; Hi, hippocampus; MF, medial orbitofrontal cortex; PP, posterior parietal cortex; Th, thalamus; VS, ventral striatum; Dotted ellipse, dorsolateral prefrontal loop; continuous ellipse, limbic loop; dotted arrow DL to CN, increase in regression coefficient c with significance levels in left dorsolateral loop ($c = 2.45$, $P < 0.05$); dotted arrow CN to GP, increase in regression coefficient c with significance levels in left dorsolateral loop ($c = 2.15$, $P = 0.05$); continuous arrow MF to VS, bilateral limbic loop (left: $c = 2.51$, $P < 0.05$; right: $c = 2.89$, $P < 0.05$) (See Colour Plate XVIII-9.1)

Physical Therapies

Electroconvulsive treatment (ECT) is by itself anticonvulsant, which suggests a reduction in cortical activity after a course of treatments (Ketter *et al.*, 1999; Post *et al.*, 2000). Using glucose uptake as a measure of cortical activity 5 days after a course of bilateral ECT in ten patients, Nobler *et al.* (2001) were able to support this hypothesis. Yatham *et al.* (2000) had previously failed to detect a significant change in five patients 1 week after a course of ECT. This is likely to be an effect of insufficient power.

Transcranial magnetic stimulation (TMS) has been used extensively to investigate cortical function in healthy volunteers and psychiatric patients (George *et al.*, 1998). A number of the simple hypotheses underlying the use of TMS in the treatment of psychiatric illness are based on the assumption that low-frequency TMS suppresses cortical activity (quenching) while high-frequency stimulation increases cortical excitability. Speer *et al.* (2000) were able to support this notion using 1- and 20-Hz stimulation over the left dorsolateral prefrontal cortex in a cross-over study of ten depressed patients. They found rCBF increases under the stimulation site and in associated paralimbic structures after 20-Hz stimulation and decreases in more restricted frontal, temporal and subcortical structures after 1-Hz stimulation. Similarly, Zheng (2000) found increases in cerebral perfusion in areas remote from the stimulation site after stimulation over the left dorsolateral prefrontal cortex.

In a parallel design study comparing 5, 10 and 20 Hz applied over the left dorsolateral prefrontal cortex in depressed patients, we found localized increases in the anterior cingulate after the first day's treatment, in combination with increased functional connectivity in the ipsilateral dorsolateral prefrontal loop and bilateral in the cingulate (limbic) loop (Figure XVIII-9.1).

In summary, recent studies have suggested an association between anatomical and functional changes in different prefrontal areas and their associated structures on one hand and disordered cognition and affect on the other. Such changes have been documented extensively in cingulate activity following successful treatment (e.g. Ebert *et al.*, 1996; Mayberg *et al.*, 1999; Volk *et al.*, 1997). Cingulate pretreatment perfusion differentiates between responders and non-responders (Mayberg *et al.*, 1997; Wu *et al.*, 1999). Furthermore, abnormalities in glial density (Drevets, 1999) and even changes in neuronal size (Rajkowska *et al.*, 1999) were found in the prefrontal cortex of unipolar patients (as discussed below). Finally, the cingulate is activated differentially in cognitive tasks (e.g. Stroop) involving attention or motivation (Whalen *et al.*, 1998), possibly denoting subregions that can be involved in specific tasks, promising a more differentiated understanding of the cognitive and emotional aspects of depression.

EFFECTS OF AGE, CHRONICITY AND TREATMENT RESISTANCE: IRREVERSIBLE AND REVERSIBLE CHANGES

Depression has been perceived as a transitory state where, on remission, brain function and altered or compromised cognition return to normal. This is no longer considered to be accurate in every case. Neuropsychological studies have discovered persistent abnormalities in cognitive performance, pertaining to the duration and severity of the illness (Abas *et al.*, 1990; Shah *et al.*, 1998). A number of imaging studies have also found potentially enduring deficits (e.g. Abas *et al.*, 1990; Shah *et al.*, 1998; Sheline *et al.*, 1999). Shah *et al.* (1998), in addition to a mere group difference, described a correlation between abnormal verbal memory and left medial temporal lobe grey matter deficits in the impaired group. Just as for certain unipolar depressed patients, structural abnormalities in bipolar depression include white-matter lesions, decreased cerebellar size, and sulcal as well as ventricular enlargement (see

Stoll *et al.*, 2000 for review). Structural MRI in 24 hospitalized bipolar patients (Strakowski *et al.*, 1999) revealed an increase in amygdala size with a similar trend in the globus pallidus, thalamus and striatum. These changes did not appear to correlate with other clinical measures, such as illness duration, medication, substance abuse, or the presence of previous episodes, although antipsychotic use would be a likely candidate.

Pathological studies have shown an excess of atheromatous disease in elderly depressed patients (Thomas *et al.*, 2001). In fact, some authors used the term 'MRI-defined vascular depression' (Krishnan *et al.*, 1997) to describe a group of elderly depressed patients with mainly later age of onset, nonpsychotic subtype, functional disability, anhedonia and a relative absence of family history (Krishnan *et al.*, 1997). Although these patients did not have a significantly worse prognosis than nonvascular depressives, a subgroup with late onset of the illness did (Krishnan *et al.*, 1998). In a large epidemiological study of cardiovascular health, small vascular lesions of the basal ganglia but not severity of white-matter lesions were associated with increased reporting of depressive symptoms as measured by the Centre for Epidemiological Studies Depression Scale (Steffens *et al.*, 1999). In a subsample, however, the MRI vascular changes in basal ganglia and non-basal ganglia structures appeared to exert their effects on depressive symptoms via the functional consequences of vascular disease, such as physical disability and cognitive impairment (Sato *et al.*, 1999). On the other hand, a case–control study of 96 elderly patients with late- and early-onset depression found the former to be associated with more white-matter hyperintensities, enlarged ventricles and hypertension (Lavretsky *et al.*, 1998).

MRI white-matter hyperintensities thus appear to be a hallmark of late-onset depression. Hyperintensities are also associated with treatment resistance (Lavretsky *et al.*, 1999; Simpson *et al.*, 1998) and future residual dysfunction, as well as cognitive decline (Hickie *et al.*, 1997; Jenkins *et al.*, 1998; Kramer-Ginsberg *et al.*, 1999) White-matter lesions seem to go with medical comorbidity, relatively independently of whole-brain and frontal atrophy, which are also more common in late-life depression (Kumar *et al.*, 2000). In particular, frontal lobe atrophy is correlated with severity of depression (Kumar *et al.*, 1998). A recent Newcastle study found that frontal white-matter changes were also more common in depressed patients with dementia. Finally, white-matter changes and frontal atrophy may be more common in depressed elderly patients with delusions (Kim *et al.*, 1999; O'Brien *et al.*, 1997a).

A combined MRI and SPECT study conducted in Edinburgh suggested greater temporal perfusion abnormalities in late- than early-onset depression and an association between cognitive deterioration and deep white-matter changes (Ebmeier *et al.*, 1997b; Ebmeier *et al.*, 1998). Similar (left medial) temporal lobe changes were reported in late-onset depression (Greenwald *et al.*, 1997), suggesting a possible link between late-onset depression and Alzheimer's disease. A large study comparing 61 depressed patients with 77 demented patients suggests, however, that temporal lobe volumetry can distinguish clearly been these two groups (O'Brien *et al.*, 1997b). Similar results were obtained using hippocampal width alone (Ebmeier *et al.*, 1997b).

Research in younger but also elderly depressed patients suggests that there may be a subgroup with long-lasting or treatment-resistant illness who show (medial) temporal lobe structural and functional changes (Shah and Ebmeier, 1998; Sheline *et al.*, 1999; Vakili *et al.*, 2000). Although there is little corroborating clinical evidence, some authors have argued that hypercortisolaemia associated with clinical depression may be responsible for the hippocampal damage observed in elderly patients. Contrary to this assumption, there is some limited evidence that temporal lobe changes are more common in late onset, i.e. shorter-lasting, depression (Ebmeier *et al.*, 1998; Lavretsky *et al.*, 1998).

Age Effects

As in younger patients, in elderly patients variation of depressive symptoms is associated with changes of brain activity in (medial) prefrontal structures. This can be shown in sleep-deprivation studies (Smith *et al.*, 1999a), in follow-up studies of depressed patients (Halloran *et al.*, 1999), in cross-sectional studies of clinical correlates in elderly depression (Awata *et al.*, 1998; Ebmeier *et al.*, 1997b) and even Alzheimer's disease (Hirono *et al.*, 1998).

Depression in children and adolescents has been attracting researchers' attention in recent years. Although the prevalence of affective disorders in childhood is now accepted to be on a par with the adult form of the illness, the contribution of the child's environment, along with cognitive and neuroanatomical developmental factors, is unclear. Data on functional brain activity in early-onset depression are consistent with findings in adults reporting left anterolateral hypoperfusion. Nonetheless, it is not certain whether the association between haemodynamic response and neural activity are analogous across the lifespan (Davidson and Slagter, 2000). Decreased frontal volume and increased choline-to-creatinine ratios in the anterior medial frontal lobe have been reported in depressed children and adolescents (reviewed in Steingard, 2000). Bipolar, just as schizophrenic, adolescents ($n = 35$) were reported to have reduced intracranial volume with enlarged ventricles and increased frontal and temporal sulci (Friedman *et al.*, 1999).

Treatment Resistance

Shah *et al.* (1998) demonstrated in 20 young patients with treatment-resistant unipolar depression, all of whom had been ill for more than 2 years, that temporal lobe structures, including the hippocampus, were reduced in grey matter density. The reduction in left medial temporal lobe grey matter density was correlated with poor performance in an auditory verbal memory task. Mervaala *et al.* (2000) examined 34 drug-resistant patients with major depression and were able to replicate findings of reduction in volume of left hippocampus. In addition, the choline/creatinine ratio in mesial temporal lobe was raised, suggesting membrane breakdown. Sheline *et al.* (1999) imaged 24 women with recurrent major depression and found that bilateral hippocampi were reduced in volume in proportion to illness duration and after controlling for age effects. Interestingly, patients also performed poorly in a verbal memory task, which may be a correlate of hippocampal reductions. Vakili *et al.* (2000) measured hippocampal volumes in 38 patients with primary depression. Although as a group patients had normal-sized hippocampi, treatment response to 20 mg fluoxetine for 8 weeks was associated with a relatively larger right hippocampus in women. Somewhat at variance with these results, a study focusing on temporal lobe epilepsy patients reported amygdala enlargement to be associated with dysthymia and depressive symptoms (Tebartz van Elst *et al.*, 1999; Tebartz van Elst *et al.*, 2000). The discrepancy may be explained by different pathology underlying mood changes in epilepsy, by divergent changes in amygdala and hippocampus, or by a different timepoint in the natural history of depression. Ketter *et al.* (2001) examined 43 treatment-resistant bipolar patients and found a contrast between cortical and subcortical glucose metabolism (18FDG–PET). Normalized effects indicating increased metabolism were reported in subcortical areas (ventral striatum and right amygdala) as well as in the posterior cortex, thalamus and cerebellum. Depressed bipolar patients also showed an absolute prefrontal, temporal and anterior paralimbic hypometabolism. Elevated metabolism in posterior cortical areas (including posterior thalamus and cerebellum) persisted beyond remission (i.e. in euthymic patients), indicating a trait marker

unique to bipolar patients. Cognitive task activation (in this case, an auditory task) may be recruiting qualitatively different circuits in patients and controls, a concern shared by all studies employing neuropsychological activation paradigms (Ketter *et al.*, 2001).

Cellular Changes

Both neuronal and glial volume changes have been reported. The latter can be a consequence of a number of factors. As a result of the chronic use of antipsychotic medication, increased volume of glial cells was noted in the prefrontal cortex of rhesus monkeys (Selemon *et al.*, 1999). Reports of laminar as well as regionally specific patterns of glial but also neuronal reduction, where neurons decreased both in size (dorsolateral prefrontal cortex) and density (orbitofrontal cortex), was observed in the prefrontal cortex of unipolar patients (Rajkowska *et al.*, 1999). By contrast, post-mortem studies in both unipolar and bipolar patients with a family history of depression have not revealed any differences in the neuronal population, while glial density was decreased; glial volume of schizophrenic patients was unchanged (Drevets *et al.*, 1997; Ongur *et al.*, 1998). Finally, age-associated glial changes in primate grey matter reached statistical significance only in the cingulate (Sloane *et al.*, 2000). It is apparent that some association between changes in glial density and pathological or restorative processes has been established in patients suffering from affective disorders. Nonetheless, it is still unclear whether these changes are adaptive, compensatory or incidental. Further, the functional effect of such changes is still undetermined.

The association between neuronal activity and the metabolic processes measured by various functional brain-imaging modalities (haemodynamic response in functional magnetic resonance imaging (fMRI), glucose utilization in FDG–PET, and so forth), is not without question. Furthermore, it is apparent that astrocytes as well as neurons may contribute to the signal changes observed in FDG–PET (Magistretti and Pellerin, 1996; Magistretti and Pellerin, 1999). In the presence of glial reduction in anterolimbic structures reported by a number of groups (Drevets *et al.*, 1998; Rajkowska *et al.*, 1999), it is unknown whether, and to what extent, glucose utilization and its associated PET signal are altered as a result.

Lithium and valproate have been suggested to have neurotrophic and neuroprotective effects. An increase of 3% in the cortical grey matter volume of bipolar patients after 4 weeks of lithium administration is attributed to neurotrophic factors rather than to cell swelling (Moore *et al.*, 2000). Mood stabilizers could be involved in the regulation of gene expression by increasing levels of mRNA for PEBP2β (polyomavirus enhancer-binding protein 2 beta subunit), which controls coding for the neuroprotective protein BCL2 (B cell lymphoma protein 2) in the frontal cortex. Increased neuronal survival and increased regeneration are all effected by BCL2 upregulation, protecting against excitotoxic damage (Chen *et al.*, 1999; Chen and Chuang, 1999). It is unclear whether recent post-mortem findings of increased neuronal numbers throughout the hypothalamus and the dorsal raphe could be related to chronic use of medication (Rajkowska *et al.*, 1999).

Small structures such as the brainstem raphe nuclei are imaged with difficulty. It is, therefore, fortuitous that ultrasound sonography can be applied using a preauricular acoustic bone window to image the mesencephalic brainstem with structures such as the red nucleus and the rostral pontine brainstem. Becker *et al.* (1995) examined 40 unipolar, 40 bipolar depressive and 40 schizophrenic inpatients, as well as 40 healthy volunteers. Reduced midline echogenicity relative to the red nucleus, which can be interpreted as structural disruption of the raphe nuclei, was found only in the unipolar depressed patients.

EXPERIMENTAL APPROACHES TO DEPRESSION: COMPLEX MODELS

Cognitive performance in depression and its relationship with the disturbance of emotion or mood have been examined in a number of contexts. Along with an investigation of cognitive impairment in subjects suffering from affective disorders, there is a large body of literature reporting different procedures of precipitating depressed or dysphoric mood in healthy subjects (mood induction). A number of conceptual questions are inherent in this literature. Above all, it is unclear to what extent sad mood is analogous with clinical depression. The transient time course of the neuropsychological impairment brought about by mood induction is usually far shorter than the experience of clinical depression. Similarly, induced mood may be of a different quality from morbid depression, although biological symptoms, such as depressive retardation, can be observed after experimental mood induction (Ebert *et al.*, 1996). The absence of biological markers often associated with depression (such as hypercortisolaemia), along with structural and functional differences, hinder direct comparison. In this sense, even if the neural systems recruited in healthy controls during mood induction are analogous to the neuroanatomical circuit activated in depressed patients at rest, the dynamics of the circuit may be different in the two conditions due to selective dysfunction in specific components or the connectivity between these. It is even possible that some of the differences between patients and controls can be explained by simple mechanisms, e.g. that depressed patients are slower to recruit the appropriate neuronal systems or their components.

Since neuropsychological studies are described in detail in Chapter XVIII-7 and XIX-8 of this book, we will focus on neuronal systems implicated by clinical and mood-induction paradigms. We will try to integrate structural and functional findings reported earlier in the chapter in an attempt to assemble some of the complex interactions between specific structures and the systems they comprise in the manifestation of affective illness.

Neuropsychological studies suggest that cognitive deficits may be the consequence of competition between cognitive and affective functional resources. If this holds true, such that neural pathways identified in the induction paradigm are analogous to those affected in the specific mood disorder they are trying to emulate, then transient sadness or elation in normal volunteers can provide a useful insight into the neural correlate of cognitive performance in mood disorders. However, covariation between the degree of depression, impaired task performance and (in-)activity of relevant functional circuits does not necessarily imply a causal relationship between the three. Thus, apparently similar short-term deficits of neuronal activity observed both in clinical depression and after mood induction could be grounded in dissimilar processes. In the long term, these may be of no consequence for healthy volunteers, but they could result in the significant deficits noted in patients with recurrent and severe depression (Shah *et al.*, 1998).

The following section includes studies emphasizing perfusion changes associated with emotional states. Most studies report impaired cognitive performance as a consequence of transient mood change, although their methods may differ greatly. Anatomical structures that are classically grouped under the definition of 'limbic lobe' are involved in circuits responsible for both cognitive function and the generation of affect. These include the cingulate cortex, hippocampus, amygdala and thalamus, as well as other prefrontal and subcortical structures. Both mood-induction and clinical studies report altered dynamics in the limbic and prefrontal circuits, comprising activation or deactivation under specific conditions, compensatory effects, and the interplay between different regions (e.g. cortical and subcortical, limbic and dorsolateral prefrontal cortex, etc). The role of the cingulate has been emphasized by authors such

as MacLean (1985). He saw the phylogenetic emergence of the cingulate cortex as fundamental to the development of maternal care in mammals. There is therefore a convergence of paradigms (Stevens and Price, 2000) between psychodynamic speculation about attachment and loss (Bowlby, 1969) and the understanding of limbic brain function in depression (Ebert *et al.*, 1996).

Such theories are now amenable to empirical testing. Lorberbaum *et al.* (1999) employed fMRI in four recently confined mothers listening to recordings of infants' cries and white noise as a control condition. Increased brain activity could be demonstrated in the anterior cingulate and right medial prefrontal cortex. These regional activations are probably not very specific, as similar areas are activated during pain and are thought to be related to affective and attentional concomitants of pain sensation (Peyron *et al.*, 2000; Schnitzler and Ploner, 2000). However, with careful experimental procedures, hypotheses about the specific role of the medial prefrontal cortex in emotion are testable, in principle.

Supporting the imaging studies in humans, there is also an animal model of congenitally helpless rats with reductions in brain activity in the dorsal frontal, medial orbital and anterior cingulate cortex, combined with increases in the subgenual cingulate (Shumake *et al.*, 2000).

Mood Induction and Neuropsychological Paradigms

Neuropsychological studies use cognitive tasks in order to recruit associated functional circuits through their component structures. Specific functional vulnerability can be described by either impaired performance of a particular task or impaired activation of characteristic circuits or their constituents. Neuropsychological tasks have been designed to probe activity in brain regions associated with them. Frontal areas are activated by tasks such as the Wisconsin card sorting test (WCST) or the Tower of London (TOL) task, both of which involve the application and manipulation of rules, adaptive skills and flexibility necessary for set shifting. Tasks that involve memory are expected to be particularly sensitive to structural and/or functional discontinuity in (mesial) temporal structures, such as the hippocampus. Finally, the involvement of subcortical structures in the experience of clinical and experimental mood is expressed by both perseverative responses (the inability to change strategies) and psychomotor slowing, experienced predominantly by melancholic patients (Austin *et al.*, 1999; Austin *et al.*, 2001). It appears that similar limbic-subcortical circuits are involved in different experimental mood-induction procedures; however, different substructures may be affected, possibly due to inconsistent methodology of experimental paradigms and the different affects involved.

Studies: Mood Induction

Baker *et al.* (1997) induced sad and elated mood in controls and examined brain activity following performance of a verbal fluency task. They reported anatomical dissociation between mood and cognitive function. Both mood states curtailed the activity normally associated with the verbal fluency task throughout the left prefrontal, premotor and cingulate cortex, as well as the thalamus. Reduced activation in the rostral medial orbitofrontal cortex and anterior cingulate was associated uniquely with sad mood.

Mayberg *et al.* (1999) proposed a simplified model of brain responses. They hypothesized a general increase in limbic-paralimbic perfusion coupled with a decrease in neocortical perfusion to accompany induction of sadness in healthy volunteers, while reciprocal changes were predicted during the resolution of dysphoric symptoms in depressed patients. These authors also observed an inverse correlation between right dorsolateral prefrontal and subgenual cingulate perfusion, supporting this notion. These finding can be interpreted as corollary evidence for the inverse relationship between depressed mood and attention.

Beauregard *et al.* (1998) studied patients' and controls' ($n = 7$) responses to mood induction. Transient sadness (triggered by film clips with emotional content) produced activation in the medial and inferior prefrontal, middle temporal cortex, cerebellum and caudate. Significantly greater activation in the left medial prefrontal cortex (Brodman's area 8) and right cingulate gyrus (Brodman's area 32) was observed in depressed patients, suggesting that these two structures may have a role in pathological sadness.

Elliott *et al.* (1997) postulated a catastrophic reaction to failure as a specific neuropsychological mechanism underlying poor performance in depression. They examined performance feedback responses in six patients and controls. Depressed patients did not share the expected activation in the medial caudate and ventromedial orbitofrontal cortex found in controls. Patients' brains were, therefore, insensitive to changes in both task and feedback conditions, consistent with the a priori hypothesis.

Schneider *et al.* (1996) found activation in a network related to performance feedback during the attempt at solving unsolvable anagrams; perfusion increased in healthy subjects ($n = 12$) in the mammillary bodies and the amygdala, and decreased in hippocampus. Solvable tasks were associated with increased perfusion in the latter. Increased frontal and temporal perfusion was associated with both conditions.

The Stroop interference task has been typically associated with activation of the cingulate gyrus (Pardo *et al.*, 1990). A different activation pattern was observed in depressed patients ($n = 11$), who failed to activate the left cingulate in comparison with control subjects. Reduced perfusion in patients' right cingulate was balanced by stronger activation of the left dorsolateral prefrontal cortex and the visual cortex (George *et al.*, 1997). A variant of the Stroop interference task (the emotional counting Stroop paradigm) showed a unique activation of the rostral anterior cingulate using fMRI in normal volunteers (Whalen *et al.*, 1998). Comparing perfusion between trials containing affective (such as murder) and neutral words, associated negative content with increased perfusion in the anterior cingulate without a change in reaction time. Performance of this form of the Stroop task compared with fixation *per se* was associated with overall decreased perfusion. These findings may suggest an association between pathological anxiety and an inability to reduce cingulate activation during task compared with fixation.

Correlation between higher global perfusion and increased cognitive demands was associated with task switching. The superior parietal cortex was reported to have a specific role in task switching, although this effect may be task specific (Kimberg *et al.*, 2000). Illness severity in this subtype correlates with increased perfusion in frontolimbic structures parahippocampal gyrus and cingulate (Ebmeier *et al.*, 1997a).

Induction of depressed and elated mood can, at times, be associated with overlapping or diverging neural circuitry. Dissociable recruitment of subcortical and cortical structures in healthy subjects ($n = 16$) during experience of both positive and negative affect was associated with specific changes in subcortical structures (especially the amygdala) but not frontotemporal structures (Schneider *et al.*, 1995). Both happy and sad mood correlated with increased blood oxygen level-dependent (BOLD) fMRI response in the left amygdala of healthy subjects ($n = 12$) (Schneider *et al.*, 1997). De Raedt *et al.* (1997) used a modified Velten procedure to induce mood both 'within and out of the realm of attention'. The latter involved a combination of dichotic listening and subliminal stimulation. Right lateral reduction in thalamic perfusion was found during both conditions compared with responses to neutral stimuli. Increased hippocampal perfusion was limited to subliminal stimulation conditions.

Divergent circuits were found in the evaluation of negative versus positive affective content. While the former was associated with increased perfusion in the right frontal gyrus and thalamus, the latter produced activation of the bilateral insula and the right inferior frontal gyrus. Neither showed associated changes in amygdala perfusion (Teasdale *et al.*, 1999). In a different study, subcortical limbic structures (amygdala, associative cortex, primary visual cortex, cerebellum) were activated through evaluation of unpleasant stimuli, while pleasant stimuli activated cortical structures (medial prefrontal cortex, dorsolateral prefrontal cortex, and the right orbitofrontal cortex, Paradiso *et al.*, 1999). Spatiotemporal differences in activation of the neuroanatomical correlate of positive and negative affect, expressed through characteristic activation of either orbitofrontal cortex or prefrontal cortex, were shown in healthy volunteers ($n = 10$). Recruitment of the medial prefrontal network in the context of negative affective content was established faster than the activation of the lateral prefrontal circuit, which was associated with positive affect (Northoff *et al.*, 2000). Finally, retrieval of episodic memories with affective content was associated with activation of anterior temporal lobe and left amygdala (Dolan *et al.*, 2000), a pattern mirrored by McGaugh and Cahill (1997) and Hamann and Adolphs (1999) in encoding of similar memories.

Hypofrontality along with specific prefrontal deficits have been reported extensively in the literature. Both depressed ($n = 6$) and control subjects performing the TOL task exhibited a pattern of deactivating the medial prefrontal cortex, superior temporal gyrus and posterior cingulate. For control subjects, increased difficulty was associated with a linear augmentation in the recruitment of the appropriate functional circuitry. By contrast, depressed patients presented with decreased activity in the rostral prefrontal cortex, caudate nucleus and anterior cingulate, without the expected compensation by the dorsolateral prefrontal cortex (Elliott *et al.*, 1997). Parietal cortex activation did not reach threshold in this experiment. Neural dissociation between cognitive and affective activation denoted specific activity-related changes in the medial frontal cortex. A reduction from baseline activity in the medial prefrontal cortex was linked to cognitive activation. Practice was associated with decreased perfusion in the medial prefrontal cortex, while performance anxiety was linked to increased perfusion in the same region. In a different experiment, elevated perfusion in the medial prefrontal cortex was reported during the anticipation of painful stimuli, and was correlated negatively with anxiety rating (i.e. increased anxiety produced a smaller reduction from baseline). Corresponding changes in flow were noted in the hypothalamus and midbrain. Thus, a decrease from baseline activity in the prefrontal cortex is thought to be a consequence of recruiting the network related to attention (Simpson *et al.*, 2001a; Simpson *et al.*, 2001b).

Systems

We conclude the chapter by discussing the neuronal circuits or systems putatively linked with affective illness. To the best of our current knowledge, there is no clear or direct correspondence between neurobiological factors and their effect on emotional experience or expression. Structural changes in areas such as the hippocampus, prefrontal cortex, cingulate or amygdala are perfect candidates for theories of dysfunctional loci. However, as we have indicated throughout this chapter, neurobiological abnormalities are not entirely predictive of functional deficits. Furthermore, dysfunction in affective disorders may involve long-term mechanisms of compensation and deterioration over time, which may not be explicit in present theories of unipolar and bipolar depression.

The limbic system is, of course, a natural candidate for many neuroimaging investigations. The evolutionary angle described earlier in this chapter (Bowlby, 1969; MacLean, 1985) places structures associated closely with environmental feedback, particularly in a social context, at the locus of a system associated with integrating internal and external states. For example, specific behaviours or neuropsychological tasks yield an associated activation in structures such as the cingulate gyrus (maternal separation cry, Stroop), amygdala (fear), hippocampus (autobiographical memories) and dorsolateral prefrontal cortex (TOL task), to name but a few. It is important to note that in brain regions not classically associated with the limbic system (and thus not salient in many investigations), such as the parietal cortex, reports of functional abnormalities are increasing (Davidson *et al.*, 1999; Drummond *et al.*, 2000; Ho *et al.*, 1996; Mayberg, 1997) Since regions of functional interest are often defined a priori in neuroimaging studies, a bias towards the well-documented structural and functional changes in limbic regions may be perpetuated.

Behavioural response to long-term stress in a social context seems to be adaptive, in the sense that initial alarm and resistance will ultimately lead to exhaustion and acceptance (Selye, 1936). Similarly, rank theories in mood disorders postulate acknowledgement of subordinate status and a 'yielding' motor response in the presence of higher rank (Stevens and Price, 2000). The limbic system is therefore assumed to unite behaviour and neuroanatomy, where conflict between external and internal input can be resolved, a role associated particularly with the cingulate. The impact on both psychomotor and prefrontal-cognitive associated capacity is inherent in these theories.

Expanding Papez's early theories of limbic circuit connectivity and function, current neuroimaging tools are instrumental in facilitating both understanding and advancement of functional circuitry in depression and mania. Careful attention to experimental design (such as activation under different experimental conditions), as well as the development of new chemical tracers and more powerful imaging technology, will contribute towards a better appreciation of the various phases associated with affective disorders. We are nearing a qualitative coupling between systems with either function or dysfunction. This is achieved by direct and indirect comparison between patients and healthy control subjects performing tasks under similar conditions. Nonetheless, quantitative knowledge regarding activity in distinct systems is, for now, sketchy.

Common to both prefrontal and limbic structures is the overlap between sensory and affective processes (LeDoux, 1996), where a visceral system provides feedback through endocrine and other neurobiological mechanisms. It is possible that inhibition by the prefrontal cortex may be involved in feedback control (not only the classically inhibitory orbitofrontal cortex but also the lateral prefrontal cortex, both denoting different strategies of associative learning) (Roberts and Wallis, 2000). Consequently, activity, or lack thereof, in frontal regions may lead to limbic hyperperfusion via nonlinear excitatory or inhibitory operational modes.

Hypofrontality has been reported extensively in different contexts. Thus, the interaction between cognitive and affective circuits with the frontal cortex has been described by neuroanatomical (Barbas *et al.*, 1999; Price *et al.*, 1996), neuropsychological and neuroimaging studies (Mayberg *et al.*, 1997; Rogers *et al.*, 1998). These circuits appear to have functional as well as structural regional specificity, whereby connectivity to other (remote) regions throughout the cortex and the subcortex are clearly defined.

The limbic system has been separated into affective and cognitive components, supported by neuroanatomical studies in humans and primates (e.g. Devinsky *et al.*, 1995; Mayberg *et al.*, 1999; Mega *et al.*, 1997; Price, 1999a). The integration of viscerosensory and affective information, yielding endocrine and motor changes (Price, 1999b) contributes to an extended network, which cannot be explicable by lesion studies alone (Frith and Dolan, 1998).

A number of compensatory mechanisms can be deduced from the extensive neuroimaging literature in the field. The amygdala and orbitofrontal cortex appear to activate in synergy, where the orbitofrontal cortex is activated more strongly in response to amygdala dysfunction (Drevets, 1999). In healthy subjects, compensatory strategies require greater activity in the prefrontal and parietal cortices to balance for decreased temporal lobe perfusion and associated performance deficits after a night of TSD (Drummond *et al.*, 2000). By contrast, Elliott *et al.* (1997) showed that depressed patients lacked an adaptive capacity, which was expressed by their inability to recruit prefrontal as well as subcortical structures during performance of the TOL task. Further, correspondence between hyperperfusion in the cognitive and affective divisions of the cingulate reported by Mayberg *et al.* (1999) could arguably have a compensatory role in depression and recovery.

Therefore, a hyperactive limbic (or 'ventral'; see Mayberg *et al.*, 1999) system was noted by a number of groups, where increased perfusion can imply higher probability for response to treatment (Mayberg *et al.*, 1997; Wu *et al.*, 1999) or, alternatively, increased likelihood of more severe illness (Austin *et al.*, 1992). In the long term, connections to temporal (amygdala, hippocampus), parietal, prefrontal and subcortical structures will help us understand the extent to which an extended network can compensate for limbic hyperperfusion.

Blood flow and glucose metabolism in subcortical structures, the dorsolateral prefrontal cortex and the cingulate have been linked extensively with affective disorders through neurological and movement disorders, lesion studies, functional activation and imaging at rest (Cummings, 1993; Mega and Cummings, 1994, Soares and Mann, 1997). Furthermore, basal ganglia and frontal lesions carry a higher probability for cognitive impairment (Rogers *et al.*, 1998; Videbech, 1997). It is apparent that subcortical involvement can be associated with increased pathophysiology (e.g. psychomotor retardation), recurrent episodes and cognitive decline (Hickie *et al.*, 1997; Hickie *et al.*, 1999; Simpson *et al.*, 1998). The anterior cingulate may play a cardinal role in this context, due to its widespread connections and functional association with the prefrontal cortex (Ebert *et al.*, 1996; Koski and Paus, 2000). This is, at present, not clearly specified, ranging from theories of a 'somatic marker' (Damasio, 1994), motivation, attention and error detection (Carter *et al.*, 1999) due to its specificity in the integration of cognition and affect. It is confounded by the structural changes in specific subgroups of unipolar and bipolar patients (Drevets *et al.*, 1997). Finally, since hippocampal volume reduction is associated with the severity of depressive illness (Sheline *et al.*, 1999) and, by association (Drummond *et al.*, 2000), transient hypoactivity in the temporal cortex, its function may be balanced by prefrontal and parietal regions.

It would be interesting to probe such functional correspondences between two or more structures in a clinical context.

To conclude, a unified theory of affective disorders is developing through the application of different imaging methods and consideration of both animal and human neuroanatomical data. Future studies will no doubt consider a larger, distributed functional network, coupled with better understanding of the long-term effects of clinical deterioration, medication and the ageing process. What emerges, is a functional interplay of brain modules that are associated with specific mood states, attention, non-specific emotional factors, and certain aspects of the neuropsychological tasks used. We now have the tools to test relevant hypotheses in the living brain. The groundwork will have to be done with fMRI and possibly TMS in healthy volunteers, although in the last analysis, studies in sufficiently large, homogeneous and representative groups of patients will be necessary. The understanding that has come from studies so far is that dimensions of mood are indeed interlinked closely with cognitive categories, and that experimental studies may necessitate our rethinking of clinical and psychological constructs.

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XVIII-10

Molecular Genetics in Mood Disorders

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INTRODUCTION

Despite intensive search for biological underpinnings, the aetiology of major mood disorders remains unknown. More work on the neurobiology of these disorders is clearly indicated. Twin and family studies have consistently demonstrated a genetic component to the disorders. Decades of research into the genetic aetiology of mood disorders provide evidence in favour of a complex mode of inheritance unlikely to be determined by single gene dysfunction, and apparently non-Mendelian patterns of inheritance. Part of the complexity of the genetic variance lies in the heterogeneity of the disorders — the depressive patient, for example, can be characterized by a variety of different symptoms and identifying features that may represent different subtypes. Different genetic mechanisms may be involved: epistasis, locus heterogeneity, allelic heterogeneity, dynamic mutations, imprinting and mitochondrial gene mutation (these issues are reviewed in Chapter XII). In terms of phenotype, there are also a variety of non-genetic factors that lessen our ability to detect genes in mental disorders: phenocopies, clinical heterogeneity, assessment bias, population stratification and lack of appropriate control groups.

Of the genetic mechanisms listed above, it is possible that more than one may be involved in the transmission of psychiatric disorders. In complex disorders the correspondence between gene and phenotype is not necessarily direct: a given genotype may give rise to a variety of phenotypes according to other genes present and environmental factors, or different genotypes may give rise to the same phenotype. Despite these complex characteristics, family- and population-based studies have provided substantial evidence that genetic factors contribute to the expression of the disorders. Recent molecular genetic approaches indicate that several chromosomal regions may be involved in the aetiology of mood disorders.

The initial molecular genetic studies of Bipolar disorder (BPAD), considered as the core phenotype of mood disorders, involved the parametric linkage studies of large families. Linkage examines the cosegregation of a genetic marker and disease in affected individuals within families; that is, the non-random sharing of marker alleles between affected members of each family (Smeraldi and Macciardi, 1995). Two genetic loci are linked if they are located closely together on a chromosome. In linkage analysis, the frequency of meiotic recombinations as an expression of the distance between marker locus and the gene under investigation is used for gene mapping (see Chapter XII). Given the difficulties inherent in detecting genes of small to modest effect using the linkage approach, the candidate gene association method offers an alternative strategy of studying genetic factors involved in complex diseases in which the mode of transmission is not known. Association between diseases and marker may be found if the gene itself, or a locus in linkage disequilibrium with the marker, is involved in the pathophysiology of the disease (Hodge, 1994). Thus, an association may imply a direct effect of the gene tested, or the effect of another gene close to the marker examined. The candidate gene approach is a useful method to investigate association between markers and disease. A candidate gene refers to a region of the chromosome which is potentially implicated in the aetiology of the disorder concerned. The possibility of false-positive results must be taken into account, as a very large number of candidate genes now exist. The probability that each of these genes is involved in the aetiology of the disorder is relatively low.

The candidate gene approach can be extended to phenotypes not directly linked to the diagnoses of mood disorders. The therapeutic effect of psychotropic drugs may be considered to investigate genetic polymorphisms (psychopharmacogenetics). In recent years, research in psychopharmacogenetics has focused on evaluating functional polymorphisms both in genes coding for drug-metabolizing enzymes and in genes coding for other enzymes or receptors involved in the mechanism of action of psychoactive drugs. In this context, the use of new technologies is rapidly evolving. Gene expression patterns in response to drug treatments can be investigated by new techniques such as DNA microarrays. Microarrays are powerful tools for investigating the mechanism of drug action by measuring changes in mRNA levels in brain tissues before and after exposure to treatment (Debouck and Goodfellow, 1999).

This chapter reviews the current methodologies and study tools used to search for molecular genetic factors in mood disorders and the chromosomal regions of interest already investigated for bipolar (BPAD) and unipolar (UPAD) mood disorders.

CANDIDATE CHROMOSOMAL REGIONS AND CANDIDATE GENES

Chromosome X

A systematic review of the literature on linkage studies in BPAD (Turecki *et al.*, 1996) indicated that the proportion of positive DNA findings is higher for X markers compared to other chromosomal regions. Mendlewicz *et al.* (1987) first reported possible genetic linkage between BPAD and coagulation Factor IX (F9) located at Xq27 in 11 pedigrees. The same genetic marker was also tested in a French pedigree where linkage was confirmed (Lucotte *et al.*, 1992). Linkage with DNA markers on the X chromosome has been excluded, however, in other pedigrees (Berrettini *et al.*, 1990; Bredbacka *et al.*, 1993; Gejman *et al.*, 1990). A study published in 1987 by Baron *et al.* (1987) demonstrated positive linkage for glucose 6 phosphate dehydrogenase (G6PD), but later results from the same author did not support this finding (Baron *et al.*, 1993), although G6PD was slightly positive for linkage in one family. In a more recent study (De Bruyn *et al.*, 1994), several DNA markers in the Xq27–28 region were tested in nine bipolar families.

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Results suggestive of linkage were found in four bipolar I (BPI) families with the markers F9, F8 and DXS52. Pekkarinen *et al.* (1995) evaluated 27 polymorphic markers on the X chromosome (Xq25–28 region) in one large Finnish family, and found the highest lod scores when using a narrower phenotype definition (BPI and BPII only). Linkage was found between a marker located on Xq26 (AFM205wd2) and BPAD. This marker is located about 7 cM centromeric to the F9 locus. The initial genome screen for BPAD in the NIMH genetics initiative of 97 pedigrees also revealed positive though small lod scores on the X chromosome (Xp22 and Xq26–28) (Stine *et al.*, 1997). All these results are extremely suggestive of X linkage and in particular, the Xq26–28 region should be considered a strong candidate for genetic studies in BPAD.

The associations between BPAD and polymorphic DNA markers in the pseudoautosomal region of the X chromosome have also been investigated. Yoneda *et al.* (1992) reported an association between the A4 allele of the marker DXYS20 in this chromosomal region in Japanese BPAD patients. This has not been replicated in European populations, however (Nöthen *et al.*, 1993; Parsian and Todd, 1994).

The MAOA and MAOB genes which code for the enzymes that degrade biogenic amines, including neurotransmitters implicated in the pathophysiology of mood disorders, such as norepinephrine, dopamine and serotonin, are both located on the X chromosome and tightly linked to each other (Xp11.12–Xp11.4). Lim *et al.* (1994a, 1995) reported a weak but significant association for three different polymorphisms of MAOA, yet not for MAOB, in a sample of 57 BPAD patients compared to population controls not assessed for psychiatric status. A weak association was found for MAOA and bipolar disorder by Kawada *et al.* (1995), but different alleles were more frequent in the patient population compared to the previous report. A series of negative reports followed, leaving the question open as to the usefulness of the MAO genes in psychiatric genetics research (Craddock *et al.*, 1995a; Muramatsu *et al.*, 1997; Nöthen *et al.*, 1995).

Chromosome 18

Berrettini *et al.* (1994) first reported linkage of BPAD to chromosome 18 DNA markers in a systematic genome survey of 22 families including 156 subjects with bipolar disorder. Although the overall lod score for the pedigree series was negative, results of two-point linkage analysis in individual families indicated possible linkage with some marker loci in the 18p11 region. Non-parametric analysis (affected pedigree member and affected sib-pair analysis) of this sample confirmed the observation of linkage (Gershon *et al.*, 1996). These results suggested the existence of a susceptibility gene in the pericentromic region that can not be fully evaluated by classic linkage analysis. These results may be of interest because genes coding for the alpha unit of a GTP binding protein involved in neurotransmission, a corticotrophin receptor gene, and RED-1 containing triplet repeats have been mapped to this region. Stine *et al.* (1996) studied 28 nuclear families for markers on chromosome 18 and also found evidence for linkage and allele sharing between sib-pairs at 18p11.22–p11.21. This study replicated the findings of Berrettini and colleagues, yet also demonstrated evidence for linkage and parent-of-origin effect in a region located on the long arm of chromosome 18, a sex-averaged distance 30 cM away. An excess of paternally but not maternally transmitted alleles from the D18S41 was observed.

In a study of five rigorously defined, high-density German families, no robust evidence for linkage for the pericentromeric region could be found, although one family showed slightly elevated lod scores under a recessive mode of inheritance for D18S40 (Maier *et al.*, 1995). Linkage to the pericentromeric region of chromosome 18 was excluded in three large Old Order Amish families (Pauls *et al.*, 1995), and in two large Belgian pedigrees (De Bruyn *et al.*, 1996). In the Belgian pedigrees, while negative lod scores were found for a marker located in the pericentromeric region, linkage and segregation analysis in one family suggested that the 18q region of the chromosome (18q21.33–q23) might contain a susceptibility locus for BPAD (De Bruyn *et al.*, 1996). Freimer *et al.* (1996), using both linkage and association strategies, reported evidence of linkage with markers in this region (18q22) in a Costa Rican pedigree with a common founder. A subsequent study further supported the interest of the 18q23 region, showing linkage among six pedigrees (Coon *et al.*, 1996). An additional study of 173 affected subjects using the multilocus affected pedigree member method demonstrated a susceptibility gene in the pericentromeric region, and multilocus analysis by the affected sib-pair method also showed evidence for linkage (Berrettini *et al.*, 1997). Yet despite slight allele sharing with two markers on chromosome 18 (D18S40 on 18p and D18S70 on 18q) in the NIMH genetics initiative BPAD pedigrees, linkage to chromosome 18 was not confirmed in this large genome scan (Detera-Wadleigh *et al.*, 1997). Furthermore, Knowles *et al.* (1998) found no evidence for significant linkage between bipolar disorder and chromosome 18 pericentromeric markers in another large sample (1013 genotyped individuals) using ten highly polymorphic markers.

Chromosome 5

Preliminary linkage data including three DNA markers on this chromosome (D5S39, D5S43 and D5S62) suggested linkage with BPAD (Coon *et al.*, 1993). Two of these markers, D5S62 and D5S43, are located on the distal region of the long arm of chromosome 5 (5q35-qter). This region contains candidate genes for mood disorders such as the alpha-1 protein subunit of the GABA A receptor (GABRA1) and the 5-HT1A receptor (5-HTR1A). These two markers, however, had exclusion lod scores in a previous study of 14 families (Detera-Wadleigh *et al.*, 1992). Similarly, strongly negative lod scores were found in a linkage study covering the 5-HTR1A locus in five pedigrees (Curtis *et al.*, 1993). The dopamine transporter gene (DAT1) is located in a different region of chromosome 5 (5p15.3). This marker has been investigated in association studies with BPAD yet no association has been found (Gomez-Casero *et al.*, 1996; Manki *et al.*, 1996; Souery *et al.*, 1996a). Kelsoe *et al.* (1996) reported possible linkage, however, between a locus near the dopamine transporter (DAT) and BPAD, under a dominant transmission model giving a modest lod score of 2.38. Replication of this finding was shown in another linkage analysis subsequently (Waldman *et al.*, 1997).

Chromosome 11

Chromosome 11 has been thoroughly investigated in mood disorders because of the presence of candidate genes involved in catecholamine neurotransmission such as tyrosine hydroxylase (TH, 11p15), tyrosinase (11q14-21), dopamine receptor D2 (DRD2, 11q22-23), dopamine receptor D4 (DRD4, 11p15.5) and tryptophan hydroxylase (TPH, 11p15). Overall, results of linkage studies indicate that the TH gene does not contribute a major gene effect to BPAD (Souery *et al.*, 1996b). A possible role for the TH gene was also examined in BPAD association studies, all on moderate to small sample sizes. Meta-analysis of the results (Furlong *et al.*, 1999) does not support the TH gene having a major role in the aetiology of BPAD, while data suggest that this candidate gene should be examined in larger samples of UPAD for which this marker may confer susceptibility to the disease.

Linkage to the dopamine 2 receptor (DRD2) has been excluded in a number of studies (Byerley *et al.*, 1990; Ewald *et al.*, 1994;

Holmes *et al.*, 1991; Kelsoe *et al.*, 1993; Nanko *et al.*, 1994). Most association studies have similarly showed no association between the gene for this receptor and BP (Craddock *et al.*, 1995b; Manki *et al.*, 1996; Souery *et al.*, 1996a).

Linkage to the DRD4 receptor gene has not been definitively excluded (Nanko *et al.*, 1994; Sidenberg *et al.*, 1994), and a few association studies have found positive results. For unipolar patients alone, an excess of short exon III repeats (2–4 repeats) has been found to be significantly more frequent than in controls (Manki *et al.*, 1996). The 7 repeat allele was associated with bipolar disorder, and an excess of allele 3 among controls in another study, yet negative results were then found in a larger sample by the same group (Lim *et al.*, 1994b). Serretti *et al.* (1998) recently reported an excess of allele 7 among mood disorder patients with delusional features, suggesting its importance in psychoses, not necessarily mood disorders.

Serotonin Markers and Mood Disorders

Dysfunction of the serotonergic system has long been suspected in major depression and related disorders. Depression can be treated successfully with selective drugs which target serotonin receptors. The serotonin transporter may also be involved in susceptibility to mood disorders and in the response to treatment with these drugs. Allelic association has been suggested between the serotonin transporter gene (located on chromosome 17q11.1–12) and UPAD (Ogilvie *et al.*, 1996). The presence of one allele of this gene was significantly associated with a risk of UPAD. This study also included a group of BPAD patients although no associations were found with this marker in this patient group compared to normal controls. This preliminary finding may add to our understanding of the possibility of polygenic inheritance in mood disorders. These findings were replicated in two different samples, again showing an association between this marker and UPAD (major depression with melancholia) (Gutierrez *et al.*, 1998a) and no association with a group of BPAD patients (Gutierrez *et al.*, 1998b). A higher frequency of the 12-repeat allele of the variable number tandem repeat (VNTR) polymorphism has been associated with mood disorders in a number of studies (Battersby *et al.*, 1996; Collier *et al.*, 1996; Rees *et al.*, 1997), with results being the most significant for bipolar subjects. The 9 repeat allele has been shown to be significantly associated with unipolar depression, on the other hand (Battersby *et al.*, 1996; Ogilvie *et al.*, 1996).

A linkage study with the functional variant of the serotonin transporter gene in families with BPAD could not exclude linkage (Ewald *et al.*, 1998b). More interestingly, a polymorphism within the promoter region of the serotonin transporter gene has been associated with treatment response to fluvoxamine, a typical serotonin reuptake inhibitor (SSRI) in major depression with psychotic features (Smeraldi *et al.*, 1998). This promising preliminary finding requires to be confirmed. The tryptophan hydroxylase (TPH) gene, which codes for the rate-limiting enzyme of serotonin metabolism, is also an important candidate gene for mood disorders and suicidal behaviour. Bellivier *et al.*, (1998b) reported a significant association between genotypes at this marker and BPAD; no association was found with suicidal behaviour. In a previous study in depressed patients suicidal behaviour has been associated with one variant of this gene (Mann *et al.*, 1997).

Chromosome 4

Possible candidate genes on chromosome 4 include the dopamine receptor D5 (DRD5) and the alpha adrenergic 2C receptor (ADRA2C) genes. Using both linkage and sib-pair methods to evaluate six families, positive results have been found for the loci for DRD5 and ADRA2C (Byerley *et al.*, 1994), although this was not fully replicated in a later study with a larger sample size (Blackwood *et al.*, 1996). Linkage was found between a locus on chromosome 4p (D4S394, 4p16) and BPAD in the latter study, yielding a robust two-point lod score of 4.1 under a model that allowed for heterogeneity. Although the region of 4p16 contains the genes for both DRD5 and ADRA2C, specific markers for these genes were not significant for linkage in this study. Although linkage was not found in the NIMH initiative for any region on chromosome 4, modest elevation of allele frequencies was found for markers on both arms of the chromosome, D4S2397 and D4S391 on 4p and DS1647 4q (Detera-Waldeigh *et al.*, 1997).

Other possible candidate genes mapped to chromosome 4 include those coding for protein subunits of the heteromeric GABA A receptor: the alpha 2 protein subunit (GABRA2) and the beta 1 subunit (GABRB1), located at 4p13–12. This region was excluded for linkage in a large family in the Blackwood *et al.* (1996) study, but perhaps could be examined more fully.

Chromosome 21

Straub *et al.* (1994) detected linkage with the locus for liver-type phosphofructokinase enzyme (PFLK) on chromosome 21 (21q22.3) in one large, multigenerational bipolar family. Initial results from 47 families assessed in this genome survey did not support linkage with this marker, however. Follow-up linkage analysis of an extended sample of 57 families on the 373 most informative individuals, using markers less than 2 cM apart, found evidence of linkage (lod score 3.35) at marker D21S1260, which is 5 cM proximal to PFKL (Aita *et al.*, 1998). Linkage between bipolar disorder and the PFLK region has also been confirmed in other studies (Ewald *et al.*, 1996, Smyth *et al.*, 1997), although negative results have also been reported (Byerley *et al.*, 1995; Vallada *et al.*, 1996). Further confirmation of linkage to this region with a large sample size, and a later report of excess allele sharing of a cluster of markers within a 9 cM interval on 21q (D21S1254, D21S65, D21S1440 and D21S1254), were found in the NIMH Genetics Initiative bipolar pedigrees (Detera-Waldeigh *et al.*, 1996, 1997). Linkage to a larger region proximal to the gene for PFKL, 21q21–22, has also been reported by a number of groups (Kwok *et al.*, 1999, LaBuda *et al.*, 1996). Taken together, these results indicate that the region of interest on 21q remains large, although no discrete locus or obvious candidate gene has been identified as of yet.

Chromosome 12

Darier's disease (keratosis follicularis), a rare autosomal dominant skin disorder associated with increased prevalence of epilepsy and mental retardation, whose gene has been mapped to chromosome 12q23–24.1 was found to cosegregate with bipolar disorder in one pedigree (Craddock *et al.*, 1994). The Darier's disease region has been investigated in several family studies in BPAD, suggesting a possible linkage (Barden *et al.*, 1996; Craddock *et al.*, 1994; Dawson *et al.*, 1995; Ewald *et al.*, 1998a). Two of these studies have been able to report significant lod scores greater than 3 (Barden *et al.*, 1996; Ewald *et al.*, 1998). To further test the hypothesis that genes containing expanded trinucleotide repeats may contribute to the genetic aetiology of BPAD, loci within this region containing CAG/CTG repeat expansions have also been investigated, but no association was found with BPAD (Franks *et al.*, 1999).

ANTICIPATION AND EXPANDED TRINUCLEOTIDE REPEAT SEQUENCES

Anticipation implies that a disease occurs at a progressively earlier age of onset and with increased severity in successive generations.

This may explain deviations from Mendelian inheritance observed in some inherited diseases. This phenomenon has been observed in several neurological diseases, including myotonic dystrophy, fragile X syndrome, Huntington's disease and spinobulbar muscle atrophy (Paulson and Fischbeck, 1996; Trottier *et al.*, 1995). Anticipation has been found to correlate with a new class of mutations, expanded trinucleotide repeat sequences. An expanded repeat sequence is unstable and may increase in size across generations, leading to an increased disease severity of the disorder. CAG repeats are detected by the repeat expansion detection (RED) method. Such unstable mutations could also be an alternative explanation in addition to environmental factors for discordance between monozygotic twins for mood disorders, where the repeat amplification might be different during mitosis in each of the two twins. A shortcoming of anticipation studies involves ascertainment bias. Earlier age of onset and/or increased severity in successive generations may be related to increased sensitivity to diagnosis in offspring of affected parents. It may appear that parents have a later age of onset compared to children, but perhaps parents with an earlier age of onset were not able to reproduce at all. Social and environmental factors in the younger generation may favour earlier detection, and expression of the disease is not necessarily related to observed repeats. Cohort effects can be controlled for in statistical analyses, however, such as examining age at onset in an entire generation compared to the difference observed between probands and parents.

Most unstable nucleotide repeat diseases showing anticipation also demonstrate imprinting, or parent-of-origin effect. Mood disorders have not yet unequivocally shown such an effect, although this does not necessarily exclude the possibility that anticipation occurs.

Evidence for anticipation has been observed in BPAD (Grigoroiu-Serbanescu *et al.*, 1997; McInnis *et al.*, 1993; Nylander *et al.*, 1994; Ohara *et al.*, 1998) and in UPAD (Engström *et al.*, 1995). Correlation between anticipation observed at the phenotypic level with the number of dynamic mutations may be the only way to confirm the implication of this phenomenon in mood disorders. One study highlighted an association between the number of CAG trinucleotide repeats and severity of BPAD illness in Swedish and Belgian patients (Lindblad *et al.*, 1995). This study, replicated subsequently in a different sample (O'Donovan *et al.*, 1995; Oruc *et al.*, 1997), showed for the first time in a major psychiatric disorder that the length of CAG repeats were significantly higher in BPAD compared to normal controls. These molecular genetic findings may indicate a genetic basis for anticipation in BPAD. However, no correlation has been found between CAG/CTG repeats and phenotypic measures of severity in a sample of 133 unrelated BPAD patients (Craddock *et al.*, 1997) and in several independent studies (Guy *et al.*, 1999; Li *et al.*, 1998; Vincent *et al.*, 1996; Zander *et al.*, 1998). This hypothesis has recently been tested in a sample of two-generation pairs with BPAD. Globally, no significant differences were found in the mean number of CAG repeats between parent and offspring generations. A significant increase in CAG repeats between parents and offspring was observed, however, when the phenotype increased in severity, i.e. changed from major depression, single episode or unipolar recurrent depression to BPAD (Mendlewicz *et al.*, 1997). A significant increase in CAG repeat length between generations was also found in female offspring with maternal inheritance, but not in male offspring. This is the first evidence of genetic anticipation in BPAD families and should be followed by the identification of loci within the genome containing triplet repeats. CTG18.1 on chromosome 18q21.1 and ERDA1 on chromosome 17q21.3 are two repeat loci recently identified (Lindblad *et al.*, 1998) which can be investigated in such study. In this study, several hundreds of candidate loci containing repeats were screened in a set of BPAD patients but expanded alleles at ERDA1 and CTG18.1 loci were found to be associated with BPAD phenotype. The authors observed that in a Swedish sample, including both unrelated and familial cases, 89% of expanded RED products correlate with expansions at these two loci and that expansion at the CTG18.1 locus was associated to the phenotype. Using the same method in a Belgian sample, Verheyen *et al.* (1999) demonstrated that 86% of the RED expansions could be accounted for by ERDA1 and CTG18.1 repeats. Expanded alleles at ERDA1 were found to be more frequent in bipolar patients. Eight CAG/CTG triplet repeats located in the 18q21.33–q23 region, identified as a candidate region in bipolar families, have been investigated in bipolar disorder by Goossens *et al.* (2000), but no expansion has been found in the bipolar family and the case-control sample investigated.

CONCLUSIONS

The physical mapping of the human genome provides an immense factory providing thousands of genes which will accelerate the identification of genes responsible for mood disorders and will contribute to significant advances in the awareness of diagnosis (diagnostic process and early recognition), pathophysiology, epidemiology and treatment issues. During the past two decades, the search for genes for mood disorders has mainly contributed to better understand and confirm the genetic complexities inherent in these disorders. The large amount of results available and the difficulty of digesting them corroborate this observation. The major contribution of these findings should be integrated in the context of the worldwide efforts to identify the thousands of genes of the human genome. The majority of these genes will be identified within the next few years. Several consistent hypotheses are currently being tested and will, hopefully, speed up the process of narrowing the important regions when the complete genome map becomes available. The most promising chromosomal regions have been localized on chromosomes 4, 5, 11, 12, 18, 21 and X. A number of candidate genes have also been investigated, some of which are directly linked to neurobiological hypotheses of the aetiology of mood disorders. In parallel, specific hypotheses have been implicated, such as anticipation and dynamic mutations. Further research should concentrate on these hypotheses and confirm positive findings through interdisciplinary and multicentre projects.

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Neonatal Developmental Neuroplasticity: A Critical Contribution from Environment

Robert M. Post

INTRODUCTION

Hubel and Wiesel won the Nobel Prize for medicine with their elegant dissection of the impact of visual deprivation on the biochemistry, physiology and microstructure of the ocular dominance columns in the visual cortex (Hubel and Wiesel, 1979). They elucidated a gradient of progressively more profound and irreversible effects in the normal development of visual pathways and processing as a function of depriving environmental input by reducing visual input in one eye to virtually zero by suturing it shut. Not only was there dysfunction and atrophy of the ocular dominance columns related to the deprived eye, but a compensatory increase in those columns synaptically related to the eye that continued to have normal visual stimulation (Wiesel and Hubel, 1965; Hubel and Wiesel, 1979, 1998).

Similar, and perhaps even more complex, interactions appear to be occurring in diencephalic, paralimbic and cortical circuits involved in mediating the normal processes of maternal infant nurturing and bonding. The lack of normal developmental trajectories in this domain have been repeatedly linked to vulnerability to depression in adults later in life (Emde *et al.*, 1965; Powell *et al.*, 1967; Brown *et al.*, 1973a, b; Breier *et al.*, 1988). Thus, the neuropsychobiology of such early environmental insults is of particular interest to the topic of this chapter, and re-emphasizes a role for environmental contingencies as well as genetic inheritance in vulnerability to affective illness.

Depression Recurrence

The work of Kendler and associates provides a particularly cogent set of examples of both gene and environmental effects. As illustrated in Figure XVIII-11.1, a variety of early environmental losses or adversities are risk factors for subsequent depressions (Kendler *et al.*, 1993). In addition, concurrent stresses and losses interact with these earlier vulnerabilities in the precipitation of affective episodes. Moreover, minor or neurotic depressions are precursors to more major depressions, and major depressions are a key risk factor for subsequent major depressive recurrences, all within the background of genetic vulnerability to the recurrent affective disorders. Whether such dual contributions would meet the more formal criteria enunciated earlier by Boomstra and Martin for gene–environment interactions remains to be delineated, and in this chapter we will use the term interactions loosely only to imply that effects of both inherited and environmental domains can be discernible.

Suicidality in Bipolar Illness

A similar interactive schema is evident in the risk factors for suicidality in patients with bipolar disorder (Leverich *et al.*, 2002a, b). A family history of severe suicides attempted or completed, as well as a family history of substance abuse, interacts with a variety of environmental experiential variables as precursors of suicide attempts (Figure XVIII-11.2). An increased incidence of suicide attempts is affected by genetics, course of illness variables, psychiatric Axis I and Axis II comorbidities, medical comorbidities, and a history of early extreme stressors and environmental adversities (physical or sexual abuse in childhood or adolescence), as well as more proximal stressors such as loss of significant others and lack of adequate access to mental and medical health care.

Thus, paradigms in unipolar depression and in suicidality in patients with bipolar illness both indicate powerful interactions of genetic and environmental variables. On the experiential side, both paradigms suggest components of episode sensitization and stress sensitization.

Episode Sensitization

Perhaps the best clinical evidence for the occurrence of episode sensitization is the study of Kessing *et al.* (1998) of more than 20 000 patients hospitalized for depression or mania. These investigators found that for both unipolar and bipolar patients, the best predictor of incidence and time to relapse was the number of prior episodes, supporting the hypothesis that episodes beget episodes. However, one has to caution that it is possible that those patients who are most vulnerable to recurrence were preselected and one may have a poor prognosis subgroup in those who have the most prior episodes and subsequent recurrences. Kessing and associates present statistical reasons to suggest that this is not the case (Kessing, 1998; Kessing *et al.*, 1998), but such a proposition could only be resolved with a randomized prospective study of adequate versus inadequate long-term prophylaxis to see whether the intervening occurrence of episodes did change the future course; this study is not really possible given the ethical constraints against inadequate prophylaxis.

The studies of Brown and colleagues (Harris *et al.*, 1986, 1987; Bifulco *et al.*, 1987) are among the most elegant in demonstrating the interaction of proximal and distal stressors in the occurrence of depression. Brown and colleagues found that a variety of risk factors in single mothers appeared to be associated with the occurrence of depression upon the occurrence of a proximal stressor. One of these factors, again related to loss of a significant other (in this case loss of one's mother in childhood), could act as a predictor of subsequent depression. This occurred with a variety of other modulating variables including lack of a confidant, having more than four children at home to care for, and lack of employment. The study of Breier *et al.* (1988) is particularly revealing in terms of the potential lasting effects of early

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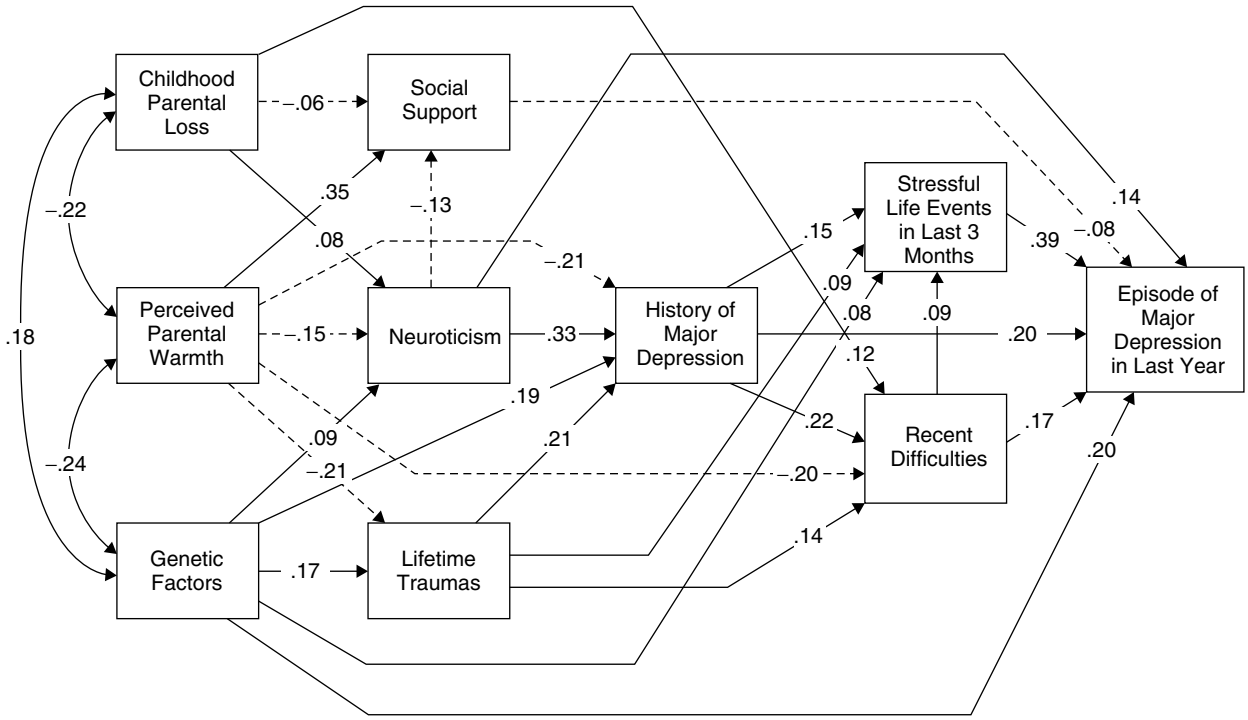

Figure XVIII-11.1 Kendler's path estimates of the best-fitting model of prediction of major depression in 1360 female twins. Early and recent life events (left, top two boxes) interacting with genetic vulnerability (left, bottom box) predispose to early mild symptoms and episodes (neuroticism box) and more major depression (middle box and last box on right). Episode occurrence further increases vulnerability to recurrence (middle box to last box on right). The values along the double-headed arrows between the independent variables (at the left side of the figure) are correlation coefficients. The values along the single-headed arrows are path or standardized regression coefficients indicating the weight of the relationships. The black single-headed arrows represent positive path coefficients. Negative path coefficients are depicted by striped paths (see Kendler *et al.*, 1993, for details). Reprinted with permission from the *American Journal of Psychiatry*, © 1993

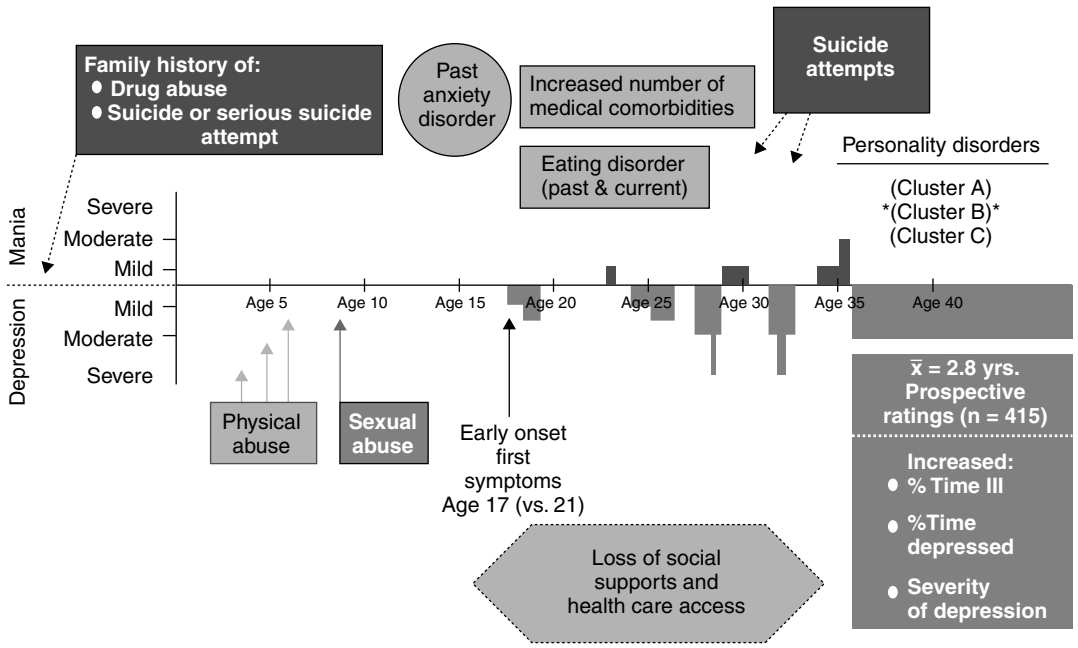

Figure XVIII-11.2 Schema of factors associated with suicide attempts in 632 patients with bipolar illness in the Stanley Foundation Bipolar Network (from Leverich *et al.*, 2002a, b)

parental loss. These investigators found that loss of a parent in childhood increased the incidence of adult depression only if the surviving parent failed to adequately substitute for the loss of the other. These data are particularly important, indicating that a variety of factors can be involved within any given risk factor as to whether or not it becomes a crucial determinant of increasing the risk of episode recurrence or of a suicidal act. The studies of Brown and colleagues in unipolar depression (Harris *et al.*, 1986, 1987; Bifulco *et al.*, 1987) and of Leverich and colleagues for suicidality in bipolar depressed patients (Leverich *et al.*, 2002a, b) re-emphasize this point, that lack of a confidant may be a particularly important mediating factor. Given a variety of stressors, the lack of availability of others to share perspectives, give their insights, and provide psychological and physical support, appears to be critically important.

Stress Sensitization

A variety of empirical investigations in affective illness suggest stress sensitization. The first (noted above) suggests that several types of early-life stressors are pertinent to later affective illness onset. In addition, numerous studies document that proximal stressors or psychosocial precipitants are more obvious in initial episodes of affective disorder than in later ones in which the episodes often occur more autonomously (reviewed in Post, 1992). Although the literature is not completely in agreement in this regard (Hammen and Gitlin, 1997), the majority of studies do suggest that early compared with later episodes are more likely to be associated with stressors. These observations thus validate Kraepelin (1921) and others' original view that:

> we must regard all alleged injuries as possibly sparks for the discharge of individual attacks, but that the real cause of the malady must be sought in *permanent internal changes*, what at least very often, perhaps always, are innate.
> in spite of the removal of the discharging cause, the attack follows its independent development. But, finally, the appearance of wholly similar attacks on wholly dissimilar occasions or quite without external occasion shows that even there where there has been external influence, it must not be regarded as a necessary presupposition for the appearance of the attack.
> Unfortunately the powerlessness of our efforts to cure must only too often convince us that the attacks of manic-depressive insanity may be to an astonishing degree *independent of external influences*. (Kraepelin, 1921, pp. 180–1)

Stress sensitization may not be unique to unipolar and bipolar disorders; evidence is emerging that it plays a role in the occurrence of the full-blown post-traumatic stress disorder (PTSD) syndrome. Several investigators have indicated that in patient populations who did develop PTSD after a given stressor compared with those who did not, there is a greater history of prior early stressors (Bremner *et al.*, 1993; Yehuda *et al.*, 1995; Breslau *et al.*, 1999). Together, these data in affective illness and in PTSD suggest that early stressors provide an area of vulnerability upon which the more proximal stressors act and interact. Should this prove to be true, the neurobiological and neuropsychological mechanisms that could underlie these types of stress sensitization phenomena deserve exploration.

EARLY MATERNAL SEPARATION

Maternal separation and parental neglect have long been recognized as potential risk factors for depression. Spitz and others studied anaclitic depression (Emde *et al.*, 1965; Harmon *et al.*, 1982), and Harlow and associates described some of the catastrophic and lasting consequences of social and environmental deprivation (Harlow *et al.*, 1965; Suomi *et al.*, 1970; Young *et al.*, 1973). Kuhn and Schanberg were among the first to demonstrate the physiological potency of even transient aberrations in the normal maternal–infant relationship (Kuhn *et al.*, 1978). They observed that the failure to show normal trajectories of growth and development in neonatal rat pups separated from their mothers could be ameliorated by the appropriate amount of touch and licking substitution (Pauk *et al.*, 1986). Separated animals did not show a failure to secrete adequate levels of growth hormone, but that growth hormone could not activate the critical enzyme for neural development, ornithine decarboxylase (Kuhn *et al.*, 1978). If neonatal rat pups were stroked lightly with a toothbrush on the ventral surface of their abdomens, they showed normal growth and normal amounts of ornithine decarboxylase activity induced by growth hormone (Pauk *et al.*, 1986). The ethological background of this interaction was determined to involve the fact that the neonate rodent has a neurogenic bladder and urinates only when the mother licks the ventral surface of the abdomen. This urine is then further licked by the mother to enhance the neonatal maternal bond at the level of smell and taste, as well as sound and touch.

Based on these preclinical observations, Kuhn and Shanberg moved to the neonatal nursery and demonstrated that properly handled premature infants grew better than those who were less handled in their incubators (Field *et al.*, 1986). At the same time, Levine and colleagues (Stanton *et al.*, 1988; Levine *et al.*, 1991) were demonstrating the consequences of single brief periods (24 hours) of maternal deprivation in neonatal rat pups, which were long-lasting alterations in behaviour and endocrinology. Some of the fundamental neurobiological mechanisms in those alterations have begun to be revealed; these animals remain hypercortisolaemic and anxious in open field tests and show a variety of changes in acute signal transduction chemicals and neurotrophic factors (Suchecki *et al.*, 1993; Rots *et al.*, 1996; Smith *et al.*, 1997; Zhang *et al.*, 1998, 2002).

For example, in rat pups separated for one day on day 11 of their life and then sacrificed on day 12, there was an increased induction of mRNA for nerve growth factor (NGF) and c-jun (Zhang *et al.*, 1998) and a decrease in hippocampal brain-derived neurotropic factor (BDNF), calcium-calmodulin kinase II (CaMKII), and nitric oxide synthase (NOS) (Zhang *et al.*, 2002; Xing *et al.*, 1998) (Figure XVIII-11.3a). In common with these changes is an approximate doubling in the rate of apoptosis observed throughout the brain, which correlates with the degree of increase in growth hormone in white matter areas of brain (Zhang *et al.*, 2002), raising the question of why increases in growth hormone might be associated with increases in cell death measured by the apoptag technique. It appears that the high-affinity receptor for NGF, the Trk-A receptor, is not adequately developed at this neonatal age and the low affinity p75 growth factor receptor predominates. When activated, the p75 receptor mediates an increase in apoptosis (Barrett, 2000). Zhang and associates (Zhang *et al.*, 2002) have observed that apoptosis occurs in both neuronal and glial cells.

Although these acute observations remain to be followed longitudinally, and the long-term consequences on brain microstructure and behaviour remain to be elucidated, they do provide another plausible mechanism (in addition to genetic deficits and other prenatal alterations) for the reported deficits in neurons or glia in selected areas of the brain in patients with schizophrenic, unipolar and bipolar affective disorders (Selemon *et al.*, 1995, 1998; Drevets *et al.*, 1998; Ongur *et al.*, 1998; Rajkowska *et al.*, 1999; Knable, 1999). In addition, they provide another plausible mechanism for the near uniform reports that patients with schizophrenia

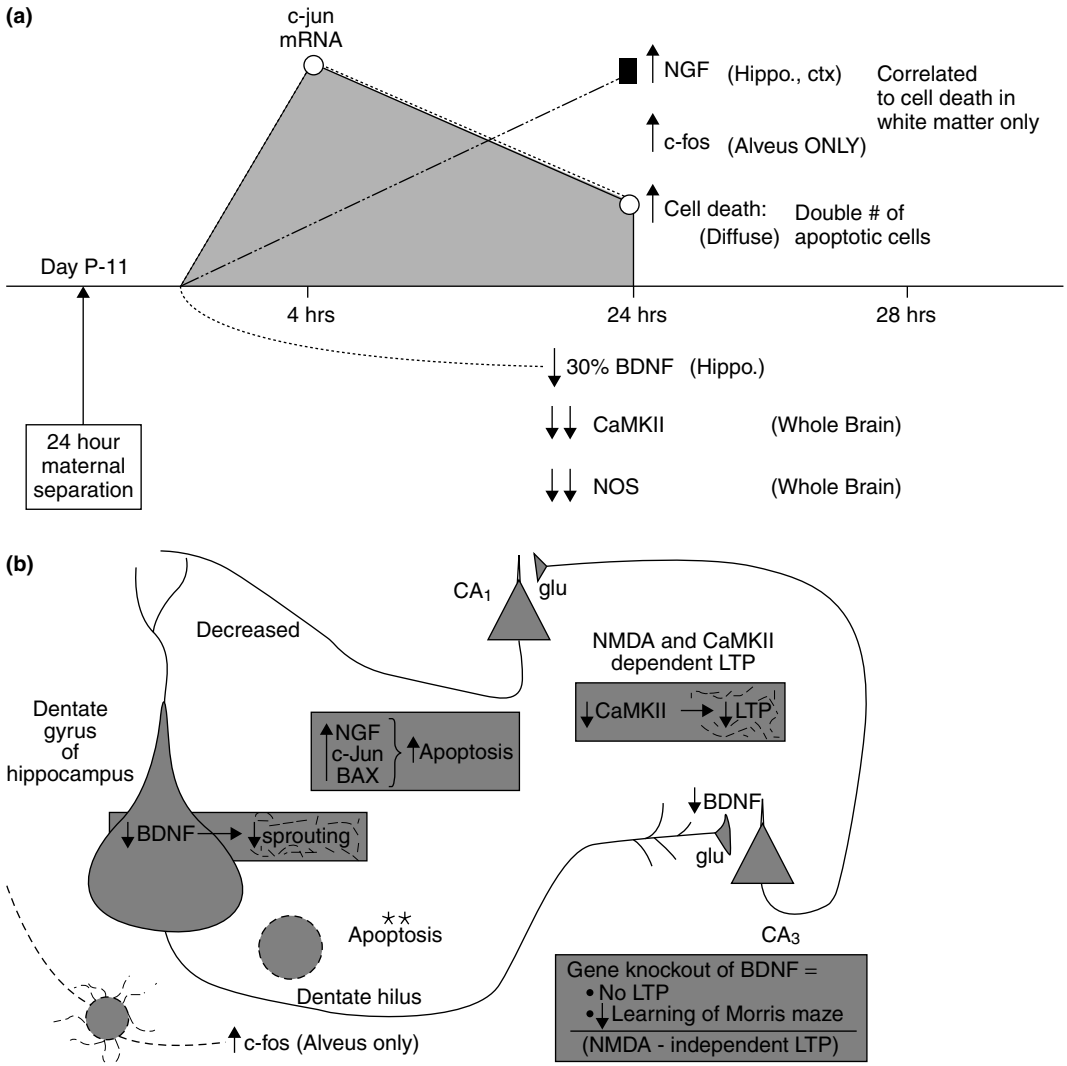

Figure XVIII-11.3 (a) Effects of maternal separation in neonatal rat pups on neurotrophins, transcription factors and gene expression (compiled using data from Zhang *et al.*, 1998, 2002). NGF, nerve growth factor; Hippo., hippocampus; ctx, cortex; BDNF, brain-derived neurotrophic factor; CaMKII, calcium-calmodulin kinase II; NOS, nitric oxide synthase. (b) Potential hippocampal effects of neonatal maternal separation. BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate; glu, glutamate; CaMKII, calcium-calmodulin kinase II; LTP, long-term potentiation

often have significantly smaller brain sizes than comparative control populations (Lawrie and Abukmeil, 1998). Although it is not clear what factors mediate such processes, the potential impact of environmental deprivation should now be added to those of genetic determinants so beautifully demonstrated in the twin studies of Weinberger and colleagues (Suddath *et al.*, 1990; Berman *et al.*, 1992; Weinberger *et al.*, 1992; Torrey *et al.*, 1995), who found that in identical twins discordant for schizophrenia, the ill twin almost always had increased ventricle size. Ventricular size was highly correlated within twin pairs, showing the effect of genetics, but additional environmental vulnerabilities appeared to be at work in increasing the risk for becoming ill in those who had another factor associated with the larger ventricular size (Suddath *et al.*, 1990).

The maternal-separation-induced decrements in hippocampal BDNF are equally interesting in relation to a number of reports of smaller hippocampal size in patients with either affective disorders (Sheline *et al.*, 1996; Bremner *et al.*, 2000) or PTSD (Bremner *et al.*, 1995, 1997; Gurvits *et al.*, 1996; Stein *et al.*, 1997), although there are notable exceptions in this regard (Hauser *et al.*, 2000; Vakili *et al.*, 2000). Could a sufficiently consistent or recurrent stress-induced decrement in BDNF early in life be associated with the relatively small hippocampal size (Figure XVIII-11.3b)? Moreover, even if the size of the hippocampus were normal, recent evidence suggests that BDNF is important to the development of normal learning and memory, as reflected in a variety of behavioural tests, and for the normal development of long-term potentiation (LTP) in the hippocampus (Korte *et al.*, 1996, 1998), the best studied model of learning and memory (Malenka and Nicoll, 1999). Similarly, animals deficient in CaMKII have impaired LTP (Silva *et al.*, 1992).

One can only wonder whether the acute decrements in these two substances (CaMKII and BDNF) after maternal separation stress could occur at a critical developmental juncture and result in sustained deficits (Figure XVIII-11.3b). Recent data of Xing and colleagues show that CaMKII amRNA is significantly decreased

in the prefrontal cortex of patients with bipolar illness compared with normal controls (Xing *et al.*, 2002). Could such a defect in this and related signal transduction systems necessary for adequate learning and memory be a factor in the many different types of evidence of frontal dysfunction in patients with bipolar illness? The fact that CaMKII is decreased in the prefrontal cortices of patients with bipolar illness, and that it can be acutely decreased in rat pups undergoing a single day of maternal deprivation stress, at least opens the possibility of both genetic and environmental influences on such a critical intracellular transduction system.

Increases and decreases in intracellular calcium are crucial to reading the input on a single neuron and translating it into synaptic and neuronal output. On the synaptic level, increases or decreases in intracellular calcium can be associated with LTP or long-term depression (LTD), respectively (Malenka *et al.*, 1989; Bolshakov and Siegelbaum, 1994), and at a cellular level can be associated with neurotrophic, apoptotic and excitotoxic effects. Thus, neural excitability as well as neural life and death are intimately related to reading intracellular calcium signals. Could a 30% deficit of CaMKII in prefrontal cortex (Xing *et al.*, 2002), one of the most critical kinases involved in calcium homeostasis based on either genetic and/or environmental determinants, account for some of the dysregulations in cognition and affect in patients with bipolar illness?

REPEATED MATERNAL DEPRIVATION: BRIEF SEPARATIONS VERSUS LONGER SEPARATIONS

The work of Plotsky and Meaney reveals a number of striking principles about the quality, quantity, timing and duration of stressors as crucial variables in determining the eventual neurobiological and behavioural outcomes. Repeated maternal deprivation stress lasting 15 minutes produces animals that are relatively hypocorticosteronaemic and are protected against age-related memory defects as adults in association with lesser degrees of hippocampal atrophy. In contrast, animals subjected to repeated maternal deprivation stress of 3 hours' duration remain anxious in the open field and are hypercorticosteronaemic throughout their lives (Plotsky and Meaney, 1993). In addition, they are more prone to alcohol- and cocaine-self-administration than their litter mate non-separated controls. Strikingly, all of these alterations are reversible with treatment with serotonin-selective reuptake inhibitors (SSRIs), although if the SSRI is discontinued, the full syndrome returns (Huot *et al.*, 2001; Meaney *et al.*, 2002).

Many of these behavioural and biochemical changes mirror those induced in the model of Barden and associates (Pepin *et al.*, 1992; Beaulieu *et al.*, 1994) in which transgenic mice were developed to have a deficient number of type II glucocorticoid receptors (Figure XVIII-11.4). These animals are hypercortisolaemic and anxious in a variety of open field tests and also normalize much of their behaviour and biochemistry with antidepressant treatment. These convergent data of a transgenic model and an environmentally-induced model emphasize not only the potential for each to affect a relatively similar behavioural syndrome, but also suggest the strong potential for genetic and environmental interactions.

Thus, one could conceptualize that an animal with a genetically low level of glucocorticoid receptors that are subthreshold for inducing hypercortisolaemia and anxious behaviours might, under the proper provocation stress, exceed that threshold and manifest new behavioural and biochemical pathologies. It is interesting that this anxious, hypercortisolaemic syndrome is driven by glucocorticoid receptor deficiencies in one instance, and by glucocorticoid excess with subsequent compensatory receptor downregulation in the other, again suggesting the possibility of multiple pathophysiological routes to similar behavioural and biochemical pathologies. An example of such common phenotypic manifestations arising from very different transmitter and receptor alterations is clearly evident in the case of diabetes mellitus, wherein one can develop the illness because of either a deficiency in the hormone insulin, or because of a variety of defects in the insulin receptor activation

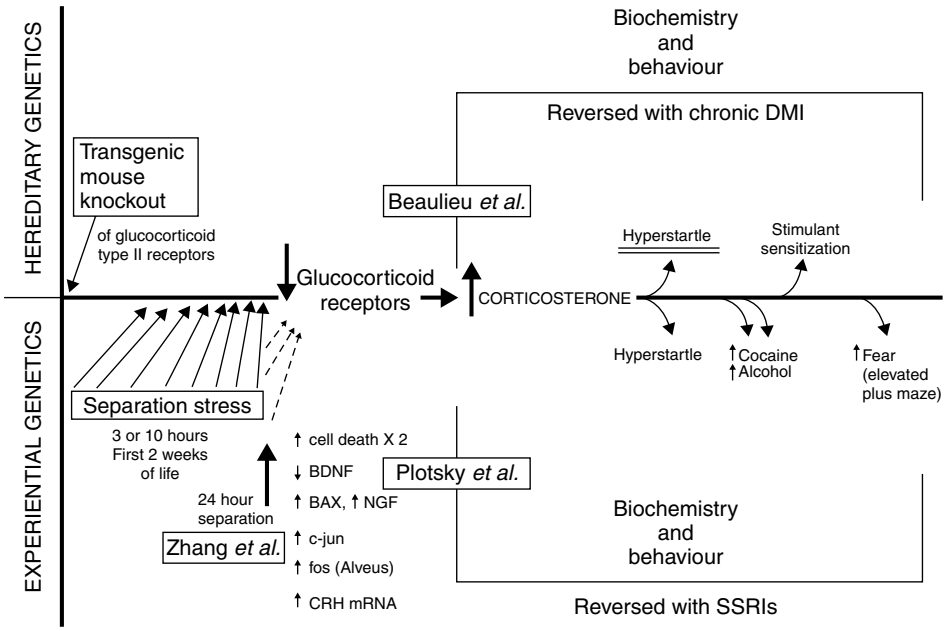

Figure XVIII-11.4 Convergent genetic and environmental models of depression. Either heredity or experiential genetics may lead to compounding behavioural and biochemical end-points similar to those seen in depression and reversible by antidepressants. DMI, desipramine; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; CRH, corticotropin-releasing hormone; SSRIs, serotonin-selective reuptake inhibitors

and its associated downstream effects (insulin resistance) despite normal to high levels of circulating insulin.

In addition to this type of mechanistic heterogeneity, the work of Plotsky and Meaney has further elucidated mechanisms of individual differences in response to maternal deprivation stress. They noted that the potential mechanism of protection against age-related memory decline in the 15-minute separated animals was that their mothers engaged in increased licking behaviour upon return of the rat pup to the litter (Liu *et al.*, 1997). Conversely, in the 3-hour deprivation experience, the mother apparently fails to recognize the previously separated neonate as her own and continues to run about the cage in a somewhat frantic and agitated manner, sometimes virtually trampling her offspring. If the mother is given a substitute litter during the time that the 3-hour deprived rat pup is removed, her behaviour remains normal upon its return, and the 3-hour separated animal does not show the typical neurobehavioural effects of repeated 3-hour separations (Anisman *et al.*, 1998). These data, again, point to crucial interactions of parent and child in the evolution of pathological behavioural and biochemical syndromes. If the brief separation is accompanied by increased licking behaviour, animals show long-term benefit. Furthermore, if the 3-hour separated animal is spared some of the traumatic effects that occur as a sequel, it is spared the long-term adverse consequences. These effects are also dependent on the time-course of neural development because animals that are separated as adolescents instead of neonates tend to show the opposite long-term behavioural and biochemical outcomes.

Given the potential potent and long-lasting effects of degree of maternal licking behaviour upon reunion, Meaney and colleagues sought to ascertain whether normal variations in maternal licking behaviour were associated with individual variability in corticosterone levels and anxiety behaviours. Indeed, they found that low-licking mothers tended to have highly anxious, highly corticosteronaemic offspring. Conversely, mothers with naturally high levels of the licking behaviour tended to have low anxious, low corticosteronaemic offspring (Liu *et al.*, 1997).

These familial traits could thus have a hereditary genetic or experientially mediated impact on gene expression. In a series of studies, these investigators elegantly demonstrated that the latter was the case. In cross-fostering studies, offspring of low-licking mothers were reared by high-licking mothers and, in fact, manifested the biobehavioural signature typical of high-licking offspring, i.e. low levels of corticosterone and low levels of anxiety. Conversely, when offspring from a naturally high-licking mother were cross-fostered by a low-licking one, high levels of corticosterone and high levels of anxiety behaviour were evident (Francis *et al.*, 1999)

Most strikingly, the signature of the second generation followed not the familial pattern, but that induced by the cross-fostering. That is, animals destined to be hypercorticosteronaemic and anxious who were cross-fostered by a high-licking mother had the opposite behavioural signature themselves as adults, and when they later had their offspring, they continued to manifest low corticosterone and anxiety. Thus, a multi-generational change in the biobehavioural signature was induced, based only on a single change in maternal rearing behaviour in one generation (Francis *et al.*, 1999). These data must give one pause about the interpretation of hypothetically hereditary genetic traits which, without the appropriate controls, may on closer examination (such as this one) turn out to depend on familial- and experience-based alterations in gene expression.

Biochemistry of Longer Separations

The neurobiological mechanisms mediating high corticosterone and high anxiety behaviours in the 3-hour separated animals are beginning to be revealed, including distinct changes in the expression of corticotropin-releasing hormone (CRH) in the amygdala and hypothalamus, and a variety of other changes in neuroendocrine and peptide set-points that are permanently altered by repeated maternal deprivation (Plotsky and Meaney, 1993). Other neurobiological changes include a decrease in tone of the inhibitory GABA/benzodiazepine system and increased corticotropin-releasing factor (CRF) and noradrenaline (NA) (Ladd *et al.*, 2000). What is clear from these studies is that some of these stress-related changes in neurobiology can be manifest over the entire life span of the organism. This appears to result from altered levels of gene transcription in critical developmental pathways which, with the proper timing, duration, quality and severity, may change neurobiological set-points in responsivity in an enduring fashion.

Thus, these data provide a plausible set of mechanisms for explaining how early environmental adversity could impart long-lasting effects on biochemistry as well as neuropsychological approaches to self, others, and the environment in parentally neglected and/or traumatized individuals. They also provide a multitude of potential compensatory mechanisms that could alter or even completely reverse this neurobehavioural signature. In the case of the briefly separated rat pup, increased evoked maternal behaviour results in long-lasting stress immunization and less decline in hippocampal function in old age (Plotsky and Meaney, 1993). It is also possible that individual differences in endogenous compensatory mechanisms could similarly play a role as to whether or not a stressor was sufficient to induce the neurochemical change.

Pathological versus Adaptive Changes in Gene Expression

The kindling model clearly elucidates the induction of endogenous compensatory mechanisms at the level of gene expression (Figure XVIII-11.5). Amygdala-kindled seizures evoke a series of alterations that appear related to the primary pathological process of kindling progression and maintenance of the pathological kindled 'memory trace'. At the same time, each seizure evokes a transient increase in a variety of endogenous anticonvulsant factors (Post and Weiss, 1992), including an increase in benzodiazepine and $GABA_A$ receptors as well as increases in neuropeptides that are potentially anticonvulsant by themselves such as thyrotropin-releasing hormone (TRH), cholecystokinin (CCK), neuropeptide Y (NPY) as well as the proconvulsant CRF.

The importance of these adaptations is indicated by the 'time-off from last seizure' effect (Weiss *et al.*, 1995; Post and Weiss, 1996). If an anticonvulsant (such as carbamazepine or diazepam) is given the day after an amygdala-kindled seizure, the drugs are highly effective in their acute anticonvulsant properties. However, if sufficient time from the last seizure is allowed to elapse (without intervening seizures being given) then the same doses of drug are no longer effective, apparently because the seizure-induced endogenous anticonvulsant mechanisms have dissipated over this period. An interval of four days or greater is necessary for carbamazepine to lose its effectiveness, and ten days for diazepam. Levels of TRH and TRH mRNA increase in the hippocampus for approximately four days after amygdala-kindled or electroconvulsive seizures, and benzodiazepine and $GABA_A$ receptor increases slightly longer.

That these data could reflect physiologically relevant endogenous anticonvulsant mechanisms is suggested by two sets of observations (in addition to the increases lasting about the appropriate length of time necessary to observe the 'time-off' effect). Wan *et al.* (1998) found that intra-hippocampal injections of TRH are indeed anticonvulsant against amygdala-kindled seizures. Moreover, in animals that have become tolerant to the anticonvulsant effects of carbamazepine or diazepam, even a full-blown seizure is insufficient to induce the increases in TRH mRNA, suggesting that loss of this and other related compensatory anticonvulsant adaptations could be

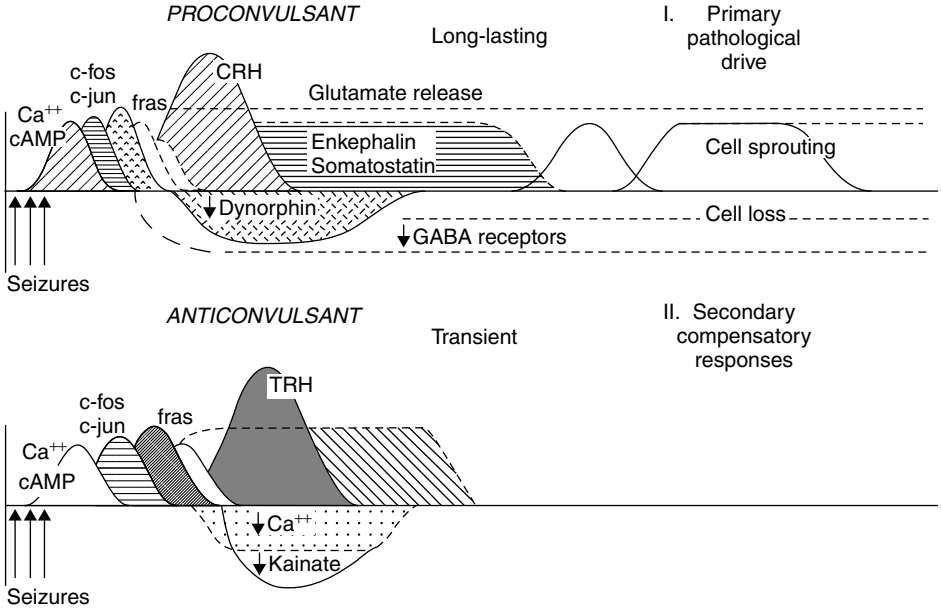

Figure XVIII-11.5 Schematic illustration of potential genomic, neurotransmitter and peptidergic alterations that follow repeated kindled seizures. Putative mechanisms related to the primary pathological drive (i.e. kindled seizure evolution) are illustrated on top and those thought to be related to the secondary compensatory responses (i.e. anticonvulsant effects) are shown on the bottom. The horizontal line represents time. Sequential transient increases in second messengers and immediate early genes (IEGs) are followed by longer lasting alterations in peptides, neurotransmitters and receptors or their mRNAs, as illustrated above the line, whereas decreases are shown below the line. Given the potential unfolding of these competing mechanisms in the evolution of seizure disorders, the question arises regarding whether parallel opposing processes also occur in the course of affective illness or other psychiatric disorders. Endogenous adaptive changes (bottom) may be exploited in the design of the new treatment strategies

linked to a loss of efficacy in instances of tolerance development as well (Figure XVIII-11.6) (Weiss *et al.*, 1995).

The idea that TRH represents an entire class of endogenous compensatory anticonvulsant mechanisms led us to follow up on the earlier observations of Prange and associates (1972) of the acute antidepressant effects of parenteral TRH. Given the evidence for TRH hypersecretion in some depressed patients based on increased levels in the spinal fluid or a blunting of the TSH response to TRH (Loosen and Prange, 1982; Banki *et al.*, 1988), we wondered whether this heightened TRH activity in depression could also represent a depression-induced endogenous antidepressant adaptation.

We thus administered 500 μg of TRH intrathecally in the context of a routine lumbar puncture (LP) in patients with refractory affective disorders and saw substantial antidepressant and antianxiety effects compared with a sham control injection (Marangell *et al.*, 1997). These data are consistent with the notion that increases in TRH observed in some depressed patients are endogenous antidepressant adaptations that, if sufficient, might normally lead to the spontaneous termination of a depressive episode. Although this proposition remains to be definitively tested and demonstrated, the data are not inconsistent with such a viewpoint.

The suggestion that changes in stress or depressive-episode-induced alterations in gene expression may either be part of a primary pathological process or a compensatory adaptation could help account for the inherent intermittency or cyclicity of the recurrent affective disorders (Figure XVIII-11.7) (Post and Weiss, 1996). One could postulate that during periods of relative wellness, the compensatory adaptions (as augmented [or not] by exogenous medications) would be sufficient to counter the underlying pathological processes.

Pathological alteration in gene expression might be driven by either those mediated by the hereditary genetic background or by experientially induced alterations. Similarly, the degree or set-point of the endogenous antidepressant mechanisms could also be determined by either genetic or environmental processes or their interaction (Figure XVIII-11.8). Thus, a predominance of depressive biological processes rather than those pushing to euthymia could be based on relatively low levels of a given compensatory neurochemical element, predisposing to, but not sufficient to result in, depressions. These could be further lowered and eroded by single or repeated episodes of extreme environmental adversity, such that they fail to exceed the threshold for adequate endogenous mechanisms maintaining euthymia.

Such a balance of pathological versus adaptive factors could also take place at the level of reaction to more proximal stressors that could influence the onset of a given affective episode. If appropriate medical and social supports are available, a stressor may not have the same pathological potency. This is most readily elucidated in the learned helplessness model, wherein the animal that receives the stressful tail shock and is able to terminate it by pressing a lever does not show helpless behaviour or the associated biochemical alterations. In contrast, the yoked control animal that receives exactly the same timing, magnitude and duration of the tail shock stress, but does not have the mechanism available for turning it off (ability to act/cope), develops the helpless neurobehavioural syndrome (Seligman and Maier, 1967; Seligman *et al.*, 1980). Thus, resistance to stress-induced behavioural changes could be built in at the level of either decreased pathological neurochemical response or increased compensatory adaptations, each mediated on either a genetic or environmental basis (or their interaction) (Figures XVIII-11.4 and XVIII-11.8). Both of these pathological or adaptive mechanisms could be enhanced or minimized with the appropriate targetted medication, or other somatic or psychotherapeutic treatment interventions.

Such a 'push–pull' model is reminiscent of the emerging perspective on the development of a variety of malignancies. In

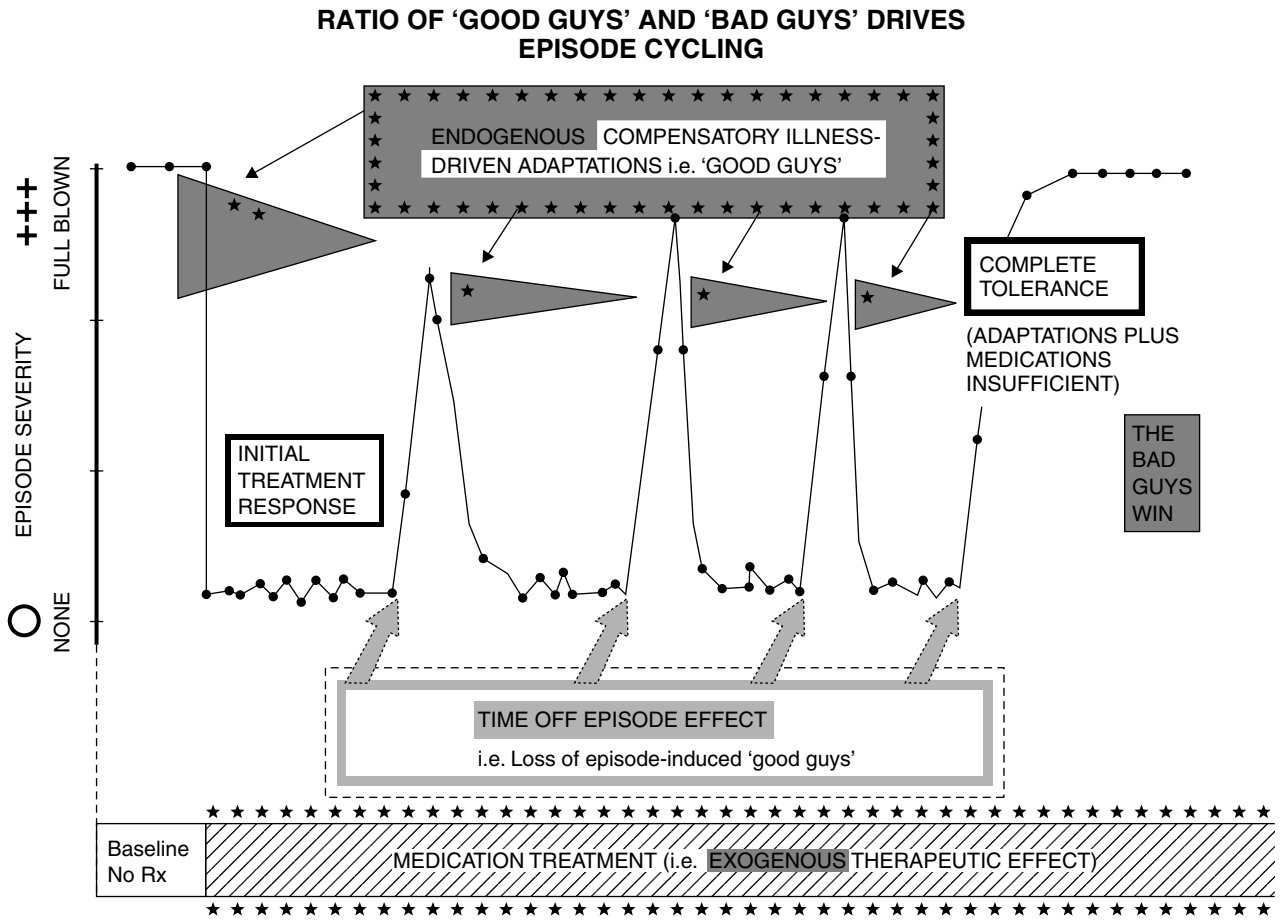

Figure XVIII-11.6 Hypothetical schema of the role of endogenous regulatory factors in the generation and progression of illness cyclicity. After an illness episode, adaptive compensatory mechanisms are induced (i.e. 'good guys'; shaded triangle with two stars), which together with drug treatment suppress the illness (initial treatment response; box). The 'good guys' dissipate with time (i.e. the 'time-off seizure' effect), and episodes of illness re-emerge. Although this re-elicits illness-related compensatory mechanisms, the concurrent drug treatment prevents some of the illness-induced adaptive responses from occurring (smaller triangles with one star). As tolerance proceeds (associated with the loss of adaptive mechanisms) faster illness re-emergence occurs. Thus, the drug is becoming less effective in the face of less robust compensatory mechanisms. The primary pathology is progressively re-emerging, driven by both additional stimulations and episodes (i.e. the kindled memory trace, or the 'bad guys') along with a loss of illness-induced adaptations. Because this cyclic process is presumably driven by the ratio of the 'bad versus good guys' at the level of changes in gene expression, we postulate that such fluctuations in the 'battle of the oncogenes' arising out of illness and treatment-related variables could account for individual patterns in illness cyclicity

the case of colon cancer, a sequence of five to more than a dozen changes in gene expression are required for the full-blown metastatic lesion (Gryfe *et al.*, 1997). These involve both increases in cellular activating, replicating and survival factors, as well as losses of neuroprotective and apoptotic (cell death) factors on the basis of multiple somatic mutations (Figure XVIII-11.9). With enough of this combination of enhanced mechanisms for cell replication and loss of compensatory tumour suppressor and cell death factors, the tumour can progress from a benign hyperplastic lesion to that of an adenoma, full-blown carcinoma, and finally, one with full metastatic malignancy. In the case of a malignancy, these positive and negative effects on gene expression enhancing cell replication and survival occur via the mechanism of somatic mutations, either genetically predisposed from the onset or arising spontaneously.

In a parallel fashion, at the level of experientially induced changes in gene expression interacting with hereditary genetic vulnerabilities, we could envision a similar cumulative progression to full-blown affective illness associated with increased likelihood of suicide attempts, treatment resistance and, ultimately, treatment refractoriness. As with the accumulating somatic mutations in the evolution of a cancer, in affective illness these might involve both increases in virulence of pathological mechanisms and, at the same time, a sequence of losing adaptive and protective mechanisms. This loss of protective factors could arise at the level of endogenous compensatory mechanisms (such as those represented by TRH), or those exogenously provided (such as those represented by some types of medications as well as social and psychotherapeutic support). Just as the animal that learns it is able to terminate the tail shock by pressing a lever does not develop the learned helplessness syndrome, if one approaches severe stressors and losses as adversities to which one can adapt (by virtue of positive distal factors derived from an optimal upbringing or by virtue of proximal factors such as new therapeutic insights), significant degrees of affective dysregulation may not occur (Figure XVIII-11.2 and Figure XVIII-11.8).

Such modulatory influences could, hypothetically, occur with appropriate psychotherapeutic intervention in a dynamic and supportive fashion, or with specific cognitive-behavioural interventions tuned to just these sets of issues and proactive problem solving toward them. However, if one has lost a parent early in life, one

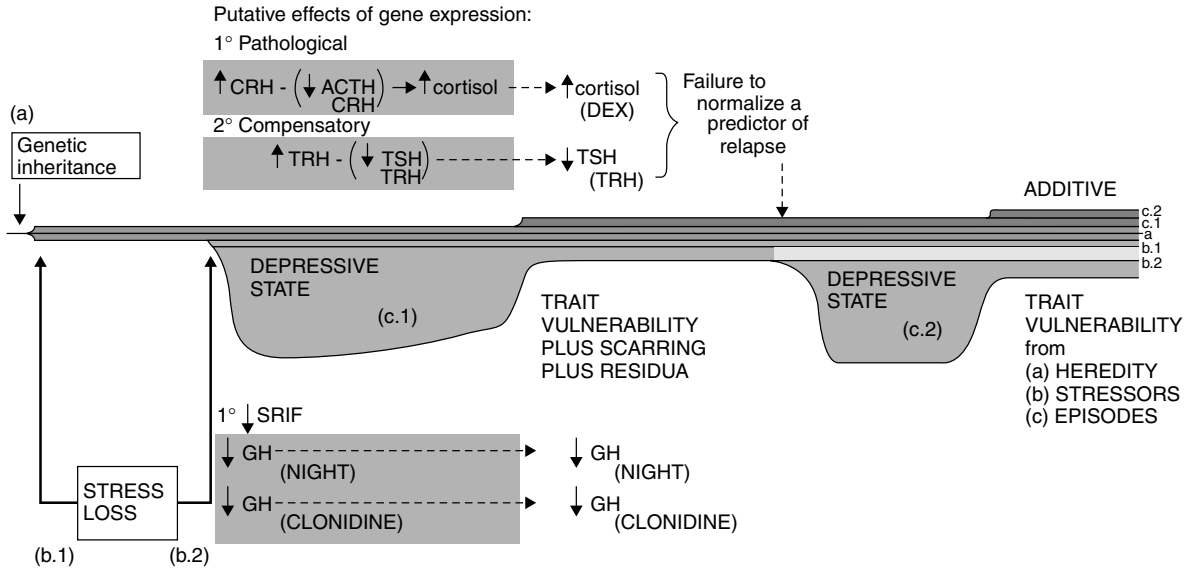

Figure XVIII-11.7 Accumulating experiential genetic vulnerability in recurrent affective illness. Schematic of how initial stressors may leave behind trait vulnerabilities (at the level of alterations in gene expression). With appropriate reactivation by stress of relevant neurobiological systems, the threshold for neuropeptide and hormonal changes associated with a depressive episode may be exceeded. These episode-related alterations may be normalized with the termination of episode but in some instances may persist and add further trait vulnerabilities toward recurrence (c) in addition to the genetic (a) and stressor (b) changes. CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropin-releasing hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; DEX, dexamethasone; GH, growth hormone; SRIF, somatostatin

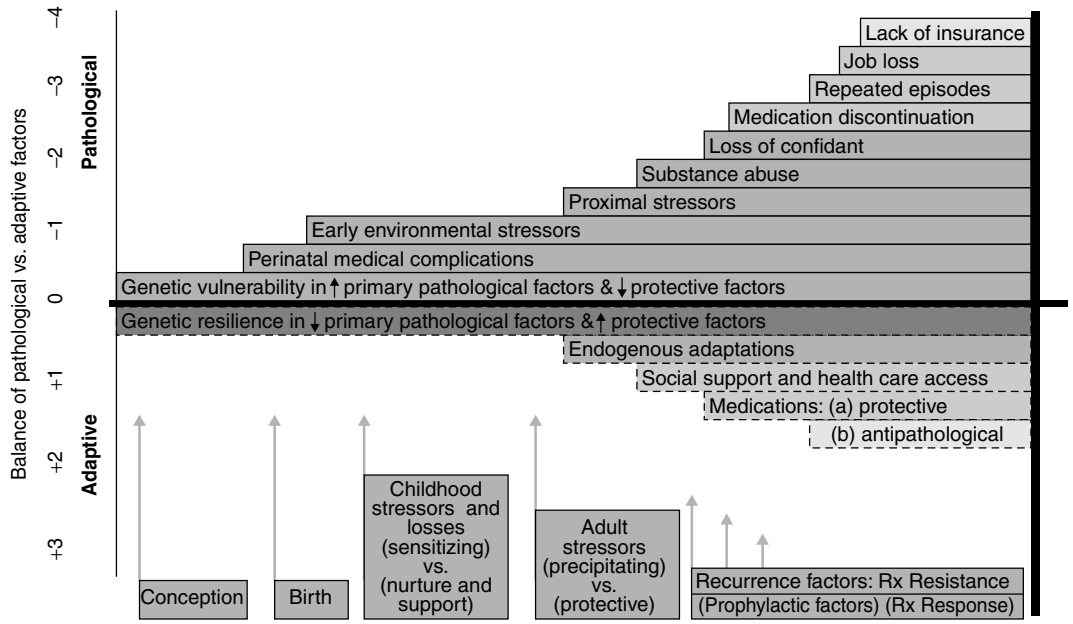

Figure XVIII-11.8 Ratio of pathological versus adaptive factors in determining illness episodes and well intervals

might have the dual liability of the loss itself and the associated failure to have the adaptive developmental benefits of that positive parental experience (in the absence of another stepping in to fill the role). If, in addition, one also loses more proximal social supports through lack of a confidant or inadequate access to health care resources, even malleable problems may appear unsolvable, particularly in the context of an inherent or experientially based set of depressive cognitions.

BRAIN MICROSTRUCTURE CHANGES: ENVIRONMENTAL AND PHARMACOLOGICAL INFLUENCES

A variety of micro- and macrostructural alterations have been described in unipolar and bipolar disorder (Figure XVIII-11.10), including altered cellular arrangements in frontal cortex and decreased glia in the subgenual part of the anterior cingulate gyrus

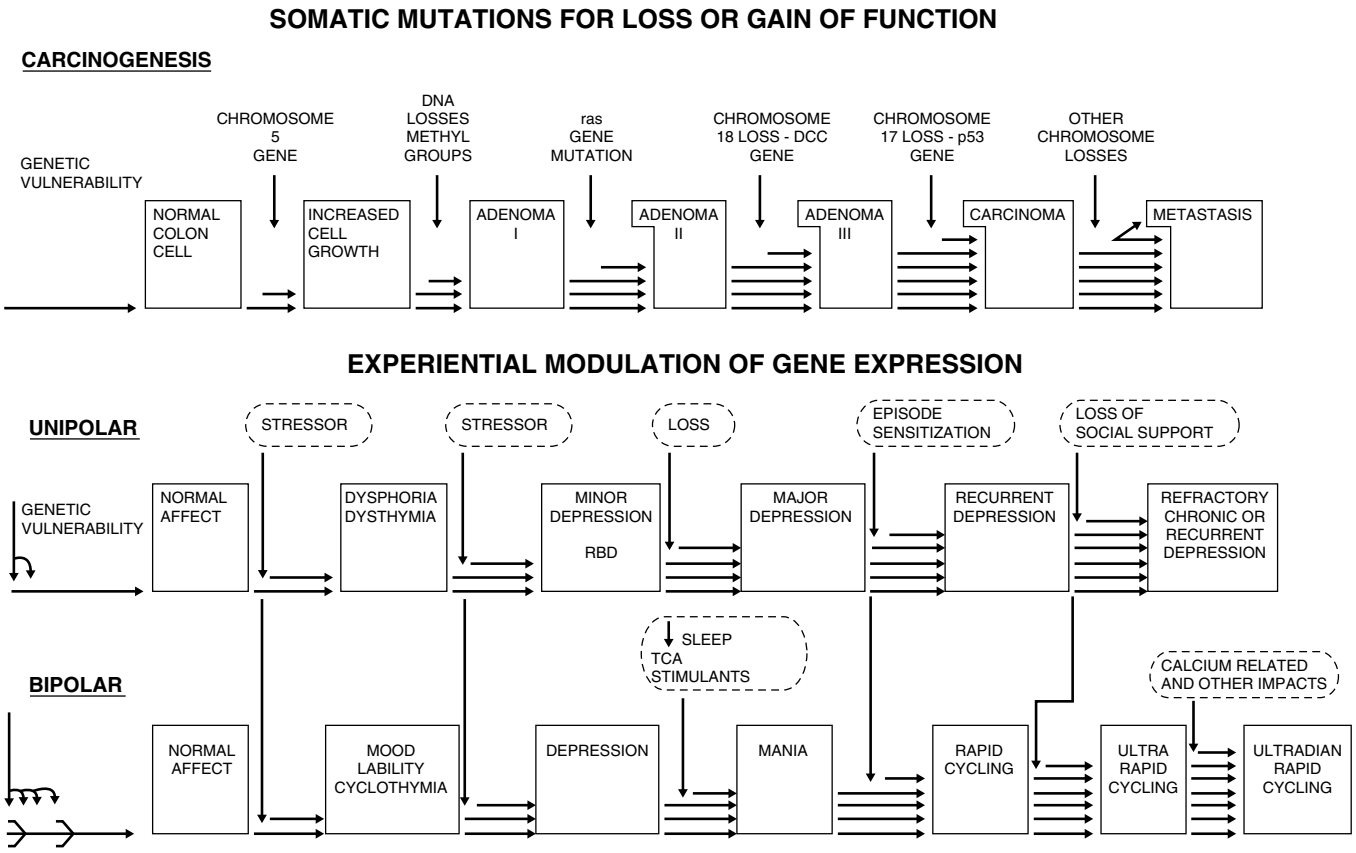

Figure XVIII-11.9 Similarities between somatic mutations in gene expression underlying carcinogenesis and experiential modulation of gene expression observed in unipolar or bipolar affective disorder. RBD, recurrent brief depression; TCA, tricyclic antidepressant

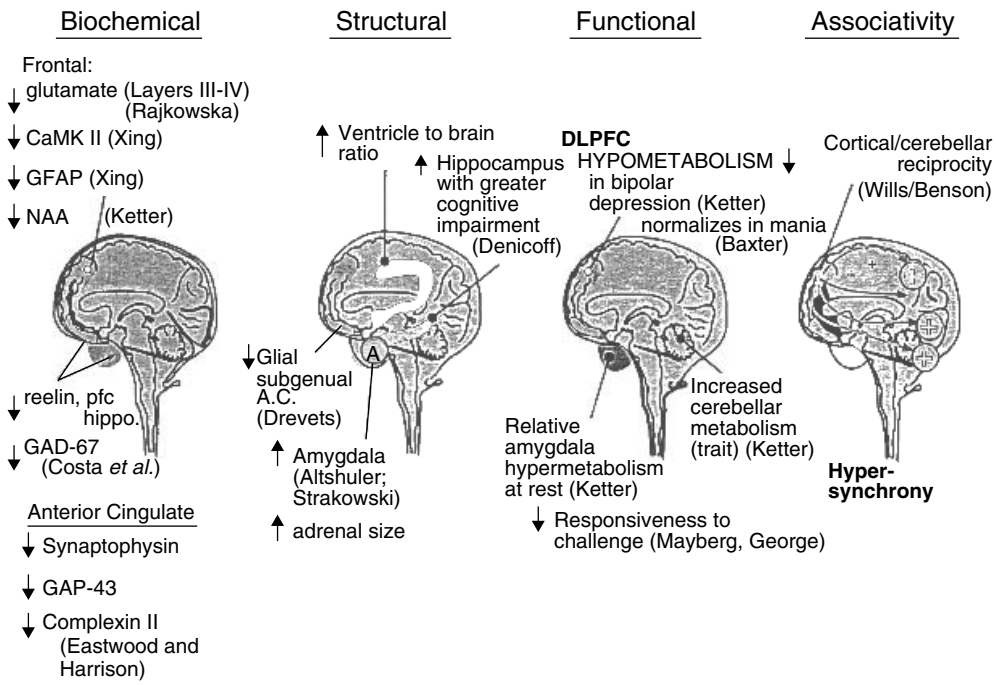

Figure XVIII-11.10 Biochemical, structural, and functional abnormalities in bipolar illness. CaMKII, calcium-calmodulin kinase II; GFAP, glial fibrillary acidic protein; NAA, *N*-acetylaspartate; pfc, prefrontal cortex; hippo., hippocampus; GAD-67, glutamic acid decarboxylase-67; GAP-43, growth associated protein-43; A.C., anterior cortex

(Drevets *et al.*, 1998; Rajkowska, 2000). Alterations in hippocampal size have been reported, as have increases in the size of the amygdala in two studies (Altshuler *et al.*, 1998; Strakowski *et al.*, 1999), but not in all cases (Pearlson *et al.*, 1997). We allude to these structural changes not because they have been definitively demonstrated, but only because they are likely to be contributing factors to the cascade of psychological and neurobiological processes that could be part of the mechanisms involved in illness vulnerability and progression. They may also be markers of high genetic vulnerability (the findings of Drevets *et al.* (1998) were limited to those patients with positive familial histories for affective disorder) or environmental impacts (not unlike the observations of Hubel and Weisel noted previously).

It may be particularly important to conceptualize even these structural alterations as potentially plastic and remediable rather than permanent and immutable. We know that environmental contingencies can be arranged to enhance cell survival, neurogenesis and the increased ratio of progenitor cells converting to neurons as opposed to glia. The latter finding is demonstrated in the studies of Kempermann *et al.* (1998) in which rearing in an enriched environment led to the increase of neural to glial ratio. Similarly, environmental adversity has been shown to decrease the rate of neurogenesis (Gould and Tanapat, 1999), which is estimated to occur in the adult rodent hippocampus at the rate of some 9000 cells per day (Cameron and McKay, 2001). Just as this neurogenesis appears bidirectionally malleable by environmental experiential mechanisms, it also appears to be alterable by pharmacology.

It is perhaps not a coincidence that the antidepressants as a class appear to induce stress counter-regulatory mechanisms at the level of gene expression for neurotrophic factors such as BDNF and neurotrophin-3 (NT3). The studies of Smith and associates at the NIMH (Smith *et al.*, 1995a, b) and those of Duman and colleagues (Nibuya *et al.*, 1995; Duman *et al.*, 1997; Duman, 1998) have revealed that antidepressants exert opposite effects to those of stress on these neurotrophic factors. Moreover, if an animal is stressed while on chronic antidepressant treatment, some of the stress-induced alterations are partially ameliorated or blocked altogether. There appears to be substantial blockade of stress-induced decreases in hippocampal BDNF when animals are co-treated with antidepressants and, concomitantly, a less robust increase in NT3 in the locus coeruleus occurs during antidepressant co-treatment compared with that during the stressor alone.

These data raise a variety of possibilities about the long-term effects of antidepressants in pharmacoprophylaxis of recurrent unipolar depression. Long-term effective prophylaxis could have multiple benefits for the individual with recurrent depression. The relative decrease in depressive episodes that occurs with a phenomenal level of statistical significance ($p < 10^{-34}$; Davis *et al.*, 1993) could spare the patient from considerable morbidity, and potential mortality from the illness by suicide. In addition, if the episode sensitization model proves valid, such pharmacoprophylaxis could alter the long-term course of the illness in a more favourable direction. To the extent that these agents exert some clinically relevant anti-stress effects at the level of gene expression on neurotrophic factors such as BDNF, antidepressants could help to lessen the impact of stressors, either as precipitating events for a depressive episode or on the microstructure of the brain as affected by the ratio of apoptotic to neurotrophic factors.

POTENTIAL NEUROTROPHIC AND NEUROPROTECTIVE EFFECTS OF LITHIUM CARBONATE

The mechanisms of action of lithium carbonate in recurrent affective disorders remain uncertain, although a host of candidate mechanisms have been postulated, as reviewed elsewhere (Post *et al.*, 2000). The latest findings on the mechanisms of lithium are of particular interest in relation to this chapter (Table XVIII-11.1). Lithium appears to exert neuroprotective effects in both a variety of *in vitro* cell culture model systems and *in vivo* in several models. Chuang and colleagues have shown that treatment with lithium reduces both the degree of neurological defect and the size of a brain infarct by approximately 50% in an animal receiving a middle cerebral artery ligation (Nonaka and Chuang, 1998). Similarly, they found that lithium reduced pathological effects of quinolinic acid injection into the striatum, a useful model for the striatal losses of Huntington's disease (Chuang *et al.*, 1999).

The groups headed by Manji and Chuang have demonstrated that lithium treatment is associated with the induction of neuroprotective factors such as Bcl2 and BDNF and decrements in cell death factors such as Bax and p53 (Chen and Chuang, 1999; Chen *et al.*, 1999; Manji *et al.*, 2000). That these alterations could be clinically relevant is revealed by several lines of evidence in addition to the *in vivo* models noted above. The changes in cell culture and in animals occur at clinically relevant concentrations of lithium, and preliminary evidence suggests that three weeks of treatment with lithium is able to increase *N*-acetylaspartate (NAA) in human brain as measured by magnetic resonance spectroscopy (Moore *et al.*, 2000a). Moreover, Moore *et al.* (2000b) demonstrated an increase in grey matter volume (using magnetic resonance imaging) in those exposed to lithium treatment compared with baseline. Thus, it remains possible that even on the level of synaptic and cellular structure of the brain, *endogenous* adaptations and *exogenous*

Table XVIII-11.1 Lithium: anti-apoptotic effects; neuroprotection; antisuicide effects (summary of data from Chuang and colleagues and Manji and colleagues)

In vitro	*In vitro* (rodent)	*In vitro* (Human)
Prevention of apoptosis in cultured neurons: Cerebellar granule cells Hippocampal (DG) cells Cortical cells	↑ Neurogenesis ↓ Size and neurological dysfunction of stroke (ligation MCA)	↓ Clinical suicide (UP & BP) ↓ Excess medical mortality (UP & BP)
Induction of cell survival factors: ↑ BCI-2; ↑ BDNF	↓ Lesion size model Huntington's chorea (Quinolinic acid in striatum)	↑ Neural integrity NAA (MRS) with 3 weeks of lithium
Inhibition of cell death factors: ↓ BAX; ↓ P53	↓ AIDS model apoptosis	↑ Neuronal volume Grey matter (MRI)

neurochemical alterations may be significant factors of short-term, if not long-term, therapeutic value.

Among the psychotropic agents used in the treatment of the recurrent affective disorders, the evidence is best for the antisuicide effect of lithium in those remaining on the drug long term (Muller-Oerlinghausen *et al.*, 1992a, b). Moreover, such long-term lithium treatment also appears to normalize the increased medical mortality that accompanies the recurrent affective disorders (Ahrens *et al.*, 1995). The data on the stroke and Huntington's disease models noted previously raise the question of whether some of lithium's effects on longevity in patients with affective illness could be mediated through cardiovascular and neuroprotective mechanisms in man similar to those demonstrated preclinically.

CONCLUSIONS

Given the multiplicity of postulated genetic and experientially driven changes in gene expression to which we have briefly alluded, one can envision the eventual use of gene therapy in reversing or ameliorating some of the key neurobiological alterations underlying vulnerability to recurrent affective disorders. However, already, currently available approaches include inhibiting or ameliorating adverse environmental experiential effects on gene expression through preventive measures and psychotherapeutic treatment, and enhancing compensatory mechanisms and minimizing primary pathological mechanisms through appropriate pharmacotherapy.

Thus, the conceptual overview espoused here is ripe for both further preclinical experimental testing and exploratory clinical therapeutic interventions. Given the virtually unlimited potential of the human brain for neuroplasticity, we look forward to increasingly effective therapeutics targetted to both pathological and adaptive processes, and ultimately, to the elucidation of the most opportune targets for gene therapy. Even when the era of gene therapy for psychiatric illness arrives, however, a role for interpersonal support and specific psychotherapeutic interventions attuned to the unique needs of patients with recurrent affective disorders will still be crucial to the successful acute treatment and long-term prevention of these otherwise recurrent disorders.

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XVIII-12

Female-Specific Mood Disorders

Meir Steiner, Edward Dunn, Leslie Born

The lifetime prevalence of mood disorders in women is approximately twice that of men. This higher incidence of depression in women is primarily seen from puberty on and is less marked in the years after menopause (Weissman and Olfson, 1995), with the exception of an additional perimenopausal blip (Kessler *et al.*, 1993). The underlying causality of this gender difference in mood-related disorders is not clear at this time. Since mood disorders occur in both men and women it is assumed that a unified basis for the development of these diseases exists. The principal constituent of this unified theory is believed to be related to genetic predisposition. Multiple environmental stressful events cause biochemical changes in a host of neuroendocrine systems and neuroanatomical areas. The genetic predisposition, which is multi-factorial, determines how stressful life events are interpreted and predicts the response, which can lead to the development of mood disorders (Heim and Nemeroff, 2001).

Notwithstanding, marked variations in the presentation of depression, comorbidity and treatment point to meaningful underlying sex differences. Women are about twice as likely as men to suffer from major depression or dysthymia (Kessler *et al.*, 1994; Weissman *et al.*, 1991). Women are prone to depressive episodes triggered by hormonal fluctuations related to reproductive events, such as during the premenstrual period, during pregnancy or the post-partum period, and around the menopause. Clinically, women present with a notably different depression symptom profile and more often develop a seasonal pattern to their depression (Ernst and Angst, 1992; Frank *et al.*, 1988; Leibenluft *et al.*, 1995; Moldin *et al.*, 1993; Whybrow, 1995). The burden of illness in women with chronic depression is profound (Kornstein *et al.*, 2000), while men may be more likely to 'forget' depressive episodes over time, a phenomenon which in turn may serve to protect them against recurrence (Ernst and Angst, 1992; Nolen-Hoeksema, 1987). Sex differences in the efficacy and tolerability of antidepressant medications is suggested by placebo-controlled and comparative studies (Kornstein, 1997, 2001; Kornstein and Wojcik, 2000). Moreover, there are marked differences between men and women in the pharmacokinetic and pharmacodynamic parameters of a number of psychotropic agents, including antidepressants (Kornstein and Wojcik, 2000; Yonkers *et al.*, 1992).

Collectively, the literature points to a higher prevalence of mood disorders in women related to an increased genetic predisposition, an increased vulnerability/exposure to stressful life events, modulation of the neuroendocrine system by fluctuating gonadal hormones, or a combination of any or all of these factors.

A biological susceptibility hypothesis has been previously proposed, to account for gender differences in the prevalence of mood disorders based on the idea that there is a disturbance in the interaction between the hypothalamic–pituitary–gonadal (HPG) axis and other neuromodulators in women (Dunn and Steiner, 2000; Meller *et al.*, 2001; Steiner and Dunn, 1996; Young *et al.*, 2000). According to this hypothesis, the neuroendocrine rhythmicity related to female reproduction is vulnerable to change and is sensitive to psychosocial, environmental and physiological factors. Thus, premenstrual dysphoric disorder (PMDD), depression with post-partum onset (PPD), and mood disorders associated with the perimenopause or with menopause may all be related to hormone-modulated changes in neurotransmitter function.

Control of mood and behaviour involves many different neurotransmitter systems, including glutamate, gamma aminobutyric acid (GABA), acetylcholine (ACh), serotonin (5-HT), dopamine (DA), noradrenalin (NA) and neuropeptides. Given the observation that prevalence and symptomatology of mood disorders is often different between males and females, it is presumed that gonadal steroid hormones are somehow involved. For example, declining levels of oestrogen in women have been associated with post-natal depression and postmenopausal depression, and the cyclical variations of oestrogens and progesterone are probably the trigger of premenstrual complaints in women with premenstrual syndrome (Fink *et al.*, 1996). The interaction between neurotransmitters and steroid hormones is extremely complex and delicately balanced. Each system appears to have a modulatory function on the other, and changes in one system may have dramatic effect on the other systems.

Glucocorticoid and gonadal steroid receptors are abundant in different areas of the brain. Gonadal steroid receptors are found in the amygdala, hippocampus, basal forebrain, cortex, cerebellum, locus ceruleus, midbrain raphe nuclei, pituitary gland and hypothalamus (Stomati *et al.*, 1998). Oestrogen receptors are also located in the preoptic area and amygdala (McEwen, 1988) and in the ventromedial nucleus and arcuate nucleus of the hypothalamus (Herbison *et al.*, 1995).

Activation of cholinergic, dopaminergic or adrenergic neurotransmitter systems can alter concentrations of cytosolic hypothalamic oestrogen receptors. Muscarinic agonists and antagonists can increase oestrogen-binding sites in the female rat hypothalamus (Lauber and Whalen, 1988). Oestrogen, progesterone and glucocorticoid receptors can also be activated by insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), transforming growth factor alpha (TGF-alpha), cyclic AMP (cAMP), protein kinase activators and by various neurotransmitters (Culig *et al.*, 1995). Thus activation of neurotransmitter systems can have a direct modulatory effect on binding of gonadal hormones in the central nervous system (CNS).

Conversely, steroid hormones can modulate neuronal transmission by a variety of mechanisms. They may affect the synthesis and/or release of neurotransmitters, as well as the expression of receptors, membrane plasticity and permeability. It has been suggested that steroid hormone receptors function as general transcription factors to achieve integration of neural information in the CNS (Mani *et al.*, 1997; Stahl, 2001a). Steroids are believed to act primarily by classical genomic mechanisms through intracellular receptors to modulate transcription and protein synthesis. This

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mechanism involves the binding of the steroid to a cytoplasmic or nuclear receptor. The hormone–receptor complex then binds to DNA to trigger RNA-dependent protein synthesis. The response time for this mechanism is on the order of several minutes, hours or days. It has also been shown that steroids can also produce rapid effects on electrical excitability and synaptic function through direct membrane mechanisms, such as ligand-gated ion channels, G-proteins and neurotransmitter transporters (Sumner and Fink, 1998; Wong *et al.*, 1996). These short-term (seconds to minutes) effects of steroids may occur through binding to the cell membrane, binding to membrane receptors, modulation of ion channels, direct activation of second messenger systems (Moss *et al.*, 1997) or activation of receptors by factors such as cytokines and dopamine (Brann *et al.*, 1995). Topical application of oestrogen or progesterone to nervous tissue has been shown to result in a rapid change in membrane potential, and sex steroids can affect membrane fluidity by modifying ion transport or receptor function (Maggi and Perez, 1985).

The role and potential relevance of oestrogen and other sex steroids to psychiatric disorders is the focus of current scientific attention. Oestrogen has been described as a 5-HT, NA, and ACh agonist, and it also modulates DA/D_2 receptors. Likewise, oestrogen may also alter the action of drugs that work on target neurotransmitters. The antidepressant properties of oestradiol have recently been demonstrated in a placebo-controlled trial of perimenopausal women with major depression (Soares *et al.*, 2001). Indeed, oestrogen is regarded by some as the 'new frontier' of potential therapeutic action in the treatment of women with mood disorders (Stahl, 2001b).

In this chapter, mood disorders across the reproductive life cycle in women will be briefly reviewed with particular attention to the impact of hormonal fluctuations during menarche, the premenstruum, pregnancy and post-partum, and the perimenopause.

MENARCHE AND MOOD DISORDERS IN ADOLESCENCE

Epidemiological studies consistently show that beginning at menarche, mood disorders are at least twice as common in women than in men. Why these gender differences exist and why they start at puberty is perhaps one of the most intriguing and least understood phenomena in clinical psychiatry (Lewinsohn *et al.*, 1998).

Prior to adolescence, the rates of depression are similar in girls and boys (or are slightly higher in boys), yet with the onset of puberty, the gender proportion of depression dramatically shifts to a 2 : 1 female to male ratio (Kessler and Walters, 1998; Lewinsohn *et al.*, 1998). In the US general population, the lifetime prevalence of major depression (MD) in adolescents and young adults (15–24 years of age) has been reported as 20.6% for females and 10.5% for males (Kessler and Walters, 1998). Lifetime rates of MD in early- as well as late-maturing girls were even higher (30% versus 22% and 34% versus 22%, respectively) when compared to 'on-time' girls.

There is conflicting opinion regarding the age at which gender differences in rates of MD emerge: researchers are divided between the 12–14 and the 15–19 year age brackets (Cohen *et al.*, 1993; Hankin *et al.*, 1998; Lewinsohn *et al.*, 1998).

An integrative theory of depression in adolescents has been introduced (Lewinsohn *et al.*, 1998), although a persuasive explanation of the sharp rise in the prevalence of depression in females after menarche has yet to be elucidated.

The onset of puberty is heralded by a growth spurt, which begins with rapid growth in height and weight typically between 7.5 and 11.5 years of age. Following this initial burst, physical growth continues at a slow pace for several years. The first sign of sexual maturation in girls is breast budding at about 10.5 years, followed by growth of pubic hair, which begins at about 11.5 years, growth of the uterus and vagina, and the enlargement of the labia and clitoris. Menstruation begins after these changes occur. Finally, axillary hair appears, hips broaden, and fat deposits increase. On average, these changes take 4–5 years; however, considerable variation exists in the sequence and tempo of these events.

In North America and Europe, the age of menarche has declined about 4 months per decade since 1850; in North America, menarche now occurs around 12.5 years of age on average (Tanner, 1968). This dramatic decline in the age at which girls reach puberty is one of the strongest examples of environmental factors that affect hormonal responses. The search to isolate the particular environmental factors involved in this acceleration, however, has been only marginally helpful. It has been suggested that urbanization has a major role in this change, as well as improvements in general health, nutrition and other sociocultural factors. But other environmental factors also seem to be implicated in the timing of menarche. Girls who are blind with some perception of light reach menarche earlier than normally sighted girls, and totally blind girls with no light perception reach puberty even earlier (Zacharias and Wurtman, 1964). Moreover, fewer girls start to menstruate during spring and summer time as compared to during seasons of reduced amounts of daylight (autumn and winter) (Bojlen and Bentzon, 1974).

The relationship between psychosocial development and physical maturation has been widely examined. Girls undergoing pubertal change are thought to experience greater distress and to be more vulnerable to stress than pre- or postpubertal girls (Caspi and Moffitt, 1991). Two parameters of pubertal change in particular have received much attention: pubertal status and pubertal timing. *Pubertal status* is defined as the current level of physical development of an adolescent relative to the overall process of pubertal change (a biological factor), usually denoted by a series of stages from prepubertal (stage I) to adult (stage V) according to Tanner (Tanner, 1962). *Pubertal timing*, on the other hand, is defined as the maturation of an adolescent relative to her peers (a psychosocial factor).

There appears to be a relatively sharp demarcated period in midpuberty when girls become more vulnerable to depression than boys. In a recent report on 1073 US children aged 9–13 years, the depression rates in girls rose significantly in mid-puberty, i.e., with the transition to Tanner stage III. In contrast, the prevalence of depression in boys *declines* from Tanner stage II (Angold *et al.*, 1998). Further, it has been determined that in girls, pubertal status (versus the age at puberty) better predicted the emergence of the sex ratio in depression rates. Thus, the onset of menarche may signal an increased but latent biological vulnerability to mood dysregulation in women (Nolen-Hoeksema and Girgus, 1994).

Although changes in affect, mood and behaviour are considered to be related to cyclic hormonal changes, studies of female adolescents and premenstrual syndrome (PMS) are inconclusive, with one study reporting no relationship between menstrual cycle phase and negative affect (Golub and Harrington, 1981) and others showing that PMS is associated with other distress factors in this age group (Freeman *et al.*, 1993; Raja *et al.*, 1992). Notwithstanding, relationships between changes in pubertal hormones and negative affect in female adolescents have been observed. For example, investigators have found that negative affect was significantly related to a rapid increase in oestradiol levels (Warren and Brooks-Gunn, 1989). Negative affect in healthy girls was also associated with higher levels of testosterone and cortisol, and lower levels of dehydroepiandrosterone sulphate (Susman *et al.*, 1991).

There is both direct and indirect — albeit limited — evidence of the involvement of the serotonergic system in the aetiology of depressive disorders in child and adolescent depression. In a comparative study of psychiatric inpatients and normal controls (aged 7–17 years), levels of whole-blood 5-HT were lowest inpatients with mood disorders (Hughes *et al.*, 1996). There is some indication of the responsiveness of children and adolescents with

MD to serotonergic but not noradrenergic agents; researchers have hypothesized that, in childhood, the serotonergic systems may mature at an earlier rate than the noradrenergic systems (Ryan and Varma, 1998). Gonadal hormones affect the production of 5-HT receptors at the transcriptional level, and the altered distribution or function of 5-HT receptor subtypes brought on by changes in the hormonal milieu at menarche may increase vulnerability to mood disorders.

It is nevertheless still unclear how the dramatic changes in the hormonal milieu associated with menarche and a host of psychosocial stressors combine to produce depressive symptoms. One possible unifying hypothesis suggests that disruption of biological rhythms, such as disturbed sleep patterns (Armitage *et al.*, 2001) or irregular menstrual cycles, together with psychosocial losses causing the disruption of social rhythms (also known as 'social zeitgebers') could trigger the onset of a major depressive episode in vulnerable individuals (Ehlers *et al.*, 1988). Another complementary theory emphasizes the neurobiology of stress and the dysregulation of affect during female biological transitions such as menarche, a transition which may be associated with changes in the reactivity of the stress system (Dorn and Chrousos, 1997). The newly fluctuating levels of gonadal hormones as well as gonadotropins, which mark the onset of menarche and the establishment of menstrual cycles, introduce a major change in the hormonal milieu to which the rest of the system has to adjust. This is the period during which the hypothalamic–pituitary–adrenal (HPA) axis has to mature and be sensitized to a variety of new feedback mechanisms. This is also the time during which the HPA axis may be more vulnerable to external psychosocial stressors, to sleep deprivation as well as to the influences of nicotine, alcohol and other drugs, resulting in a higher incidence of HPA axis dysregulation and mood instability.

Taken together, it is suggested that pubertal and other hormonal changes should be monitored prospectively along with individual, genetic, constitutional and psychological characteristics in our efforts to predict the development of negative affect during puberty (Steiner *et al.*, 2000).

PREMENSTRUAL DYSPHORIA

The inclusion of research diagnostic criteria for PMDD in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn (DSM-IV) recognizes that some women in their reproductive years have extremely distressing emotional and behavioural symptoms premenstrually (American Psychiatric Association, 1994 pp. 717–18). Through the use of these criteria, PMDD can be differentiated from *premenstrual syndrome* (PMS) which has milder physical symptoms, i.e., breast tenderness, bloating, headache and minor mood changes (World Health Organization, 1996a). PMDD can also be differentiated from *premenstrual magnification* (concurrent diagnoses of PMS or PMDD *and* a major psychiatric or an unstable medical condition) and from *premenstrual exacerbation* of a current psychiatric disorder or medical condition (Steiner and Wilkins, 1996).

Epidemiological surveys have estimated that as many as 75% of women with regular menstrual cycles experience some symptoms of premenstrual syndrome (Johnson, 1987). PMDD, on the other hand, is much less common. It affects only 3–8% of women in this group (Angst *et al.*, 2001; Johnson *et al.*, 1988; Ramcharan *et al.*, 1992), but it is more severe and exerts a much greater psychological toll. These women report premenstrual symptoms that seriously interfere with their lifestyle and relationships (Freeman *et al.*, 1985; O'Brien *et al.*, 1995).

The aetiology of PMS and PMDD is still largely unknown. That PMS and PMDD are biological phenomena (as opposed to psychological or psychosocial events) is primarily underscored by recent, convincing evidence of the heritability of premenstrual symptoms (Kendler *et al.*, 1998) and the elimination of premenstrual complaints with suppression of ovarian activity (Schmidt *et al.*, 1998) or surgical menopause (Casson *et al.*, 1990). In women with PMDD, the ovarian axis is apparently functioning normally with normal oestrogen and progesterone levels (Schmidt *et al.*, 1998). The current consensus seems to be that normal ovarian function rather than simple hormone imbalance is the cyclic trigger for biochemical events within the central nervous system and other target tissues which unleash premenstrual symptoms in vulnerable women (Roca *et al.*, 1996). This viewpoint is attractive in that it encourages investigation of the neuroendocrine-modulated central neurotransmitters and the role of the HPG axis in PMDD.

There is increasing attention to the metabolite of progesterone, allopregnanolone, in the manifestation of premenstrual symptomatology. Current evidence suggests that progesterone and progestogens are not likely to be effective in the treatment of premenstrual syndromes (Wyatt *et al.*, 2001). Allopregnanolone, on the other hand, is thought to modulate GABA receptor functioning and produce an anxiolytic effect (Rapkin *et al.*, 1997); quantitative differences in progesterone and allopregnanolone levels between PMS subjects and controls have been examined. The findings to date, however, are contradictory (Bicikova *et al.*, 1998; Girdler *et al.*, 2001; Monteleone *et al.*, 2000; Rapkin *et al.*, 1997; Schmidt *et al.*, 1994; Wang *et al.*, 1996). A recent study using an animal model, though, is more promising. In a progesterone-withdrawal paradigm designed to mimic PMS and post-partum depression in female rats, Smith and colleagues have found that decreased levels of allopregnanolone lead to increased production of the $\alpha 4$ subunit of the $GABA_A$ receptor. This changes the sensitivity of the $GABA_A$ receptor to endogenous ligands, resulting in symptoms associated with PMS (Smith *et al.*, 1998).

The role of the female sex hormones in premenstrual symptomatology has been considered of central importance. Notwithstanding, attention by some has shifted from a focus on oestrogen and progesterone to the role of androgens in premenstrual dysphoria.

Early investigations of androgens have suggested that women with PMS or PMDD have elevated levels of serum testosterone in the luteal phase compared with controls (but still within the normal range), which may contribute primarily to the symptom of irritability (Dunn *et al.*, 2001; Eriksson *et al.*, 1992). This hypothesis of increased androgenicity is backed by both animal and human studies of androgens and irritability and/or aggression. Androgens promote sexual drive in humans, and also have been tentatively linked with mood (e.g. depression and premenstrual irritability) and impulsive behaviour (e.g. compulsions and binge eating). Enhanced serotonin availability (e.g. with the use of selective serotonin reuptake inhibitors; SSRIs), on the other hand, is associated with reduction in irritability, depression and impulsive behaviour, as well as reduced libido. An inverse relationship between serotonin and androgens, and their effects on human behaviour has been proposed; the behavioural effects of androgens may be therefore partly mediated by a reduction in serotonin activity (Eriksson *et al.*, 2000).

Reduction of premenstrual dysphoria with androgen antagonists in women with PMS who showed higher mean levels of total testosterone in the late luteal phase also lends support to the idea of increased androgenicity (Burnet *et al.*, 1991; Rowe and Sasse, 1986). Others, however, have not observed differences in plasma testosterone in comparisons of women with or without PMS (Dougherty *et al.*, 1997), and one study has reported significantly *lower* total and free testosterone plasma levels in a sample of 10 women with PMS (Bloch *et al.*, 1998). Further comparative studies of women with PMS and PMDD are therefore required.

An alternative strategy to measuring various hormone plasma levels in an attempt to discern the aetiology of PMDD has been to search for endocrine abnormalities that have been repeatedly

associated with various other forms of psychopathology. The main advantage of this approach is its potential to help further our understanding of PMDD as well as its relation to other psychiatric disorders. The current literature suggests that thyroid dysfunction may be found in a small group of women with premenstrual symptoms but that PMDD should not be viewed as a masked form of hypothyroidism (Korzekwa *et al.*, 1996; Schmidt *et al.*, 1993).

Of the neurotransmitters studied to date, increasing evidence suggests that 5-HT may be important in the pathogenesis of PMDD (Steiner *et al.*, 1997; Rapkin, 1992). PMDD shares many features of other mood and anxiety disorders linked to serotonergic dysfunction. In addition, reduction in brain 5-HT neurotransmission is thought to lead to poor impulse control, depressed mood, irritability and increased carbohydrate craving — all mood and behavioural symptoms associated with PMDD.

The serotonergic system is in close reciprocal relationship with gonadal hormones. In the hypothalamus, oestrogen induces a diurnal fluctuation in 5-HT (Cohen and Wise, 1988), whereas progesterone increases the turnover rate of 5-HT (Ladisich, 1977).

More recently, several studies concluded that 5-HT *function* may also be altered in women with PMDD. Some studies used models of neuronal function (such as whole-blood 5-HT levels, platelet uptake of 5-HT, and platelet tritiated imipramine binding) and found altered 5-HT function during all phases of the menstrual cycle (Ashby *et al.*, 1988; Bixo *et al.*, 2001; Rapkin, 1992; Steege *et al.*, 1992). Other studies that used challenge tests (with L-tryptophan, fenfluramine, buspirone, *m*-chlorophenylpiperazine) suggested abnormal serotonin function in symptomatic women but differed in their findings as to whether the response to 5-HT is blunted or heightened (Bancroft and Cook 1995; Bancroft *et al.*, 1991; FitzGerald *et al.*, 1997; Rasgon *et al.*, 2001; Su *et al.*, 1997; Steiner *et al.*, 1999). Acute tryptophan depletion (suppressing brain 5-HT synthesis) was significantly associated with exacerbation of premenstrual symptoms, in particular irritability (Menkes *et al.*, 1994). Additional evidence suggesting the involvement (although not necessarily aetiological) of the serotonergic system has emerged from treatment studies: drugs facilitating serotonergic transmission, such as SSRIs, are very effective in reducing premenstrual symptoms. These studies imply, at least in part, a possible change in 5-HT transporter/receptor sensitivity in women with premenstrual dysphoria (Steiner and Born, 2000).

The current consensus is that women with premenstrual dysphoria may be behaviourally or biochemically sub- or supersensitive to biological challenges of the serotonergic system. It is not yet clear whether these women present with a trait or state marker (alternatively, both conditions could be possible) of premenstrual syndromes.

The reciprocal relationship between the serotonergic system and the gonadal hormones has been identified as the most plausible target for interventions. This notion has recently gained further support in a very elegant study showing that an SSRI (fluoxetine) reduces sex-steroid-related aggression in female rats in a paradigm which is proposed as an animal model of premenstrual dysphoria/irritability (Ho *et al.*, 2001). Thus, beyond the conservative treatment options such as lifestyle and stress management, and the more extreme interventions that eliminate ovulation altogether, the SSRIs are emerging as the most effective treatment option for this population (Steiner and Born, 2000).

POST-PARTUM DEPRESSION

The specific link and the uniqueness of psychiatric disorders precipitated or triggered by pregnancy or childbirth have recently been acknowledged by the American Psychiatric Association (1994, pp. 386–7). Based primarily on the work of the Task Force on DSM-IV (Purnine and Frank, 1996) the manual now has a course-specific designation 'post-partum onset', which can be applied to both psychotic and non-psychotic post-partum mental disorders. Thus, major depressive disorders, bipolar disorders (manic and depressed), schizoaffective disorders, and psychotic disorders (not otherwise specified) will have the qualifier 'with post-partum onset'.

Post-partum 'blues' is considered the most mild of the post-partum mood disturbances; its prevalence has been reported to be 26–85%, depending on the diagnostic criteria used (Stein *et al.*, 1981). The symptoms of this syndrome typically begin within the first week following childbirth, peak on the fifth day and resolve by the twelfth day post-partum. Symptoms include dysphoria, mood lability, crying, anxiety, insomnia, poor appetite and irritability. The mood disturbance characterizing post-partum blues is considered transient and insufficient in and of itself to cause serious impairment of a woman's functioning (O'Hara *et al.*, 1991). In some women, however, the disturbance may persist beyond the initial post-partum period, leading to more serious PPD (Cox *et al.*, 1993).

Epidemiological studies of the nature, prevalence and course of an episode of major PPD have found that 10–15% of women exhibit depressive symptoms in the first weeks following delivery (Carothers and Murray, 1990; Pop *et al.*, 1993), and that the great majority of these depressive episodes resolve spontaneously within three to six months (Cooper and Murray, 1995; Cox *et al.*, 1993). The symptom profile of PPD resembles that of a major depressive episode experienced at other times in life, but it is unique in its timing and in that it always involves at least the mother–baby dyad and in most cases an entire family unit.

Post-partum psychosis is much more rare and more severe than either depression or the blues. It has a prevalence of approximately 1 in 500–1000 births, and a rapid onset within the first few days to two weeks post-partum (Brockington *et al.*, 1982). Post-partum psychosis, believed to be in most cases an episodic presentation of a manic-depressive illness, severely impairs the affected woman's ability to function. In the most extreme cases, the risks of suicide or infanticide are high (Millis and Kornblith, 1992), requiring admission to a psychiatric hospital (Kendell *et al.*, 1987).

Pregnancy and childbirth have an enormous combined psychological, physiologic and endocrine effect on a woman's body and mind. Since the changes in mood coincide with these profound changes in hormones and other humoral agents related to pregnancy and childbirth, a causal link has been supposed probable (Steiner, 1998). In the animal kingdom, maternal behaviour is mediated by hormonal and neurochemical changes associated with reproduction (Rosenblatt *et al.*, 1988). In animals, it has been suggested that the various neuromodulators be divided into groups which define their proposed role in maternal response: primers — most important during late pregnancy (e.g. steroid hormones and prolactin); triggers — released during parturition (e.g. oxytocin); and modifiers — of oxytocin release (e.g. beta-endorphins, other neurotransmitters) (Keverne and Kendrick, 1994). There is, of course, considerable scope for interactions between these changes and varying repertoires of maternal behaviour across different species (Fleming and Corter, 1988) and the relevance to human behaviour is as yet unclear.

The peak in mood disturbance during the blues at around the fifth day post-partum coincides with extreme hormonal fluctuations that are a natural consequence of parturition. These hormones act within the central nervous system at a variety of limbic sites known to be involved in emotional responses, arousal and reinforcement. Only a handful of studies have attempted to measure these changes, especially in gonadal hormones and prolactin. To date, most of the results do not seem to correlate strongly with changes in mood and are inconsistent. Serum allopregnanolone levels were shown to be lower in women experiencing post-partum blues (Nappi *et al.*, 2001), whereas serum testosterone levels have been

weakly correlated with depression and anger in the first post-partum days (Hohlagschwandter *et al.*, 2001). A rapid fall in progesterone showed a weak but significant relationship to the development of the blues in one study (Harris *et al.*, 1994) but not in another (Heidrich *et al.*, 1994).

Similarly, increased plasma cortisol levels correlated with the blues and with PPD in one study (Okano and Nomura, 1992) but not in others (O'Hara *et al.*, 1991; Smith *et al.*, 1990). Preliminary results suggest that natural-killer-cell activity is lower in post-partum dysphorics and that this decrease is related to higher levels of cortisol (Pedersen *et al.*, 1993a). In contrast, negative or false-positive results with the dexamethasone suppression test do not correlate with mood changes, indicating that the HPA axis is physiologically hyperactive post-partum ('ceiling effect') and measurements along this axis as an indicator for depression in this population are probably invalid (O'Hara *et al.*, 1991; Smith *et al.*, 1990; Steiner *et al.*, 1986). The HPA, rather than the HPG, axis may in fact play a unique role in human maternal behaviour. Euthymic new mothers with positive maternal attitudes and high levels of cortisol post-partum exhibit the highest level of post-partum maternal approach behaviour (Fleming *et al.*, 1987). None of the other hormones measured (oestradiol, progesterone, testosterone and thyroid indices) were correlated with any of the maternal behaviours measured (Fleming *et al.*, 1987, 1995). These results suggest that cortisol does not induce maternal behaviour directly but it probably facilitates maternal attitudes, which may then be expressed as emotions and/or behaviour.

Thyroid dysfunction has been implicated in mood disorders and it has been suggested that transient thyroid dysfunction following childbirth is associated with PPD (Pederson *et al.*, 1993b). In some women, pregnancy and the post-partum period are associated with pathological changes in thyroid function. A review of the literature in this area clearly indicates the possibility that a subgroup of women with PPD have a basis for the depressed mood in thyroid disorder. More specifically, in some women depressive symptoms are associated with positive thyroid antibody status during the post-partum period (Harris *et al.*, 1992). It is believed that 1% of all post-partum women will show a mood disorder associated with transient thyroid dysfunction, and treatment of the thyroid condition must be part of the management.

The direct and/or indirect effect of the rate of the post-partum withdrawal of some of the other major hormones and neuromodulators involved is nevertheless still intriguing. It has been suggested that women who experience a more rapid beta-endorphin withdrawal are more prone to mood changes (Smith *et al.*, 1990). A sharp fall in circulating oestrogen concentrations after delivery has been associated with acute onset of post-partum psychosis (Wieck *et al.*, 1991). These changes are believed to be the triggers to a cascade of changes at central and peripheral monoamine centres. Very preliminary data suggest an increased sensitivity of dopamine receptors in acute post-partum psychosis (Wieck *et al.*, 1991) and an abnormality in alpha$_2$ adrenoceptor sensitivity associated with the blues (Best *et al.*, 1988). Changes in sensitivities of serotonergic receptors have been documented in PPD (Hannah *et al.*, 1992), but not in women with the blues (Katona *et al.*, 1985).

More recently it has been hypothesized that PPD may be caused by transient hypothalamic corticotropin-releasing hormone (CRH) suppression (Magiakou *et al.*, 1996). The HPA axis is progressively hyperactive throughout pregnancy, with increasing levels of circulating CRH of placental origin and decreasing levels of CRH-binding protein. Both these phenomena, together with the elevated levels of oestradiol of pregnancy, which also stimulate the HPA axis, particularly during the third trimester, contribute to the elevated levels of CRH, ACTH and cortisol (Cizza *et al.*, 1997). After parturition the source of placental CRH is removed, and together with the post-partum oestrogen withdrawal, which is further prolonged by breastfeeding (Kim *et al.*, 2000), may lead to a prolonged state of HPA axis hypoactivity. Indeed, it has been demonstrated that in a subgroup of women with PPD, the suppression of the HPA axis was more severe and lasted longer than that of women who had no post-partum mood instability (Magiakou *et al.*, 1996).

CRH has been associated with the neurobiology of stress and depression (Chrousos and Gold, 1992). PPD also appears to be a state of central CRH dysregulation. With the additional established evidence of direct oestrogenic regulation of the CRH gene expression (Vamvakopoulos and Chrousos, 1993) it is therefore not surprising that oestrogen has been proposed as a treatment for PPD (Ahokas *et al.*, 2001; Gregoire *et al.*, 1996; Sichel *et al.*, 1995). In the only double-blind, placebo-controlled study published to date, a 3-month course of 200 μg per day of 17ß-oestradiol significantly improved the clinical symptoms of severely depressed women post-partum (Gregoire *et al.*, 1996). Unfortunately further research on the role of oestrogen therapy for PPD has not yet emerged. Similarly, progesterone has been widely used for the treatment of post-natal depression but without controlled trials (O'Brien and Pitt, 1994).

The role of hormone replacement therapy is of interest beyond the realm of the post-partum period and, as discussed in the next section, is of major relevance during the perimenopausal and menopausal years and beyond.

The reciprocal relationship between the serotonergic system and gonadal hormones has not as yet been studied during pregnancy or in post-partum women. However, preliminary results from studies in post-partum rats indicate that 5-HT receptor changes in the limbic area are negatively correlated with progesterone levels (Glaser *et al.*, 1990). It is argued that post-partum withdrawal of gonadal hormones may cause changes along the serotonergic cascade, which may lead to a mood disorder in vulnerable or genetically predisposed women. (It should therefore be possible to treat the disturbance by 'adjusting' the levels of the hormone (the trigger) (Henderson *et al.*, 1991) or by reversing the sensitivity (predisposition). Results from some preliminary studies on preventative interventions with lithium prophylaxis (Cohen *et al.*, 1995; Stewart *et al.*, 1991) and with SSRIs (Appleby *et al.*, 1997; Stowe *et al.*, 1995) are very encouraging. Since mood disorders associated with childbearing have different times of onset in different women and are heterogenous in their presentation, concomitant measurements of the changes over time in gonadal hormones and the biochemical changes in the monoamine system are crucial.

Further evidence of a biological component of post-partum mood disorders comes from family and family history studies. It has been suggested that women with a history of post-partum depression are differentially sensitized to mood-destabilizing effects of gonadal steroids (Bloch *et al.*, 2000). A study of women with post-partum mood disturbances and their first-degree relatives found that at least one family member met criteria for a past or present psychiatric disorder in 71% of the cases for which the information was available. Positive histories for MD and alcoholism were found in 48% and 30% of these families, respectively (Steiner and Tam, 1999). Further analysis of these data revealed an interesting gender distribution of psychiatric disorders in the first-degree relatives of the post-partum women. A female : male ratio greater than 2 : 1 was found in relatives with a past or present diagnosis of MD; in the case of alcoholism, a male : female ratio of 4 : 1 was evident. This lifetime prevalence of mood-related disorders in the first-degree relatives of women presenting with post-partum mood disorders is much higher than in the population at large and may indicate potential genetic or familial components of the disorders.

Despite the fact that most animals share the same physiological events at parturition, the differences in behavioural response between humans (as well as other primates) and non-primate mammals are remarkable. The differences between primates and non-primates are mainly in the organization of social structures, the

complex influences of the family unit, and the constant exposure of all members of a group to the young. It is therefore easy to assume that, in humans, even thinking about children may be sufficient to stimulate maternal responsiveness. The psychosocial literature to date has advanced several psychological and social stress factors as potential aetiological theories of primary non-psychotic PPD. These factors include lack of social support, negative life events, occupational instability, lack of prior experience with children, unplanned pregnancy and antenatal 'pessimism', dissatisfaction with the marital relationship (or being unmarried), and a poor relationship between the affected woman and her own mother (Murray *et al.*, 1995; Paykel *et al.*, 1980).

In summarizing these studies, no unifying conclusion can be reached, and it is impossible at this stage to translate any of these results into predictive, diagnostic, therapeutic, prognostic or preventative applications. It seems more likely that an intrinsic abnormal reaction to some of the hormonal changes, rather than the changes themselves, is responsible for the disorder. If the psychobiological factors (or their interactions) responsible for the emotional disorders associated with childbearing could be elucidated, our understanding of the aetiology not only of PPD but also of a wider range of psychiatric disorders might be enhanced.

PERIMENOPAUSE, MENOPAUSE AND BEYOND

The transition into menopause is a major hormonal event and is associated in many women with both physical and psychosocial symptoms. The term perimenopause describes the period immediately before the menopause — from the time when the hormonal and clinical features of approaching menopause commence until the end of the first year after menopause (World Health Organization, 1996b).

The physiological hallmark of the transition into menopause is gradual oestrogen depletion. In the 1960s and 1970s 'depletion' was equated with 'deficiency' and menopause, representing a state of oestrogen deficiency, was therefore considered a medical disorder warranting treatment. A famous quotation from that era highlights this approach: 'It sometimes seems as if the only thing worse than being subjected to the raging hormonal influences of the female cycle is to have those influences subside' (Parlee, 1976). The notion of universal hormone replacement for *all* menopausal women was so rampant that the WHO convened a special session and eventually came out with a consensus statement to counter the above, which read: 'Menopause is part of the normal aging process which in itself does not require therapeutic intervention. The health status of women during this period is not recognized as being a simple endocrine-deficiency state which could or should be corrected by attempting to create for each woman a premenopausal normal environment' (WHO, 1981).

Changes most commonly associated with oestrogen depletion (and/or unpredictable fluctuations) include vasomotor symptoms such as hot flushes and night sweats (Freedman, 2000; Guthrie *et al.*, 1996), urogenital dryness/atrophy causing dyspareunia as well as an increased risk over time of osteoporosis and cardiovascular disease (Mitchell and Woods, 1996). The relationship between the perimenopause/menopause and mood disorders is less well understood. Epidemiological data indicates that the majority of postmenopausal women do not experience prominent symptoms of depression, but a higher than expected prevalence of depressive-like symptoms has been observed in peri- and postmenopausal women attending gynaecological clinics (Avis and McKinlay, 1991; Schmidt and Rubinow, 1991).

It is unclear as to whether there is decline in new-onset episodes of major depression in females of this age group, as suggested by the Epidemiologic Catchment Area study, a finding not supported by data from the National Comorbidity Survey. The role of sociocultural factors and demographic differences have been the focus of much study but the results are controversial (Anderson *et al.*, 1987; Hay *et al.*, 1994).

Some cross-cultural differences are nevertheless noteworthy: Japanese women experience very few physical as well as emotional symptoms around menopause. It has been proposed that these findings are indicative not only of cultural and demographic differences but also reflect the influence of biological, genetic and nutritional/dietary factors (Lock, 1994; Nagata *et al.*, 1998).

The most prevalent mood symptoms during the perimenopause include irritability, tearfulness, anxiety, depressed/labile mood, lack of motivation/energy, poor concentration and interrupted sleep. These symptoms have been linked to predictable fluctuations in oestradiol, especially abrupt withdrawal from very high erratic levels, rather than to times when levels are slowly and gradually declining (Prior, 1998).

Several lines of evidence point to the link between oestrogen depletion/deficiency and mood disorders in vulnerable or predisposed women. Oestrogen has direct effects on the CNS in areas which are not strictly relevant to reproduction. For example, oestrogen regulates synaptogenesis, has a general trophic effect on cholinergic neurons and stimulates a significant increase in 5-HT_{2A} binding sites in areas which are involved in regulating both mood and cognition. It is therefore not surprising that oestrogen has been shown to improve psychological functioning and well-being in nondepressed postmenopausal women (Ditkoff *et al.*, 1991; Palinkas and Barret-Connor, 1992) and that oestrogen replacement therapy (ERT) has a positive effect on mood states (Zweifel and O'Brien, 1997). The ability of oestrogen to act as a 5-HT agonist/modulator is of particular significance. Oestrogen not only increases the number of 5-HT_{2A} receptor binding sites but also increases 5-HT synthesis, uptake and 3H-imipramine binding; it decreases 5-HT_1 receptor binding sites and 5-HT transporter mRNA and increases the prolactin response to 5-HT agonists — all in line with antidepressant-like action (Biegon and McEwen, 1982; Fink *et al.*, 1996; Halbreich *et al.*, 1995; Pecins-Thompson *et al.*, 1998; van Amelsvoort *et al.*, 2001). The clinical relevance of these effects to the pathophysiology of women-specific mood and anxiety disorders remains to be determined.

The strongest evidence to date for oestrogen's ability to improve mood and cognitive functioning comes from studies in young surgically menopausal women treated with ERT (Sherwin, 1988; Sherwin and Suranyi-Cadotte, 1990). It is encouraging to note that several very preliminary studies seem to indicate the beneficial effects of combining ERT with SSRIs in the treatment of postmenopausal depressed women (Schneider *et al.*, 1997). Preliminary evidence also indicates the efficacy of transdermal 17ß-oestradiol alone in the treatment of perimenopausal women with major and minor depression (Schmidt *et al.*, 2000; Soares *et al.*, 2001).

Oestrogen specifically maintains verbal memory in women and may prevent or forestall the deterioration in short- and long-term memory that occurs with age (Sherwin, 1999a). There is also evidence that oestrogen may have a role in the prevention and treatment of Alzheimer's disease (AD). Theoretically, oestrogen could be the perfect anti-Alzheimer's treatment (Garcia-Segura *et al.*, 2001). Oestrogen has the properties of an antioxidant, can modify inflammatory response, increases growth of ACh neurons, can affect amyloid precursor protein cleavage, inhibits ApoE levels, stimulates glucocorticoid levels, increases glucose utilization and increase cerebral blood flow. Unfortunately the clinical data to date are somewhat mixed: the estimated risk of AD decreases significantly in women who have been on long-term ERT (Kawas *et al.*, 1997; Paganini-Hill and Henderson, 1994) but others have reported only 50% reduction in incidence (Waring *et al.*, 1999), with some benefit in early-onset AD only and some protection against further deterioration (Costa *et al.*, 1999) whereas others have seen no beneficial effect at all (Mulnard *et al.*, 2000).

The use of ERT continues to be controversial, with the risk of breast and endometrial cancer in long-term users still looming. At the same time, the search for the perfect selective oestrogen receptor modulator (SERM) is ongoing. The 'ideal' SERM would have negative receptor activity on breast and endometrial cells and positive receptor activity on bone, cardiovascular and brain. So far there is evidence that raloxifene is effective in preventing osteoporosis and has protective cardiovascular properties and also seems to reduce the risk for breast cancer (Cauley *et al.*, 2001; Delmas *et al.*, 1997) but its effect on cognitive function in humans has not been established. There is some indication that it may lower the risk of decline in attention and memory (Yaffe *et al.*, 2001) and in animals there is some indication that raloxifene plus oestradiol induces neurite outgrowth to a greater extent than raloxifene or oestradiol alone (Nilsen *et al.*, 1998).

Progesterone, which in the past has been promoted by some as an antidepressant, by itself can not only cause depression but seems also to reverse the oestrogen-induced receptor expression. Progestogens also have potent anaesthetic properties and dampen brain excitability; they also increase the concentration of monoamine oxidase, the enzyme that catabolizes 5-HT in the brain, whereas oestrogen decreases the enzyme, thereby increasing the concentration of 5-HT (Luine *et al.*, 1975; Sherwin, 1999b).

Testosterone is also an extremely important psychoactive compound and its relevance to women's well-being is just beginning to be recognized (Tuiten *et al.*, 2000).

While we are awaiting results of the ongoing long-term prospective studies with ERT and SERM, it is important to recognize that depressive symptoms are a significant risk factor for mortality in older women (Whooley and Browner, 1998). Whether depressive symptoms are a marker for, or a cause of, life-threatening conditions remains to be determined. Nevertheless, treatment for depression may not only enhance the quality of life but may also reduce mortality in this population.

CONCLUSION

The complex integration of the neurotransmitter and steroid hormone systems implies that circulating steroid hormones from peripheral endocrine glands can directly regulate brain function and modulate behaviour. Regulation occurs through a variety of mechanisms, including, for example, direct interaction with or upregulation of specific receptors on neuronal cells. Thus, the hormonal milieu surrounding a neuronal cell will, in part, determine the response of that cell to various stimuli.

Adrenal and gonadal steroids regulate the transcription of most of the major neurotransmitter systems.

Steroid hormones also have direct effects on neuronal cell function by non-genomic mechanisms influencing the sensitivity and responsiveness of the neurons.

Levels of oestrogen and progesterone vary significantly across the female lifespan. At puberty there is an increase in oestrogen and initiation of cyclic and diurnal variation in oestrogen production. The sudden appearance of higher levels of oestrogen in puberty alters the sensitivity of the neurotransmitter systems. Behaviours such as moodiness, irritability and conflicts with parents around this time may in part reflect this increased sensitivity. The constant flux of oestrogen and progesterone levels continues throughout the reproductive years. The neurotransmitter systems are thus constantly being attenuated or amplified. PMS and PMDD may be the result of an altered activity (or sensitivity) of certain neurotransmitter systems. Pregnancy and delivery produce dramatic changes in oestrogen and progesterone levels as well as significant suppression along the HPA axis, possibly increasing vulnerability to depression. Finally, at menopause, oestrogen levels decline while pituitary LH and FSH levels increase. The loss of modulating effects of oestrogen and progesterone may underlie the development of perimenopausal mood disorders in vulnerable women.

Since these hormonal changes occur in all women, it seems safe to speculate that the development of mood disorders requires more than just fluctuating levels of hormones, but also a genetic predisposition. These as yet unidentified genetic 'defects' probably relate to subtle alterations in number and function of various receptors and enzymes and to subtle structural and anatomical differences in the CNS. These differences caused by genetic polymorphism, combined with the flux in the hormonal milieu, determine how the system reacts to multiple environmental stresses and predicts the development of mood disorders. Further research into this complex system is needed to identify specific genetic markers which might help us better understand how the balance between oestrogen, progesterone, testosterone and other steroid hormones affects neurotransmitter function.

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XVIII-13

Therapeutic Armamentarium

Robert H. Howland and Michael E. Thase

INTRODUCTION

Currently, a wide variety of therapies are available for the treatment of mood disorders. These therapies have been studied mainly in major depressive disorder and bipolar I disorder, although they also are commonly used in clinical practice for the treatment of such related mood disorders as dysthymia, minor depression, cyclothymia and bipolar II disorder (Howland, 1991; Howland and Thase, 1993). The diversity of the many antidepressant and antimanic therapies that are effective likely reflects the underlying pathophysiological heterogeneity that exists among the mood disorders (Howland and Thase, 1999b), although seemingly different therapies also may share common therapeutic mechanisms of action. For a significant proportion of mood disorder patients, the available standard antidepressant and antimanic therapies are not entirely effective, contributing to the development and clinical use of novel treatments. In this chapter we review standard antidepressant and antimanic therapies for mood disorders. We also review novel pharmacological and non-pharmacological therapies that are being studied and sometimes used for the treatment of major depressive and bipolar disorders.

TREATMENT OF DEPRESSION

Antidepressant Drugs

During the 1950s, the clinical effects of tricyclic antidepressant (TCA) and monoamine oxidase inhibiting (MAOI) drugs were first discovered (Crane, 1957; Kuhn, 1958). Since the 1980s, numerous antidepressant drugs unrelated to the TCAs and MAOIs have been developed and studied for the treatment of depression (Kent, 2000; Sampson, 2001).

Tricyclic Antidepressants (TCAs)

After their discovery, the TCAs were the cornerstone of pharmacotherapy for depression for nearly three decades (Nelson, 2000). The most commonly used TCAs, so called because of their cyclic chemical structure, include imipramine, desipramine, amitriptyline and nortriptyline. Doxepin, trimipramine and protriptyline are less commonly used. Clomipramine is approved for use in the United States only for the treatment of obsessive-compulsive disorder, but it is a highly effective antidepressant. The antidepressant effects of the TCAs are believed to be primarily due to their inhibition of the reuptake of the neurotransmitters norepinephrine and serotonin (Richelson, 2001), although they and other antidepressant drugs also directly or indirectly affect second-messenger systems (Popoli *et al.*, 2000; Vaidya and Duman, 2001) that are implicated in the neurobiology of depression (Duman *et al.*, 1997). Aside from clomipramine, the TCAs are more active on noradrenergic reuptake inhibition. They also potently block several important postsynaptic neurochemical receptors, which account for most of their typical side effects. Dry mouth, constipation, blurred vision, sinus tachycardia, urinary retention and memory dysfunction are due to cholinergic receptor blockade. Sedation and weight gain are primarily related to histaminic receptor blockade, although serotonergic and anticholinergic effects may also contribute to the weight gain associated with the TCAs and other types of antidepressant drugs (Fava *et al.*, 2000). Dizziness, hypotension and reflex tachycardia are caused by their blockade of α-1 adrenergic receptors. The TCAs also have quinidine-like effects on cardiac conduction, which can cause serious cardiotoxic effects, including arrhythmias and conduction abnormalities, and can be fatal with an overdose as small as 7–10 times a daily therapeutic dose.

As a group, the TCAs do not differ significantly in their relative antidepressant efficacy (Thase, 1997). Two TCAs, nortriptyline and desipramine, are secondary amine metabolites of amitriptyline and imipramine, respectively, and they are often preferred because of less pronounced anticholinergic side effects. Placebo-controlled studies have found TCAs to be effective in adult and geriatric patients, but not in younger age groups (Brent *et al.*, 1995). Interestingly, compared to men and to postmenopausal women, there is evidence that premenopausal women respond less well to TCAs than to other antidepressant drugs (Kornstein *et al.*, 2000; Thase *et al.*, 2000a). Higher TCA doses are often associated with greater therapeutic benefit, but this is limited by their greater side effect burden. Measuring plasma levels of imipramine, amitriptyline, desipramine and nortriptyline is clinically useful for achieving a therapeutic response (Bernstein, 1995). Because of their side effects and potential lethality, the use of TCAs has been gradually supplanted by newer generation antidepressants. The TCAs, however, can be quite effective in treating patients who do not respond to other drugs, and they remain especially effective treatments for more severe melancholic depressions (Thase and Rush, 1995).

Monoamine Oxidase Inhibitors (MAOIs)

The pharmacology and presumed mechanism of action of the MAOIs is distinctly different from all other antidepressants (Kennedy *et al.*, 2000). The MAOIs phenelzine, tranylcypromine and isocarboxazid irreversibly and non-selectively inhibit the enzyme monoamine oxidase (MAO), which degrades norepinephrine, serotonin and dopamine, thereby increasing the availability of these neurotransmitters and likely contributing to their antidepressant effects (Richelson, 2001), although they may also directly or indirectly affect second-messenger systems (Popoli *et al.*, 2000; Vaidya and Duman, 2001) that are implicated in the neurobiology of depression (Duman *et al.*, 1997). Typical side

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effects of the MAOIs include hypotension, dizziness, sedation, weight gain, dry mouth and sexual dysfunction. Phenelzine is more often associated with these side effects than is tranylcypromine, whereas tranylcypromine may be more likely to cause insomnia. Because the MAOIs inhibit MAO in the gut, severe hypertension can occur when foods containing the amino acid tyramine (which stimulates the release of norepinephrine) are ingested. As a result, patients treated with MAOIs must follow a tyramine-free diet. In addition, use of the MAOIs is associated with potentially severe and fatal interactions with various other drugs, including antidepressants, narcotics and sympathomimetics.

Because of their side effect profile and dietary restrictions, the MAOIs are not commonly used in clinical practice. However, comparative studies have found the MAOIs to be more effective than the TCAs and other antidepressants in the treatment of atypical depression (Jarrett *et al.*, 1999; McGrath *et al.*, 2000) and bipolar depression (Thase and Sachs, 2000). In addition, the MAOIs have consistently been found to be effective in 40–60% of patients who do not respond to other antidepressants (Thase *et al.*, 1995). Like the TCAs, higher doses (within the therapeutic range) also are associated with a greater antidepressant effect if tolerable.

Some MAOIs are relatively selective for the Type A or Type B subforms of MAO (Lotufo-Neto *et al.*, 1999). Selegiline is a selective inhibitor of MAO-B at low doses, but there is little evidence that it has antidepressant effects at these doses. At higher doses, however, selegiline loses its selectivity and has greater antidepressant effects. A selegiline transdermal application system is currently being developed, which avoids peripheral MAO-A inhibition in the gut and permits greater systemic selegiline levels without the risk of a tyramine reaction, potentially making it a more viable antidepressant therapy (Barrett *et al.*, 1997; Mahmood 1997; Sacktor *et al.*, 2000). By contrast, moclobemide and brofaromine are reversible inhibitors of MAO-A (RIMAs). The RIMAs have demonstrated antidepressant effects, are much better tolerated than the TCAs and older MAOIs, and do not require any dietary restrictions, although there is some evidence that they may be slightly less effective compared to TCAs and older MAOIs (Amrein *et al.*, 1997; Lotufo-Neto *et al.*, 1999; Parker *et al.*, 2001; Philipp *et al.*, 2000).

Heterocyclic Antidepressants

Problems with the tolerability and safety of the TCAs and MAOIs led to interest in developing alternative antidepressant drugs (Bernstein, 1995). These pharmacologically diverse drugs include amoxapine, maprotiline and trazodone (Richelson, 2001). Amoxapine is a TCA-like compound that is related to the antipsychotic drug loxapine, and can have similar extrapyramidal side effects (e.g. dystonia, akathesia, parkinsonian symptoms, and possibly tardive dyskinesia). As a result, it has not been a commonly used antidepressant, although it is sometimes used as a 'monotherapy' for the treatment of psychotic depression (Wheeler Vega *et al.*, 2000). Maprotiline is a tetracyclic drug that primarily blocks the reuptake of norepinephrine, but is rarely used because of a higher risk of seizures and cardiac toxicity compared to other antidepressants. Trazodone is a phenylpiperazine compound that has weak effects on monoamine reuptake, but does block postsynaptic serotonin (5-HT2) receptors. Trazodone has been used almost exclusively in lower doses as a safe and effective non-habit forming hypnotic, often in combination with other antidepressants. Higher doses are usually needed to treat severe depression and have been associated with severe hypotension and, rarely, the development of priapism.

Serotonin Reuptake Inhibitors (SRIs)

The SRIs have become the most commonly used class of antidepressants for the treatment of depression around much of the world (Gram, 1994). These drugs include fluoxetine, paroxetine, sertraline and citalopram (Sampson, 2001). A fifth SRI, fluvoxamine, is approved in the United States as a treatment for obsessive-compulsive disorder, but also is an effective antidepressant. As a class, the SRIs are chemically dissimilar, which accounts for various pharmacologic differences (e.g. different half-lives, active metabolites and hepatic enzyme inhibitory effects). Despite the pharmacologic differences, these drugs have a similar mechanism of action, which is their potent and specific inhibition of serotonin reuptake (Richelson, 2001), although they also directly or indirectly affect second-messenger systems (Popoli *et al.*, 2000; Vaidya and Duman, 2001) that are implicated in the neurobiology of depression (Duman *et al.*, 1997). Typical side effects of the SRIs include nausea, diarrhoea, insomnia, nervousness, headache and sexual dysfunction. Additional clinical experience with the SRIs also suggests that some patients may complain of sweating, fatigue and weight gain (Fava *et al.*, 2000). They do not have significant cardiac effects, and are much safer than the TCAs and MAOIs in overdose. Several of the SRIs also can inhibit various hepatic enzymes, which are important for drug metabolism and can lead to clinically significant drug interactions, but this limitation has not lessened the utility of the SRIs (Thase, 1997).

As a group, the SRIs do not differ significantly in their relative efficacy or typical side effect profiles, although there may be subtle differences in the degree of certain side effects (Kelsey and Nemeroff, 2000). There is some evidence that patients who do not respond to or cannot tolerate one SRI may do better after switching to an alternative SRI (Howland and Thase, 1999a). Comparative studies with the TCAs have generally found similar efficacy rates, although indices of tolerability usually favour the SRIs. There continues to be debate about whether the TCAs are more effective among patients with more severe melancholic depressions (Perry, 1996; Thase and Rush, 1995). Unlike the TCAs, placebo-controlled studies have found the SRIs to be effective in treating depression in adolescents (Emslie *et al.*, 1997). Moreover, compared to men and to postmenopausal women, premenopausal women may respond relatively better to SRIs than to other antidepressant drugs (Kornstein *et al.*, 2000; Thase *et al.*, 2000a). The SRIs are generally considered to be safe for use during pregnancy (Ericson *et al.*, 1999).

Atypical Antidepressants

The term 'atypical antidepressants' is used to group the remaining antidepressant drugs (bupropion, venlafaxine, nefazodone, mirtazapine and reboxetine), which are chemically unrelated to each other and to the TCAs, MAOIs or SRIs (Preskorn, 1995; Kent, 2000).

Bupropion

Bupropion is a novel antidepressant that belongs to the aminoketone class of drugs (Golden and Nicholas, 2000). Its mechanism of action is uncertain, although bupropion appears to facilitate or modulate the effects of norepinephrine and dopamine. Bupropion has virtually no effect on serotonergic, cholinergic, histaminic or other adrenergic systems (Richelson, 2001). As a result, it lacks the usual side effects seen with the TCAs, MAOIs or SRIs. Typical side effects include restlessness, insomnia, nausea, headache, constipation and tremors. Bupropion is not associated with sexual dysfunction or weight gain and does not have significant cardiac effects. Bupropion is a modest inhibitor of the hepatic isoenzyme CYP450 2D6, but is not notorious for clinically significant drug interactions. High doses (greater than 450 mg per day) are associated with an increased risk of seizures, but within the manufacturer's recommended dose range the rate of seizures does not differ from other antidepressant drugs.

Comparative studies of bupropion and other antidepressants in depression have not found any significant differences in efficacy,

but it has a different tolerability profile (especially with regard to sexual and weight effects) (Spier, 1998). It is sometimes combined with SRIs to lessen sexual dysfunction or augment antidepressant effects (Howland and Thase, 1999a). Bupropion may not be as effective in the treatment of severe anxiety states associated with depression (e.g. obsessive-compulsive or panic anxiety), but might be especially useful in anergic, atypical, seasonal or bipolar depressions (Sachs *et al.*, 1994; Thase, 1997).

Venlafaxine

Venlafaxine is a novel antidepressant belonging to the phenylethylamine class of drugs (Beauclair *et al.*, 2000). The primary mechanism of action is the inhibition of serotonin and, at higher doses, norepinephrine reuptake (Richelson, 2001). Typical side effects include nausea, headache, dizziness, dry mouth, constipation, loss of appetite, nervousness and insomnia (Kent, 2000). Some patients may complain of fatigue, sweating and sexual dysfunction. A small proportion of patients may develop increased blood pressure at high doses, but it does not have any other significant cardiac effects. It also does not have any clinically significant effect on hepatic metabolic enzymes and appears to be relatively safe in an overdose.

Some studies directly comparing venlafaxine to several different SRIs have found it to be more effective and more likely to lead to a full remission, especially at higher doses (Thase *et al.*, 2001a). There also is some evidence that venlafaxine may have a more rapid onset of action compared to other antidepressants, but this has not been well studied (Thase *et al.*, 2001c). In addition, other studies have found it effective in the treatment of refractory depression, including patients who had not responded to various antidepressants from different classes (Thase *et al.*, 2000b).

Nefazodone

Nefazodone is a novel antidepressant that, like trazodone, belongs to the phenylpiperazine class of drugs (Garlow *et al.*, 2000). Its primary mechanism of action may be blockade of postsynaptic serotonin-2 receptors. It has very weak inhibitory effects on reuptake of norepinephrine and serotonin (Richelson, 2001). Typical side effects include sedation, dry mouth, nausea, headache, constipation and dizziness (Kent, 2000). Compared to SRIs and TCAs, it is not associated with sexual dysfunction or weight gain (Ferguson *et al.*, 2001; Sussman *et al.*, 2001). Although nefazodone is a chemical analogue of the antidepressant trazodone, it is much less sedating initially and has not been found to cause priapism. Hypotension may occur rarely at high doses and rare cases of hypatotoxicity have been reported, but it does not have any other significant cardiac effects and seems to be relatively safe in an overdose. Nefazodone can inhibit the hepatic isoenzyme CYP450 3A4, which has some potential for drug–drug interactions (Thase, 1997).

Comparative studies have not found any significant difference in efficacy between nefazodone and SRIs or TCAs in the treatment of depression (Feighner *et al.*, 1998). Across 6–8 weeks of treatment, nefazodone appears to have more beneficial effects (than SRIs) on insomnia associated with depression. Nefazodone is effective in treating anxiety symptoms in depression, and its side effect profile (especially its sexual and weight effects) may be preferable for some patients compared to the SRIs (Sajatovic *et al.*, 1999).

Mirtazapine

Mirtazapine is a novel tetracyclic antidepressant belonging to the piperazinoazepine class of drugs (Claghorn, 2000). It has a very complicated presumed mechanism of action, involving blockade of several different adrenergic and serotonergic receptors, with the net effect of increasing serotonin and norepinephrine transmission (Richelson, 2001). It also blocks postsynaptic histamine receptors, which contributes to its most common side effects (i.e. sedation and weight gain). Compared to the SRIs, it is less likely to cause nausea, headache and sexual dysfunction (Kent, 2000). In some patients, it can cause dry mouth, constipation and dizziness. It does not have significant cardiac effects, or any clinically significant drug interactions, and it appears to be relatively safe in an overdose.

Comparative studies with other antidepressants generally have not found any significant differences in efficacy (Fawcett and Barkin, 1998; Guelfi *et al.*, 2001), although several recent studies found mirtazapine to have a more rapid onset of action compared to SRIs (Thase *et al.*, 2001c). Its advantage over the SRIs with respect to gastrointestinal and sexual effects must be counterbalanced against its greater degree of sedation and weight gain. However, it may have more rapid and beneficial effects on sleep and anxiety in many patients, compared to the clinical effects of other antidepressants.

Reboxetine

Reboxetine is a novel antidepressant compound, not yet approved for use in the United States, that is unrelated to the TCAs, MAOIs, SRIs or other atypical antidepressant drugs (Healy and Healy, 1998). It is a potent and selective norepinephrine reuptake inhibitor and does not bind to serotonin, dopamine, cholinergic or histamine receptors (Richelson, 2001). As a result, reboxetine is not associated with sedation, weight gain or sexual dysfunction (Kent, 2000). Typical side effects include dry mouth, constipation, insomnia, increased heart rate and urinary hesitancy/retention (in men). It does not have significant cardiac effects, does not have any clinically significant effect on hepatic metabolic enzymes, and appears to be relatively safe in an overdose. Comparative studies with other antidepressants generally have not found any significant differences in efficacy, although the side effect profile differs (Massana, 1998; Versiani *et al.*, 2000).

Antidepressant Psychotherapies

Historically, before the discovery of effective antidepressant compounds, long-term psychodynamic therapy was considered the treatment of choice for non-psychotic depressions. This approach, although helpful to patients in many ways, was never studied to determine if it is effective in treating the core symptoms of depression. During the past 30 years, psychological models of depression have been used to develop short-term highly-structured forms of psychotherapy specifically for treating depression (Wells and Giannetti, 1990). Compared to traditional forms of psychotherapy, several of these depression-focused psychotherapies have been studied in clinical trials and found to be as effective as pharmacotherapy for the treatment of depressed outpatients (Persons *et al.*, 1996; Rush and Thase, 1999). Depression-focused psychotherapies should not be considered for treatment of psychotic or bipolar forms of depression without concomitant pharmacotherapy, although there is developing evidence that the use of specific psychosocial interventions together with pharmacotherapy may lead to a better outcome in bipolar disorder (Frank *et al.*, 2000; Miklowitz *et al.*, 2000).

Interpersonal psychotherapy (IPT) is a time-limited form of psychotherapy that focuses on difficulties in the patient's intimate and vocational relationships (Klerman *et al.*, 1984). IPT has been shown to be an effective and easily used intervention in the treatment of depression (Klerman and Weissman, 1993; Markowitz, 1998). Some studies have found it to be as effective as medication among outpatients with mild to moderate levels of depression (Persons *et al.*, 1996; Weissman and Markowitz, 1994). Interestingly, a recent functional brain imaging study of patients with major depression treated with paroxetine or IPT found that regional brain metabolic abnormalities tended to normalize with either treatment (Brody *et al.*, 2001). There also is developing evidence that IPT may be beneficial for the depressive phase of bipolar disorder (Swartz and Frank, 2001).

Various behavioural therapy (BT) approaches have been developed and studied in the treatment of depression, such as activity scheduling, self-control techniques and social skills training (Becker *et al.*, 1987; Rush and Thase, 1999). A number of controlled studies have found BT to be effective among outpatients with depression. Some evidence suggests that BT may be as effective as antidepressant medications in depression (Hersen *et al.*, 1984; Rush and Thase, 1999).

Cognitive behavioural therapy (CBT) is another time-limited form of psychotherapy that is based on the theory that depression is invariably associated with automatic negative thoughts about oneself, the world and the future (Beck *et al.*, 1979; Clark and Fairburn, 1997). CBT is the best studied form of psychotherapy for major depression and dysthymia (Howland, 1996a; Persons *et al.*, 1996). Many studies have found it to be as effective a treatment for major depression as TCAs (Hollon *et al.*, 1992). Several controlled studies have found that the efficacy of CBT may be limited in more severe depressions, but this finding has not been observed by a number of other studies (Rush and Thase, 1999). Cognitive Behavioural Analysis System of Psychotherapy (CBASP) is a modified form of CBT that incorporates some principles of IPT (McCullough, 2000). CBASP was recently found to be as effective as nefazodone in the treatment of chronic depression (Keller *et al.*, 2000). There also is some evidence that CBT may be helpful for depression in bipolar disorder, but this deserves further study (Patelis-Siotis, 2001).

Other Standard Drug Therapies for Depression

Despite the availability and effectiveness of a large number and variety of antidepressant drugs, some important problems exist in the treatment of depression (Howland and Thase, 1997). First, all antidepressants have a delayed onset of action. Second, a significant minority of patients do not respond to an initial choice of drug, and the majority of patients who do respond show only a partial improvement in their depression. Third, a large proportion of patients who have an initially good response to an antidepressant will suffer a subsequent relapse or recurrence despite adequate ongoing treatment. Finally, many patients who respond to an antidepressant drug will have intolerable side effects. As a result, many different drugs have been studied and/or used in clinical practice to accelerate the antidepressant response, improve partial- or non-responders, treat depressive relapses or recurrences, or alleviate noxious side effects (Howland and Thase, 1999a).

Lithium, buspirone (a 5-HT1A receptor agonist), pindolol (a beta-adrenergic receptor antagonist that also blocks presynaptic 5-HT1A receptors) and stimulant drugs have been used to accelerate the response to antidepressants (Howland and Thase, 1999a). With the exception of pindolol, which has been shown to be effective in some placebo-controlled studies (Bordet *et al.*, 1998), these approaches have not been adequately studied under controlled conditions.

Lithium is the most extensively studied and best established augmentation strategy for treatment-resistant depression (Bauer and Dopfmer, 1999; Baumann *et al.*, 1996). Augmentation with many other drugs is commonly done, but has little empirical support (Thase *et al.*, 1998). These include the use of buspirone, pindolol, anticonvulsants such as valproate, atypical antipsychotics such as risperidone and olanzapine, the analgesic tramadol (which also inhibits the reuptake of serotonin and norepinephrine), the opioid buprenorphine, and psychostimulants such as methylphenidate, pemoline and modafanil (Bodkin *et al.*, 1995; Howland and Thase, 1999a; Menza *et al.*, 2000; Shelton *et al.*, 2001b). Combining different antidepressants (e.g. SRIs with TCAs, bupropion, mirtazapine or nefazodone) also has been a common clinical practice for treatment-resistant depression. Psychotic depression does not respond well to antidepressant monotherapy (with the exception of amoxapine), and combining antidepressants and antipsychotics is considered the treatment of choice (Wheeler Vega *et al.*, 2000). Many of these augmentation strategies or drug combinations have been employed in the management of patients who have depressive relapses or recurrences while taking antidepressants, but none have been sufficiently studied to clearly demonstrate their efficacy (Howland and Thase, 1999a).

Psychostimulant drugs, because they affect dopamine and norepinephrine transmission, may be useful in treating the apathy and sexual dysfunction that sometimes occur during antidepressant treatment, especially with the SRIs, but have not been well studied for this indication (Howland and Thase, 1999a). These drugs include modafanil, methylphenidate, pemoline, amphetamine, amantadine, bromocriptine, pramipexole, ropinirole and pergolide (Zajecka, 2001). Other drugs that have been used for treating antidepressant-associated sexual dysfunction include cyproheptadine, yohimbine, buspirone, bethanechol, bupropion, mirtazapine and nefazodone (Ferguson *et al.*, 2001).

Novel Drug Therapies for Depression

Abnormal regulation of the hypothalamic–pituitary–adrenal (HPA) axis, leading to persistent elevations in cortisol levels, has been implicated in the pathophysiology of depression (Reus *et al.*, 1997). A number of open-label and controlled studies of drugs that suppress or inhibit HPA axis function, such as dexamethasone, aminoglutethimide, metyrapone, ketoconazole and mifepristone, have shown promise in the treatment of major depression, including refractory depression (Belanoff *et al.*, 2001; Brown *et al.*, 2001; Murphy *et al.*, 1993, 1998; Thakore and Dinan 1995; Wolkowitz *et al.*, 1999). Other drugs acting on the HPA axis (e.g. CRH or glucocorticoid receptor antagonists) are currently being developed for study in depression and other psychiatric disorders (McQuade and Young, 2000).

Several lines of evidence have suggested an important role for gonadal and adrenal steroid hormones in mood regulation. Women are especially vulnerable to mood disturbances during premenstrual, post-partum and perimenopausal periods (Epperson *et al.*, 1999). There also is evidence that mood disturbances may be associated with decreased steroid hormone levels in men (Margolese, 2000; Seidman and Walsh, 1999). In addition, steroid hormones act on specific receptors in the brain and affect neuronal function and neurotransmission. Considerable evidence suggests that oestrogen may improve mild mood symptoms in perimenopausal women (Epperson *et al.*, 1999; Joffe and Cohen, 1998), but there is less consistent evidence that oestrogen is an effective monotherapy for major depression (Halbreich, 1997; Morrison and Tweedy, 2000; Saletu *et al.*, 1995). There also is inconsistent evidence of the antidepressant effects of testosterone in men (Grinspoon *et al.*, 2000; Seidman *et al.*, 2001). Several recent placebo-controlled studies, however, found that oestradiol was effective for post-partum and perimenopausal major depression, suggesting important mood differences among oestrogen preparations (Ahokas *et al.*, 2001; De Novaes-Soares *et al.*, 2001). Some, but not all, studies have found that oestrogen or testosterone may augment the effects of antidepressant drugs in major depression (Amsterdam *et al.*, 1999; Schneider *et al.*, 1997; Seidman and Rabkin, 1998; Shapira *et al.*, 1985). Finally, there is developing evidence that adrenal steroid hormones such as dehydroepiandrosterone (DHEA) have significant antidepressant effects in older men and women (Bloch *et al.*, 1999).

Thyroid hormone also has been studied and used in the treatment of depression, especially as an augmentation strategy in refractory depression (usually triiodothyronine; T3) (Thase *et al.*, 1998).

The antidepressant use of thyroid hormone is based on the known association between depression and hypothyroidism as well as evidence that the hypothalamic–pituitary–thyroid axis affects neuronal function and neurotransmission in the brain (Howland, 1993). Thyroid augmentation has been studied almost exclusively in TCA non-responders, and these studies have suggested that it may be more effective in women, perhaps because of their higher risk for thyroid disease (Howland and Thase, 1999a).

Melatonin is a pineal gland hormone that regulates circadian sleep cycles. Although melatonin has not been adequately investigated in controlled trials of major depression, some evidence suggests that it has antidepressant effects (Wetterberg, 1998). Moreover, it also may be a clinically useful adjunct together with antidepressants, because of its potentially beneficial effects on disturbed circadian sleep–wake rhythms found in mood disorders (Wirz-Justice, 1995).

Although most psychotropic drugs have direct effects on neurotransmitter reuptake sites or neurotransmitter receptors, they may also have direct or indirect effects on second-messenger signal transduction systems, which ultimately mediate the synthesis of various protein products that lead to their therapeutic benefit (Chen *et al.*, 1999; Vaidya and Duman, 2001). Inositol, a precursor of the phosphatidyl-inositol second-messenger system, has been found effective in unipolar and bipolar depression in some, but not all, studies (Chengappa *et al.*, 2000; Levine *et al.*, 1995, 1999; Nemets *et al.*, 1999). Omega-3 fatty acids, which have an inhibitory effect on neuronal signal transduction systems, have been studied as a mood stabilizer in bipolar disorder. One recent study found that they had relatively greater antidepressant than antimanic effects (Stoll *et al.*, 1999).

Numerous herbs and plant extracts are known to have psychotropic effects, and historically many have been used therapeutically for the treatment of depression, anxiety and other emotional maladies (Fugh-Berman and Cott, 1999). Indeed, the use of herbs, nutritional supplements and other unorthodox homeopathic therapies has increased tremendously in recent years, but unfortunately many of these products have not been well studied with respect to their safety or efficacy. Two exceptions to this are studies of St John's wort and S-adenosylmethionine (SAMe) in the treatment of major depression. St John's wort (*Hypericum perforatum*), a wild flowering plant, has been used since ancient times to treat a variety of nervous conditions, and has become a very commonly used contemporary treatment for depression and anxiety. The most common adverse effects of St John's wort include photosensitivity, headache, dry mouth, dizziness, constipation and other gastrointestinal problems. St John's wort also is a potent inducer of hepatic metabolic enzyme systems, which may lead to decreased levels of various drugs (especially protease inhibitors, cyclosporine, theophylline, warfarin, digoxin and oral contraceptives). A meta-analysis of randomized trials in outpatients with mild to moderate levels of depression concluded that St John's wort was significantly superior to placebo and comparably effective to standard antidepressant drugs, although many of these trials were considered to have serious methodological flaws (Linde *et al.*, 1996). A recent large multicentre randomized study in patients with more severe and chronic symptoms of major depression did not find any difference between St John's wort and placebo (Shelton *et al.*, 2001a). Results from another large randomized study did not find any significant difference among St John's wort, sertraline, or placebo (Hypericum Depression Trial Study Group, 2002). The dietary supplement SAMe is an amino acid compound that is essential for methylation processes in the nervous system, which are important for neurotransmitter synthesis and second-messenger system function (Reynolds and Stramentinoli, 1983). The most common adverse effects are insomnia, nervousness, headaches and mild gastrointestinal problems, and it is not known to interact adversely with other drugs (Fava *et al.*, 1995). A meta-analysis of randomized trials in depressed outpatients found that SAMe was more effective than placebo and as effective as TCAs (Bressa, 1994), but it has not been compared to newer generation antidepressants and it has not been well studied in more severe forms of depression (Echols *et al.*, 2000).

Other Somatic Therapies for Depression

Electroconvulsive Therapy (ECT)

Historically, ECT has been considered the most effective treatment for major depression, and has also been shown to be highly effective for the treatment of severe melancholia, psychotic depression, bipolar depression, catatonia associated with mood disorders and treatment-resistant depression (Swartz *et al.*, 2001; Thase and Rush, 1995; Thase and Sachs, 2000; Wheeler Vega *et al.*, 2000). Because of its relative effectiveness, tolerability and safety, ECT is considered to be especially appropriate for geriatric patients and pregnant women, who might otherwise be at risk for adverse pharmacological effects (Kelly and Zisselman, 2000). ECT involves the passage of a brief electrical current through the brain to induce a generalized seizure lasting about 30–90 seconds, and is performed under general anaesthesia and muscle relaxation using short-acting drugs. A typical course of ECT consists of 6–12 treatments given three times weekly, although some patients might require a greater number of treatments. One of two electrode placements are used for ECT: unilateral non-dominant hemisphere placement or bilateral placement. Most patients are treated initially with unilateral ECT. Bilateral ECT usually is reserved for patients who have shown a minimal response after six unilateral treatments, or for patients with a history of a poor response to prior courses of unilateral ECT, a history of a positive prior bilateral ECT response, or extremely severe symptoms (Bailine *et al.*, 2000). Aside from the potential adverse effects and risks associated with general anaesthesia, the most common adverse effects of ECT include transient changes in heart rate and blood pressure, postictal and postanaesthetic confusion, memory impairment, muscle soreness, nausea and headaches. There is no convincing evidence that acute or long-term treatment with ECT causes brain damage. Increased efficacy and side effects of ECT are correlated with higher intensities of the electrical current, and efficacy and side effects are also somewhat greater with bilateral ECT (McCall *et al.*, 2000; Sackeim *et al.*, 2000). The acute clinical benefits of ECT are usually time-limited. For this reason, patients at risk for a depressive relapse should receive longer-term continuation treatment with pharmacotherapy or ECT after completing a course of acute ECT (Sackeim *et al.*, 2001a). However, patients who failed to respond to adequate trials of antidepressant medication prior to ECT have a higher risk of relapsing with pharmacotherapy after successful ECT and therefore should preferentially receive continuation treatment with ECT (Sackeim, 1994). Moreover, there is some evidence that continuation ECT is more effective than medications alone in preventing relapse and recurrence in depression (Gagne *et al.*, 2000).

Repetitive Transcranial Magnetic Stimulation (rTMS)

By contrast with ECT, rTMS is a novel non-invasive method for causing focal non-electrical stimulation of the brain (George *et al.*, 1999). In rTMS, a high-intensity electrical current is passed through an electromagnetic coil on the scalp. Rapidly turning the current on and off generates repetitive pulses of a magnetic field that can be focused on particular regions of the brain. Slow frequency stimulation with rTMS tends to inhibit neurons, whereas fast stimulation is excitatory. The most common adverse effect is headache, but seizures have been reported. Adverse cognitive effects of rTMS also are uncommon. Short-term placebo-controlled

(using sham rTMS) studies of fast rTMS focused on the left dorsolateral prefrontal cortex have found it effective in major depression (George *et al.*, 1997, 2000a), although there is evidence that slow rTMS focused on the right prefrontal cortex also is effective (Klein *et al.*, 1999). The results from studies using rTMS in treatment-resistant depression are mixed (Berman *et al.*, 2000; Loo *et al.*, 1999). A recent open study found that ECT may be somewhat more effective than rTMS, especially among patients with psychotic depression (Grunhaus *et al.*, 2000). Unfortunately, the long-term clinical efficacy, tolerability and safety of rTMS in mood disorders is unknown.

Vagus Nerve Stimulation (VNS)

The vagus nerve (cranial nerve X) is a parasympathetic nerve composed of afferent (carrying sensory information from the viscera) and efferent (regulating parasympathetic autonomic function) fibres. Animal studies found that VNS has behavioural and anticonvulsant effects, which led to the study and approved clinical use of VNS as a treatment for refractory partial-onset seizures in epilepsy (George *et al.*, 2000b). VNS involves the surgical implantation of a pacemaker-like programmable pulse generator, which is connected to and intermittently stimulates the left cervical vagus nerve. Studies in epilepsy patients also found that VNS had positive effects on mood symptoms. Brain imaging and neurochemical studies have shown that VNS activates various limbic regions and affects various neurotransmitters. This work led to interest in studying VNS in depression. An open-label pilot study using VNS in a group of very chronic and highly treatment-resistant patients with major depression reported a response rate of approximately 40% (Rush *et al.*, 2000). VNS was very safe and generally well tolerated, similar to its safety and side effect profile in epilepsy (Sackeim *et al.*, 2001b). Longer-term follow-up of these patients has found that the antidepressant response to VNS increases over time and continues to be well tolerated (Marangell *et al.*, 2002). Results of a recently completed large multicentre randomized controlled study of VNS in treatment-resistant major depression have not yet been reported.

Acupuncture

Acupuncture, like other complementary and alternative therapies, is becoming a more popular medical treatment for depression (Ulett *et al.*, 1998). Several controlled studies comparing 'active' acupuncture to 'sham' acupuncture and/or to antidepressant medication have reported some antidepressant benefit (Allen *et al.*, 1998; Luo *et al.*, 1998; Roschke *et al.*, 2000). Although these studies suffer from various methodological problems, the results suggest that acupuncture might be considered a safe and well tolerated alternative treatment for some depressed patients that warrants further study. More research clearly is needed to establish the safety and efficacy of acupuncture in major depression and other mood disorders. Moreover, because different procedures are used in the application of acupuncture, additional work is needed to determine the most effective acupuncture procedure in depression.

Psychosurgery

In the absence of effective pharmacological therapies, psychosurgery was widely accepted and commonly practised during the 1940s and 1950s for the treatment of schizophrenia, mood disorders and anxiety disorders. Although somewhat effective, the early surgical procedures were rather primitive (i.e. the prefrontal lobotomy crudely severed white matter connections between the prefrontal cortex and the rest of the brain) and had significant complications (e.g. bleeding, seizures, frontal lobe syndrome and death). However, the refinement of stereotactic neurosurgical methods, together with a better understanding of the neuroanatomical pathophysiology of psychiatric disorders, has led to more selective interventions with improved clinical outcomes and reduced morbidity and mortality. The most common currently performed neurosurgical procedures are anterior cingulotomy, subcaudate tractotomy, limbic leukotomy and anterior capsulotomy, which involve selective lesioning of particular brain regions using magnetic resonance imaging-guided stereotactic techniques. Psychosurgery is considered a treatment of last resort, for patients with chronic, severe and debilitating illnesses that have not responded to any available treatment. Short-term and long-term studies have found that one-third to two-thirds of patients with intractable depression show clinically significant improvement after psychosurgery, with low rates of surgical and neurological complications and minimal adverse effects on intellectual, personality and behavioural functioning (Binder and Iskandar, 2000; Spangler *et al.*, 1996).

Non-Somatic Therapies for Depression

Mood disorders are commonly characterized by clinical and EEG sleep disturbances. Hence, disturbed sleep is not only a symptom of depression, but may also reflect abnormal underlying biological processes that regulate sleep–wake cycles and are relevant to the pathophysiology of mood disorders (Wirz-Justice, 1995). Approximately 30–50% of depressed patients have a significant but transient antidepressant effect from total sleep deprivation, but this positive benefit is often lost with a full night of sleep (Wu *et al.*, 1999). Serial partial sleep deprivation, whereby patients stay awake daily from 2 a.m. to 10 p.m., has been used to achieve a more sustained antidepressant response, but this has not been especially effective. Sleep deprivation followed by pharmacotherapy may be more effective (Benedetti *et al.*, 1999; Smeraldi *et al.*, 1999). In addition, some evidence suggests that sleep deprivation may accelerate the response to antidepressant drugs and may be a useful augmentation strategy for antidepressant partial responders (Thase and Rush, 1995). Patients with seasonal depression or bipolar depression may be somewhat more sensitive to the antidepressant effects of sleep deprivation. Indeed, sleep deprivation may precipitate mania in patients with bipolar disorder.

Phototherapy (bright light therapy) was studied and introduced as a treatment for seasonal affective disorder, primarily winter depressions (Wirz-Justice, 1995). Phototherapy typically involves the daily morning use of 1–2 hours of bright light (2000 lux or more) (Terman *et al.*, 2001). The most common adverse effects are irritability and headache. Phototherapy also may be beneficial for patients with non-seasonal depression or bipolar depression (sometimes precipitating hypomania or mania), and has been used to accelerate or augment the effects of antidepressant drugs (Kripke, 1998; Thase and Rush, 1995). A positive response to sleep deprivation predicts a positive response to phototherapy, and the combination has been used to obtain a more sustained antidepressant effect (Colombo *et al.*, 2000; Fritzsche *et al.*, 2001). An alternative form of phototherapy is dawn simulation, which uses a low intensity light that gradually increases in illuminance before the subject awakens. This has been found to be as or more effective than bright light therapy in some studies (Avery *et al.*, 2001).

A novel approach in the treatment of winter depression is the use of a negative ion generator as an antidepressant therapy (Terman and Terman, 1995). In this method, an electronic negative ion generator is used to produce negative ions in the ambient air. Patients use the device at home for approximately 30 minutes. A

controlled trial comparing low-density and high-density negative ion generation found a greater antidepressant effect with the high-density treatment. This method is currently being compared to phototherapy and dawn simulation in a randomized trial of winter depression.

Clinical Issues in the Treatment of Depression

Given the availability of a wide variety of effective antidepressant therapies, how does one choose among these treatments? Because of the delay in onset of action of treatment, adherence to treatment is very important. Hence, patient preference is important to consider. Patients who strongly favour medication or psychotherapy should be offered treatment as such. The choice of psychotherapy will depend, of course, on the availability of a therapist trained in the use of a particular therapy model. The choice of medication also will depend on such factors as patient preference, past treatment history, family treatment history, clinical symptoms, health and side effect profile (Thase, 1997).

The goal of antidepressant treatment should be an optimal outcome; that is, the absence of any depressive symptoms along with a complete recovery of psychosocial function. This type of outcome is referred to as remission. About 50% of patients will have a significant response to the first-choice treatment, which is defined as a 50% or greater decrease in their symptoms of depression. Of these responders, however, only about one-half to one-third attain a full remission. Hence, a significant number of depressed patients are left with residual or persistent symptoms despite adequate antidepressant treatment (Rush and Trivedi, 1995). For these patients, switching to an alternative antidepressant drug or therapy, or combining different antidepressant treatments (e.g. pharmacotherapy and psychotherapy) may lead to a full remission.

The treatment of depression, whether with pharmacotherapy or psychotherapy, is now provided in three phases, each having different therapeutic goals (Thase and Kupfer, 1996). The acute phase, which typically lasts 6–12 weeks, refers to the initial treatment period, where the treatment is administered with the goal of full symptom remission. The continuation phase, which typically lasts 4–9 months, follows acute phase treatment and is recommended for all patients. The goal of continuation phase treatment is to prevent early relapse and to allow further symptomatic and psychosocial functional improvement. The goal of maintenance phase treatment is to prevent further recurrences of depression among patients who have a high risk of developing depression again. Such risk factors include three or more previous episodes of major depression, chronic depression, residual or persistent symptoms despite adequate treatment, and severe or disabling episodes of depression. The length of maintenance treatment will depend on the number of risk factors, and may range from a year to even life-long treatment.

For patients who do not have a satisfactory response to their initial antidepressant treatment, various strategies have been advocated (Thase and Rush, 1995). Switching to a different treatment (e.g. an alternative antidepressant drug or psychotherapy) is recommended for patients who have shown no response at all or for those who have intolerable side effects (Howland and Thase, 1999a). Augmentation (the strategy of adding a second medication to an antidepressant drug) may be most useful in patients who have shown a partial response to treatment (Thase *et al.*, 1998). Although combining pharmacotherapy and psychotherapy is commonly recommended, there is no clear evidence that the combination is more effective than either modality alone in the treatment of uncomplicated depressions (Thase *et al.*, 1997). Complicated depressions (e.g. very severe depression, treatment-resistant depression, chronic forms of depression, and partially remitted depression), however, may respond better to the combination of psychotherapy and pharmacotherapy (Fava *et al.*, 1997; Keller *et al.*, 2000; Paykel *et al.*, 1999; Thase *et al.*, 2001b).

TREATMENT OF BIPOLAR DISORDER

Antimanic Drugs

Lithium

Compared to other antimanic therapies (McElroy and Keck, 2000; Sachs and Thase, 2000), lithium is the best studied short-term and long-term treatment for bipolar disorder in adults (Baldessarini and Tondo, 2000; Maj, 2000) and in children and adolescents (Kowatch *et al.*, 2000). There is some evidence that patients with mixed episodes (mania together with depression) or a rapid-cycling pattern of illness may be less responsive to lithium (Montgomery *et al.*, 2000; Post *et al.*, 2000), but this has not been definitively proven (Baldessarini *et al.*, 2000). Although its antimanic effects are relatively greater than its antidepressant effects, it is the best studied and established pharmacological treatment for bipolar depression (Nemeroff *et al.*, 2001; Zornberg and Pope, 1993), and is less likely than antidepressants to be associated with the development of hypomania, mania, mixed states or rapid-cycling (Montgomery *et al.*, 2000; Post *et al.*, 2001; Thase and Sachs, 2000). There also is clear evidence that long-term treatment with lithium is associated with a reduced risk of suicide in bipolar and unipolar disorders (Coppen, 2000; Tondo and Baldessarini, 2000), an effect that has not been demonstrated with other antimanic therapies (Goodwin, 1999).

Although lithium has been used clinically for more than three decades, its precise mechanism of action is uncertain. Developing evidence, however, strongly suggests that lithium has direct and indirect effects on various components of second-messenger signal transduction systems, including G proteins, adenylyl cyclases, protein kinase C and the phosphoinositide cycle, which have been implicated in the neurobiology of bipolar disorder (Manji *et al.*, 1995, 1999). Typical adverse effects of lithium include gastrointestinal irritation, sedation, tremors, polyuria, polydipsia, oedema, acne, psoriasis and weight gain (Watson and Young, 2001). Careful serum level monitoring is required, as lithium toxicity can be serious (affecting cardiac, renal and central nervous system function) and potentially life threatening. Chronic lithium therapy is associated with the development of hypothyroidism, which is easily treated with thyroid hormone replacement. Lithium treatment is associated with teratogenic effects (i.e. cardiac anomalies). With careful clinical monitoring, lithium can be used safely with anticonvulsants, antipsychotics and antidepressants, but adverse interactions can occur with drugs that affect renal functioning (e.g. diuretics, non-steroidal anti-inflammatory drugs, calcium channel antagonists and ACE inhibitors).

Anticonvulsant Drugs

Lithium has been the standard treatment for bipolar disorder for nearly 30 years, but a significant proportion of patients do not respond to or cannot tolerate it (Watson and Young, 2001). As a result, various anticonvulsant drugs have been actively investigated and are increasingly used in clinical practice in the treatment of bipolar disorder (Dunn *et al.*, 1998; Keck *et al.*, 2000; Sachs *et al.*, 2000). There is now abundant evidence of the effectiveness of two anticonvulsants, divalproex and carbamazepine (Dunn *et al.*, 1998; McElroy and Keck, 2000). Divalproex is now considered by many physicians to be the most appropriate first-line treatment. For other patients, these anticonvulsants are useful second-line

or adjunctive treatments for patients who are intolerant of or do not respond to lithium (e.g. those with rapid-cycling or mixed states). Long-term treatment with anticonvulsants has not been as well studied as lithium in adults (Bowden *et al.*, 2000b; Sachs and Thase, 2000) or in children and adolescents (Kowatch *et al.*, 2000). More recently, a variety of newer anticonvulsant drugs have become available for clinical use, including lamotrigine, topiramate, gabapentin, oxcarbazepine, tiagabine, zonisamide and levetiracetam (Tatum *et al.*, 2000). Among these, lamotrigine, topiramate and gabapentin have been of particular clinical and research interest as potentially promising treatments for bipolar disorder (Dunn *et al.*, 1998). Surprisingly, oxcarbazepine, which is a better tolerated pharmacological analogue of carbamazepine, has been studied more extensively than many other anticonvulsants in the treatment of bipolar disorder, but is not yet commonly used in clinical practice (Dunn *et al.*, 1998; Teitelbaum, 2001). Tiagabine, zonisamide and levetiracetam, structurally and pharmacologically novel drugs unrelated to other anticonvulsants, have not been systematically investigated in bipolar disorder (Dunn *et al.*, 1998; McElroy and Keck, 2000; Tatum *et al.*, 2000). Like lithium, the therapeutic mechanism of action of anticonvulsants in bipolar disorder is uncertain. Recent studies, however, have suggested that carbamazepine, divalproex and perhaps other anticonvulsant drugs have direct and indirect effects on various components of second-messenger signal transduction systems (Chang *et al.*, 2001; Manji *et al.*, 1996, 1999).

Divalproex has been intensively studied in the treatment of bipolar disorder. Double-blind placebo-controlled studies have found it to be as effective as lithium in acute mania (Bowden *et al.*, 1994). Divalproex has not been as well studied in the long-term treatment of bipolar disorder, but there is evidence from a number of open studies that it has prophylactic benefits (Bowden *et al.*, 2000b; Sachs and Thase, 2000). The results from a recently completed double-blind placebo-controlled study suggested that it was somewhat more effective than lithium on some clinical measures, especially with regard to depressive symptoms, but there were no overall significant differences in outcome among patients treated with divalproex, lithium or placebo (Bowden *et al.*, 2000a). Open-label studies have shown it to be effective in bipolar depression, although it likely has relatively greater antimanic than antidepressant effects and is less likely than antidepressants to be associated with the development of hypomania, mania, mixed states or rapid-cycling (Montgomery *et al.*, 2000; Thase and Sachs, 2000; Young *et al.*, 2000). Mixed states, rapid-cycling and a poor lithium response tend to predict a positive response to divalproex (Swann *et al.*, 1997). The most common dose-dependent side effects are nausea, sedation and tremor. Transient hair loss can occur, and weight gain is common with long-term use. Blood dyscrasias, typically thrombocytopaenia, are rare. Mild transient hepatic enzyme elevations may occur, but hepatotoxicity is rare. Reports of severe hepatitis have been described primarily among young children with epilepsy taking valproic acid together with other anticonvulsants. There is some concern that women treated with divalproex may be at risk for developing polycystic ovarian syndrome, based on studies in epilepsy, but this has not been clearly demonstrated in mood disorder patients. Divalproex treatment is associated with teratogenic effects (i.e. neural tube defects) (Ferrier, 2001). In general, divalproex appears to be better tolerated than lithium or carbamazepine. Drug interactions are less common with divalproex compared to carbamazepine, because it is a weak inhibitor of hepatic enzymes, but it can increase serum levels of carbamazepine, lamotrigine and TCAs. Aspirin and naproxen can increase levels of divalproex, whereas carbamazepine can decrease its levels. Therefore, use of these drugs together with divalproex may require dose adjustments. Divalproex also can be used safely and effectively in combination with lithium, other anticonvulsants, antipsychotics and antidepressants.

Carbamazepine has been the most extensively studied anticonvulsant in bipolar disorder, although it has not been formally approved for this indication (McElroy and Keck, 2000). Double-blind placebo-controlled studies have found it to be effective in the acute and prophylactic treatment of mania (Keck *et al.*, 2000). Most controlled comparative studies have found that carbamazepine is as effective as lithium, though some have found it slightly less effective (Bowden *et al.*, 2000b). The results from controlled studies also have shown that it is effective in bipolar depression (Thase and Sachs, 2000). Similar to lithium, however, it may be relatively more effective in mania than in depression (Sachs *et al.*, 2000). Patients with mixed mania, rapid-cycling or a poor response to lithium often respond well to carbamazepine (Montgomery *et al.*, 2000). Because carbamazepine induces its own metabolism, periodic dose increases may be needed during treatment. Serum levels may therefore be especially useful to assess compliance, distinguish between poor and rapid drug metabolizers, and maintain patients at a steady-state level during long-term treatment. Sedation, dizziness, ataxia and diplopia are the most common dose-dependent side effects; weight gain frequently occurs during long-term use. Many patients develop benign skin rashes, mild leukopaenia or transient hepatic enzyme elevations, but the Stevens–Johnson syndrome, agranulocytosis, aplastic anaemia and hepatitis are rare complications. Although monitoring blood counts is recommended, this is not needed as frequently as has been suggested in the past. Carbamazepine treatment is associated with teratogenic effects (Ferrier, 2001). Because carbamazepine induces hepatic enzymes, drug interactions are common. It can significantly decrease levels of other anticonvulsants (including divalproex and lamotrigine), antipsychotics, benzodiazepines, TCAs and oral contraceptives. Erythromycin, cimetidine, divalproex, fluoxetine, fluvoxamine and calcium channel antagonists can increase carbamazepine levels. Therefore, concurrent use of these drugs with carbamazepine may require dose adjustments. Carbamazepine has been found to be relatively safe and often effective when combined with lithium or valproate, although the combinations are more likely to cause side effects than monotherapy with any of these drugs alone.

Lamotrigine has pharmacological effects that are very different from those of divalproex and carbamazepine (McElroy and Keck, 2000). The published literature on the use of lamotrigine in bipolar disorder is limited compared to divalproex and carbamazepine, but is very promising. Open-label reports have found that lamotrigine is effective alone or in combination with other antimanic drugs in patients with bipolar disorder, including many who were treatment-resistant or had rapid-cycling (Calabrese *et al.*, 1999a). Double-blind placebo-controlled studies have also found it effective in bipolar disorder (Frye *et al.*, 2000), especially in bipolar depression (Calabrese *et al.*, 1999b) and rapid-cycling (Calabrese *et al.*, 2000). The most common adverse effects are nausea, headache, dizziness, ataxia and diplopia. A benign rash occurs in approximately 10% of patients, and more severe rashes occur in 0.5–1.0%; the Stevens–Johnson syndrome also has been reported. Rashes tend to occur more commonly when higher starting doses are used or the dose is rapidly increased, and when it is used together with divalproex (which inhibits its metabolism). Lamotrigine does not affect hepatic metabolism. Carbamazepine can increase its metabolism, while divalproex decreases it.

Topiramate is a structurally and pharmacologically novel drug unrelated to other anticonvulsants (McElroy and Keck, 2000). The published literature on the use of lamotrigine in bipolar disorder is very limited compared to divalproex, carbamazepine and lamotrigine. Open-label reports have found that lamotrigine may be effective in combination with other antimanic drugs in patients with bipolar disorder, including many who were treatment-resistant (Chengappa *et al.*, 1999; Marcotte, 1998; McElroy, *et al.*, 2000). Unfortunately, the results from one double-blind placebo-controlled study reported only limited benefit with topiramate in mania

(Chengappa *et al.*, 2001). The most common adverse effects are somnolence, dizziness, anxiety, ataxia, speech difficulties, cognitive impairment, anorexia and weight loss.

Gabapentin also has pharmacological effects that differ from all other anticonvulsants. The published literature on the use of gabapentin in bipolar disorder is limited to case reports, uncontrolled open-label studies and two placebo-controlled trials. The open-label reports found that gabapentin was effective alone or in combination with other mood stabilizers in about 60–90% of patients, including those with treatment-resistant or rapid-cycling bipolar disorder (Ghaemi and Goodwin, 2001; McElroy and Keck, 2000). However, the double-blind studies did not find a significant drug–placebo difference (Frye *et al.*, 2000; McElroy and Keck, 2000). Sedation, dizziness and ataxia are the most common adverse effects. Gabapentin is not metabolized, does not affect hepatic metabolism, does not have significant drug interactions, and is not associated with hepatic or haematologic abnormalities. Because it is renally excreted, lower doses may be required in patients with renal disease, but serious toxicity in such patients is unlikely compared to lithium. The combination of gabapentin and other psychotropic drugs is generally well tolerated.

Antipsychotic Drugs

Typical Antipsychotics

For many years, the use of the older typical antipsychotic drugs has been a common clinical practice for the treatment of acute mania and psychotic mania (Keck *et al.*, 2000; Sachs and Thase, 2000). These drugs have a relatively rapid effect on manic and psychotic symptoms, psychomotor agitation and sleep. Most controlled studies have been conducted using chlorpromazine or haloperidol, which had antimanic effects comparable to lithium, divalproex and carbamazepine (McElroy and Keck, 2000). Typical antipsychotics are associated with parkinsonian side effects, akathesia and tardive dyskinesia. They also may be associated with the development of depression in some patients. One advantage of typical antipsychotics is that they are generally considered to be safe for use during pregnancy, and would be an appropriate treatment alternative to lithium or anticonvulsants in pregnant women with bipolar disorder. Another potential advantage is that two of the typical antipsychotics (haloperidol and fluphenazine) have long-acting depot formulations, which may be useful for maintaining compliance during long-term treatment for some patients (Sachs and Thase, 2000).

Atypical Antipsychotics

Because of their clinical effects and side effect profile, atypical antipsychotics have gradually supplanted use of the typical antipsychotics, even in the treatment of bipolar disorder (Sachs *et al.*, 2000). They are less likely to cause extrapyramidal side effects or tardive dyskinesia, and they appear to have more positive effects on mood (Dunayevich and McElroy, 2000; Ghaemi and Goodwin, 1999; Guille *et al.*, 2000). Olanzapine is the best studied and most established atypical antipsychotic for the acute treatment of mania (Bhana and Perry, 2001), and also may be effective for mixed states and rapid-cycling (Meehan *et al.*, 2001; Tohen *et al.*, 1999, 2000). There is evidence from controlled trials that risperidone is effective in the acute treatment of mania when added to ongoing antimanic drugs, but it has not been studied as a monotherapy in placebo-controlled trials (McElroy and Keck, 2000; Sachs *et al.*, 2000). Small open-label reports have suggested some benefit for quetiapine in treatment-resistant bipolar disorder, but there are no controlled studies (Dunayevich and Strakowski, 2000; Ghaemi and Katzow, 1999; Zarate *et al.*, 2000). One randomized double-blind placebo-controlled study of ziprasidone found it effective in bipolar patients with mania or mixed episodes (McElroy and Keck 2000). Clozapine has been reported in open-label studies to be effective in bipolar disorder, especially in treatment-resistant cases, but has not been studied in controlled trials (Dunayevich and McElroy, 2000; Ghaemi and Goodwin, 1999; Guille *et al.*, 2000). The clinical use of clozapine is limited, however, by its side effect profile and the risk of agranulocytosis. Compared to lithium and some anticonvulsants, none of the atypical antipsychotics has been well studied for long-term treatment (Bowden *et al.*, 2000b; Sachs and Thase, 2000; Vieta *et al.*, 2001). Sedation and weight gain are a problem with some of these drugs (e.g. olanzapine, quetiapine and clozapine), although ziprasidone is associated with weight loss. These drugs also are generally well tolerated when combined with other psychotropic drugs.

Novel Drug Therapies for Bipolar Disorder

Considerable evidence suggests that lithium and anticonvulsant drugs have direct and indirect effects on various components of second-messenger signal transduction systems, which may underlie their therapeutic mechanism of action (Chen *et al.*, 1999). As a result, other drugs that affect second-messenger systems have been studied in bipolar disorder. Calcium channel antagonists, which affect intracellular calcium metabolism, have been studied in bipolar disorder (Hollister and Garza Trevino, 1999; Post *et al.*, 2000), with some evidence of efficacy using nimodipine in rapid-cycling (Pazzaglia *et al.*, 1998), but mixed results with verapamil (Dose *et al.*, 1986; Janicak *et al.*, 1998). Omega-3 fatty acid, which has an inhibitory effect on neuronal signal transduction systems, has been studied as a mood stabilizer in bipolar disorder, but may have relatively greater antidepressant than antimanic effects (Stoll *et al.*, 1999). A small open-label study of the protein kinase C inhibitor tamoxifen showed a significant antimanic effect (Bebchuk *et al.*, 2000).

Various cholinomimetic drugs, which stimulate cholinergic pathways (Cummings, 2000), have been found in small open-label studies to have antimanic effects, including physostigmine, RS 86, and donepezil (Burt *et al.*, 1999; Davis *et al.*, 1978; Krieg and Berger, 1986). Other studies using choline or phosphatidylcholine, which are precursors of acetylcholine, also have suggested some antimanic effects (Leiva, 1990; Stoll *et al.*, 1996). These approaches may work in the treatment of mania by readjusting the hypothesized 'adrenergic-cholinergic imbalance'. Alternatively, these drugs may work by inhibiting phosphatidylcholine second-messenger systems (Chen *et al.*, 1999).

A number of reports have suggested that the adjunctive use of high doses of thyroid hormone may be effective in treatment-resistant bipolar disorder, including rapid-cycling, refractory mania and intractable depression (Bauer and Whybrow 1990; Bauer *et al.*, 1998; Baumgartner *et al.*, 1994).

Other Somatic Therapies for Bipolar Disorder

As discussed previously, ECT and rTMS have demonstrated efficacy in the treatment of major depression, including bipolar depression. Surprisingly, there is some evidence that these treatments also are effective in the treatment of mania. A double-blind controlled trial of right versus left prefrontal cortex fast rTMS found greater antimanic effects with right-sided stimulation, which would be consistent with the known antidepressant effects of left-sided stimulation (Grisaru *et al.*, 1998). ECT also has been used in the treatment of refractory mixed states and rapid-cycling, as well as catatonia associated with bipolar disorder (Keck *et al.*, 2000; Mukherjee *et al.*, 1994). Finally, sleep deprivation and phototherapy have been used as adjunctive therapies for rapid-cycling (Sachs *et al.*, 2000).

Clinical Issues in the Treatment of Bipolar Disorder

Bipolar disorder is a chronically recurrent phasic illness. As a result, acute treatments often are phase-specific (Sachs *et al.*, 2000). For acute bipolar mania, lithium, anticonvulsants and antipsychotics are clearly effective. High-potency benzodiazepines (such as lorazepam or clonazepam) or low-potency sedating antipsychotics (such as chlorpromazine or thioridazine) can be used adjunctively to promote sleep, which will have an additional therapeutic antimanic effect. For refractory mania, antimanic drug combinations, atypical antipsychotics, novel anticonvulsants, calcium channel antagonists, cholinomimetics, omega-3 fatty acids, thyroid hormone and ECT can be useful.

For bipolar depression, lithium and anticonvulsants can have antidepressant effects. They are the preferred initial treatment because they are less often associated with the development of hypomania, mania, mixed states or rapid-cycling. Antidepressant drugs can be effective, but may induce hypomania, mania, psychosis, mixed states or rapid-cycling (Altshuler *et al.*, 1995). Although there is some clinical evidence that the risk of antidepressant-induced mania may differ among antidepressant drugs, this has not been clearly established (Boerlin *et al.*, 1998; Howland, 1996b). The dose and/or serum level of antimanic drugs should be optimized if antidepressants are used. For treatment-resistant bipolar depression, atypical antipsychotics, dopamine agonist drugs, sleep deprivation, phototherapy and ECT are potentially effective alternatives. Mixed states and rapid-cycling, which are more common in women than in men, predict a poorer response to most treatments and a poorer long-term prognosis (Montgomery *et al.*, 2000). In these situations, anticonvulsants, atypical antipsychotics, thyroid hormone, calcium channel antagonists, sleep deprivation, phototherapy and ECT may be effective, along with minimizing or avoiding the use of antidepressants.

Virtually all patients with bipolar disorder will require longer-term continuation and maintenance therapy to prevent relapse or recurrence of mood episodes (Sachs and Thase, 2000). For many patients, it is prudent to continue acute treatments indefinitely if their tolerability and efficacy are acceptable. Changes in symptoms (such as depression, hypomania or psychosis) during long-term therapy can usually be effectively managed by careful titration of ongoing antimanic, antipsychotic and/or antidepressant treatments. Significant symptoms unresponsive to titration of current therapies can be treated by adding an appropriate symptom-specific therapy. Because of the inherent mood instability in bipolar disorder, however, medication changes or additions should be done gradually while closely monitoring the patient's clinical condition. For example, adding or discontinuing antidepressant drugs are both associated with the development of mania (Goldstein *et al.*, 1999). Therefore, adjusting the antidepressant dose (rather than abruptly stopping the drug) while maximizing the antimanic drug dose and/or serum level would be an appropriate strategy for managing antidepressant-associated mania.

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XIX

Anxiety Disorders

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XIX-1

Animal Models of Anxiety Disorders

Frederico G. Graeff and Hélio Zangrossi Jr

INTRODUCTION

The last decades have witnessed a growing interest in the use and development of animal models in psychiatry. The demand for these models has been motivated by the ethical, methodological and economical constraints that restrain the study of neurobiological processes in human subjects. It is undeniable that animal experimentation has contributed to advances in psychopharmacology and to our understanding of brain mechanisms involved in psychiatric disorders. Suffice to remember that benzodiazepine anxiolytics have been discovered because of the taming effect of chlordiazepoxide observed in *Cynomolgus* monkeys by Randall (Randall *et al.*, 1960). Also, hypotheses on the role of serotonin (5-HT) in anxiety were based on experimental work with conflict tests in rats and pigeons (Robichaud and Sledge, 1969; Graeff and Schoenfeld, 1970; Stein, Wise and Berger, 1973) as well as with aversive electrical stimulation of the rat dorsal periaqueductal grey matter (DPAG) (Kiser and Lebovitz, 1975; Schenberg and Graeff, 1978, Schütz, de Aguiar and Graeff, 1985).

Early animal models of anxiety were developed when behaviourism was the main conceptual framework within experimental psychology, and before classifications of psychiatric disorders split pathological anxiety into distinct nosological entities (see later). These animal models of anxiety rely on either inhibition of ongoing behaviour elicited by conditioned stimuli that predicted unavoidable electric shock or on suppression of rewarded responding by electric-shock punishment. The first type of model is based on principles of associative or Pavlovian conditioning, and the second on instrumental or operant conditioning. Punishment tests also suggest clinically derived constructs that emphasize the role of inner conflict in pathological anxiety, and this may be the reason why these tests became known as conflict models. Early pharmacological analysis showed conflict models to have a higher predictive value than conditioned suppression and, as a result, punishment tests became paradigmatic for assaying anti-anxiety drugs (Kelleher and Morse, 1968).

Surprisingly, classical conflict tests failed to consistently detect the anxiolytic action of drugs that act primarily on the neurotransmission mediated by serotonin (5-HT), such as buspirone and ritanserin (Handley *et al.*, 1993; Griebel, 1995). Such false-negative results undermined the general confidence in conflict models, although many arguments may be summoned to their defence, as for instance the time course of drug action. Unlike benzodiazepine anxiolytics, the newer drugs need several weeks of continuous administration to become clinically effective, initial doses being sometimes anxiogenic (Nutt, 1991). Why, then, should one expect single administration of these agents to be anxiolytic in animal models? In spite of this, it became generally accepted that conflict tests were good only for anxiolytics that acted primarily on the neurotransmission mediated by γ-aminobutyric acid (GABA), which is the case for barbiturates and benzodiazepines.

A theoretical shift from behaviourism to ethology has also contributed to the discredit of conflict models, which have been criticized because of their artificiality and the confounding influence of appetitive drives, such as hunger and thirst, and of pain (Treit, 1985). As a result, a search for ethologically based animal models of anxiety has begun. The most widely used animal model of anxiety resulting from this trend has been the elevated 'X' or 'plus' maze, which is based on the natural fear that rats have for elevated, open spaces, represented by the two open arms of the apparatus (Handley and Mithani, 1984; Pelow and File, 1986). Countless drugs have been assayed in this model, and the results obtained summarized in several comprehensive reviews (e.g., Handley *et al.*, 1993; Rodgers, 1994; Griebel, 1995). Disappointingly, this model also failed to detect non-benzodiazepine anxiolytics. To do this, ethological analysis of behavioural items shown by the animals while exploring the elevated plus-maze has been added to the procedure (Cruz, Frei and Graeff, 1994; Rodgers and Johnson, 1995). However, with this modification one of the main advantages of the test, which is simplicity, is lost.

As these developments were taking place in basic research, the split of anxiety disorders into distinct diagnostic categories, a trend that was initiated by the DSM III classification of psychiatric disorders (American Psychiatric Association, 1980), became accepted worldwide. Even though present-day psychiatric classifications cluster symptoms empirically, they constitute a necessary starting point for systematic research. It is hoped that the evidence thus obtained will either validate or modify the original categories.

The following categories from the DSM IV classification of psychiatric disorders (American Psychiatric Association, 1994) will be considered in the present analysis. (1) Generalized anxiety disorder (GAD), a state of excessive anxiety or apprehension lasting for more than six months. Neurovegetative symptoms are often present, but relatively minor. (2) Panic disorder (PD), characterized by recurrent panic attacks, either unexpected or associated with particular situations. Panic attacks are sudden surges of intense fear or terror, desire of fleeing and feeling of imminent death, going crazy or loosing control. These subjective symptoms are accompanied by major neurovegetative changes, such as palpitation, hypertension, dyspnoea, difficulty in deep breathing, sweating, urge to void the bladder and increased peristalsis. This leads to worry about the next attack or anticipatory anxiety, and avoidance of places where a panic attack would be embarrassing. Ultimately, generalized avoidance or agoraphobia may ensue. Nevertheless, agoraphobia sometimes occurs without panic attacks. (3) Obsessive–compulsive disorder (OCD) characterized by intrusive, distressing thoughts (obsessions) and/or stereotyped or ritualized behaviour (compulsions) that must be performed in order to alleviate intense anxiety. (4) Specific phobias, which are irrational fears of either objects (animals, blood, pointed instruments) or situations (heights, closed environments). (5) Social

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phobia, or marked anxiety experienced in social situations, such as speaking in public or going to parties or class rooms.

Given this background, it is no longer acceptable to look for animal models of anxiety, in general. Instead, models that represent specific anxiety disorders are due. Animal models of psychopathology have been evaluated according to criteria of predictability of drug response, analogy or face validity, and homology or theoretical construct validity (Treit, 1985; Willner, 1991). As Willner (1991) pointed out, 'Earlier attempts to develop criteria for validating animal models of human behaviour have tended to be concentrated largely on the assessment of face validity. The identification of two further categories reflects two ways in which the literature has developed in recent years. First, there has been a considerable expansion in the literature dealing with the pharmacological exploitation of animal models, much of which contributes to the assessment of predictive validity. Second, there has been significant growth in our understanding of the psychological mechanisms underlying psychopathological states, and examination of construct validity provides a convenient way of bringing animal models into contact with this very relevant literature'.

Indeed, the predictive criterion alone may be insufficient to characterize a test as an animal model of anxiety, since correlation between drug response in the model and in the clinics may happen, despite differences in brain mechanisms of pathophysiology and drug effect (e.g., drug-induced catalepsy and schizophrenia). The analogy criterion in itself may also be misleading, since in different species similar behaviours often serve different adaptive functions and, conversely, distinct behaviours may lead to the same goal. For instance, rat probe-burying behaviour is attenuated by anxiolytics (Treit, 1985), although it does not resemble any symptom of GAD. However, this behaviour is one of the defensive strategies of the rat's repertoire, and thus may be theoretically related to anxiety (see later).

It may be concluded that only homology qualifies animal models as representative of psychopathologies. Yet this conclusion does not look very promising, considering the (purposefully) non-theoretical stance of present-day psychiatric classifications and the scant knowledge available on how psychiatric symptoms are generated and on the neural events that underlie different animal models of anxiety. Nevertheless, we have to face the challenge or, otherwise, are bound to create theoretically shallow animal models, which are likely to generate more noise than information.

In order to assess homology, theories that encompass both pathophysiology and animal behaviour indexes have to be constructed. In this regard, evolutionary psychology and psychiatry provide a sound theoretical framework (see, e.g., McGuire and Troisi, 1998). Charles Darwin himself laid the ground for this line of thought in his book *The Expression of Emotions in Man and Animals* (Darwin, 1872) by advocating that behavioural, no less than physical, characteristics would be acquired as a result of selective pressure exerted by biological evolution. This view justifies the use of animals belonging to the phylogenetic line that resulted in humans to study the neurobiology of psychological phenomena, to the extent that brain properties be conserved along evolution. Since several environmental constraints are similar across species, or even genera and families, many adaptations are species-general, among which stand the basic emotions. Although emotional expression varies from one species to another, functional behavioural classes, such as escape and avoidance from danger or approach sources of food, remain the same and constitute the basis for the classification of basic emotions (Nesse, 1990). No wonder that the neural substrate of such emotions is conserved along biological evolution. On this basis, Panksepp proposed that basic emotions, such as rage, fear, panic and expectancy, are represented by inborn neural networks that co-ordinate behavioural strategies allowing animals to deal with enduring environmental (including social) challenges (Panksepp, 1982).

Fear and anxiety are emotional states with high adaptive value, rooted as they are in defensive reactions displayed in situations of threat to the physical integrity or survival of the organism. The sources of danger are manifold, including predators, environmental stimuli or situations, such as height, illumination, painful stimuli, novel places or objects, and confrontation or competition with animals from the same species. To deal with these challenges, animals generally use one of four basic defensive strategies, namely escape, immobilization, defensive attack and submission (Adams, 1979; Marks, 1987; Blanchard and Blanchard, 1988). The decision to choose one particular strategy takes into account several factors, such as the characteristics of the environment, distance from the threatening stimulus and previous experience with the stimulus and/or environment. Thus, in a highly discernible situation of threat, as in the confrontation with an approaching predator, the animal will preferentially escape, if an exit route is available. In turn, the animal will become tensely immobile (freezing) if the predator is not in close proximity and/or escape is impossible. In this case, being motionless decreases the probability of detection or attack by the predator. On the other hand, in a situation of close proximity or contact with the predator, vigorous flight or defensive threat and attack toward the predator may be more efficient in deflecting predatory action. Alternatively, certain species, including birds, fishes and some mammals, may display a state of tonic immobility, feigning death, by means of which the animals may decrease the predator's interest (Marks, 1987). Finally, submission postures are often observed in confrontation with conspecifics. By acting submissively, the individual may inhibit the attack by a socially dominant animal. However, submissive responses hardly ever inhibit predatory attack (Adams, 1979).

The evolutionary approach views anxiety disorders as pathologies of defence (Panksepp, 1982; Marks, 1987; Marks and Nesse, 1994; Graeff, 1994; Hofer, 1995; Stein and Bouwer, 1997; Gray and McNaughton, 2000; Blanchard *et al.*, 2001). For instance, specific phobias, GAD and PD may be related to predatory aggression. In this regard, the work carried out by Robert and Caroline Blanchard on predatory defence in rats and mice (Blanchard and Blanchard, 1988; Blanchard, Griebel and Blanchard, 1997) classified the behavioural strategies displayed according to the level of threat, namely potential or uncertain, distal and proximal. The first level evokes cautious exploration and hesitant behaviour aimed at risk assessment. The second induces either directed escape or immobility. Finally, when the predator is very close or in contact with the prey, either vigorous flight or defensive aggression occurs. To this ethoexperimental analysis, Gray and McNaughton (2000) have added elements of learning theory, including conditioned stimuli that signal either punishment or loss of an expected reward (frustration) as elicitors of anxiety (first level of defence). The same authors have also highlighted the importance of approach-avoidance conflict and of the direction of response to distinguish anxiety from fear. Thus, anxiety exists only when there is a tendency to approach the source of danger. When only the escape or avoidance component exists there is fear. Indeed, pharmacological data show that active avoidance and escape responses are remarkably resistant to anxiolytic drugs, in contrast to risk-assessment and punished suppressed responding (Gray and McNaughton, 2000).

In a synthesis of experimental evidence from several research fields, the above levels of defence have been related to different emotions and to distinct anxiety disorders (Graeff, 1994; Gray and McNaughton, 2000). Table XIX-1.1 summarizes this view, which provides the theoretical framework presently used to qualify an animal model as representative of GAD, PD and specific phobias, respectively.

Following the same evolutionary approach, social phobia has been related to submissive displays that are expressed by subordinates in social interaction with dominant animals of the same

Table XIX-1.1 Levels of defence, main neural substrate, related emotion and anxiety disorder, and their drug sensitivity

Danger	Defence reaction	Critical brain structures	Emotion	Disorder	Drug sensitivity
Potential (Conflict)	Risk-assessment/behavioural inhibition	Septo-hippocampal system/amygdala	Anxiety	GAD	Anxiolytics Antidepressants
Anticipated (CS)	Freezing	Amygdala VPAG	Anticipatory anxiety		
	Avoidance	Amygdala	Conditioned fear	Specific phobias	None
Distal (US)	Escape	Medial hypothalamus	Unconditioned fear	Specific phobias	None
Proximal (US)	Flight/freezing	DPAG	Panic	PD	Antidepressants

Key: CS, conditioned stimulus; US, unconditioned stimulus; VAPG, ventral periaqueductal grey; DPAG, dorsal periaqueductal grey; GAD, generalized anxiety disorder; PD, panic disorder (Modified from Graeff, 1994 and Gray and McNaughton, 2000).

species (Stein and Bouwer, 1997). Nevertheless, to our knowledge this hypothesis has not been explored to generate animal models of this disorder.

Finally, the most frequent compulsive behaviours found in OCD, washing and checking, have been related to self-grooming and territorial checking routines that many animal species perform to protect themselves against micro-organisms and intruders, respectively (Rapoport, 1991).

The subsequent sections will focus on pharmacological evidence that is relevant to the validation of selected models of each psychiatric disorder. Because the aim of this article is to relate animal models and psychiatric disorders, only drugs that have been evaluated in clinical assays will be considered. Whenever pertinent, there will be mention of evidence from studies using direct intervention on the brain.

MODELS OF GENERALIZED ANXIETY DISORDER

As pointed out earlier, the ethoexperimental analysis of defence strategies against predatory attack made by the Blanchards (Blanchard and Blanchard, 1988; Blanchard *et al.*, 1997) led them to relate GAD to behaviours that occur in response to potential threat, chiefly the so-called risk-assessment behaviour. Gray and McNaughton (2000) also view 'behaviours that occur in the context of potential threat as paradigmatic of anxiety'. But they suggest that 'the critical feature for such behaviours was the approach-avoidance conflict inherent in the "potential threat" situation rather than the potentiality of threat as such'. They also broadened the notion of potentially threatening stimuli to include 'novelty stimuli of certain kinds, signals of punishment, and signals of reward omission, in addition to the paradigmatic innate anxiety stimuli'. Gray and MacNaughton (2000) have further suggested that anxiety has a cognitive component — approach-avoidance conflict — managed by the septo-hippocampal system, and an affective component — aversion — accomplished by the uppermost structure of the brain defence ('fight/flight/freezing') system, the amygdala.

It follows that conflict tests would rank first in homology regarding GAD, since they would engage both the septo-hippocampal system and the amygdala (Gray and MacNaughton, 2000). Because of the shortcomings analysed in the introduction of this paper, classical conflict tests are seldom used for assessing anxiolytic drug action. However, a modification of the latter, inhibitory avoidance in the elevated T-maze (Graeff, Viana and Tomaz, 1993), eliminates most of the objections raised against punishment models, since animals are not deprived, and aversion for an open, elevated arm of the maze replaces electric foot-shock. Also, the light-dark transition model (Crawley and Goodwin, 1980), in which rats or mice are placed inside an experimental chamber divided into two compartments, one bright and another dark, may be viewed as a conflict test: the naïve animal tends to explore both compartments of the box, but the entrance to one of them is punished by exposure to bright light.

Also at the first level of homology are models that involve risk-assessment behaviour determined by potential threat from innate fear stimuli, such as in the anxiety/defence test battery (Blanchard and coworkers, 1993, 2001) and a component of the mouse defence test battery (Griebel, Blanchard and Blanchard, 1996a).

In anxiety models such as conditioned suppression (Estes and Skinner, 1941) and fear-potentiated startle (Davis, 1986) an exteroceptive stimulus predicts unavoidable foot-shock, causing, respectively, suppression of rewarded responding and increased amplitude of the acoustic startle response. Therefore, there is no approach-avoidance conflict and according to Gray and McNaughton (2000) the septo-hippocampal system is not involved. Therefore, the behavioural inhibition seen in these situations would rely entirely on the amygdala. Indeed both the amygdala and the ventral periaqueductal grey (VPAG) have been shown to be critical for the expression of conditioned freezing (Davis, 1992; Fanselow, 1991). Since these tests activate the affective, but not the cognitive component of anxiety (Gray and McNaughton, 2000), they only partially fulfil the theoretical requirements for a model of GAD. It is more likely that these models represent anticipatory anxiety, which like GAD is also sensitive to anxiolytic drugs (Table XIX-1.1). A remark about fear-potentiated startle is in order. Although the anxiety index is the enhancement of the startle response, this is directly proportional to the degree of freezing produced by the conditioned aversive stimulus (Leaton and Borszcz, 1985). Therefore, fear-potentiated startle may be viewed as behavioural inhibition in disguise.

There are also anxiety models that involve defensive aggression — usually threat — provoked by an approaching danger stimulus. A typical example is the primate defensive reaction evoked by the experimenter's gloved hand. Despite the historical importance of this model — in particular the discovery of the anxiolytic action of chlordiazepoxide (Randall *et al.*, 1960) — they are little used today, due to its high cost and low feasibility. Nevertheless, a rodent model that measures defensive behaviour against a conditioned aversive stimulus, the shock-probe-burying test (Pinel and Treit, 1978) deserves attention.

Social interaction in young rats has a pharmacological profile suggestive of GAD (File, 1995). Nevertheless, it seems to involve many variables, and the theoretical construct underlying the test has not been fully clarified. Therefore, this model will not be addressed here.

In the following subsections, the main models of GAD are discussed in detail. The mouse defence test battery and the elevated T-maze will be analysed in a separate section, because these tests are intended to model PD, in addition to GAD.

The Anxiety/Defence Test Battery

The anxiety/defence test battery (A/DTB) was developed in order to measure drug effects on risk-assessment behaviours (Blanchard and coworkers, 1993, 2001). In this model, measures of risk-assessment and inhibition of non-defensive behaviour are evaluated in rats submitted to three situations representing potential threat associated with a predator. In the first situation, the 'proxemics/activity test', the rat's location in the test chamber is measured after non-contact exposure to a cat. It has been shown that after a 5-minute encounter with the predator, rats avoid locations near the cat compartment. In the second condition, the 'eat/drinking test', the frequency and duration of two non-defensive behaviours, feeding and drinking, are measured during and after a 5-minute non-contact exposure to a cat. In the presence of the predator these behaviours are inhibited, being gradually resumed over time. Finally, in the 'cat odour test', the frequency and duration of risk assessment expressed towards an object saturated with cat odour are measured. The indexes used are flat-back approach and stretch-attend posture, as well as the frequency and time spent by rats in direct contact with the cat odour source (for further details on these tests see Blanchard and coworkers, 1990, 1993).

The effects of different classes of anxiolytics in the A/DTB are reviewed elsewhere (Blanchard and coworkers, 1993, 1997, 1998). Overall, benzodiazepines and buspirone-like drugs decrease avoidance from the area close to the cat compartment and counteract inhibition of feeding and/or drinking after cat exposure. These drugs interfere with the expression of risk-assessment behaviours in a bi-directional way. They increase risk assessment when the baseline expression of these behaviours in the control group is low or absent, due to enhanced freezing behaviour. However, they decrease risk assessment in less aversive situations, in which a considerable level of risk assessment is already being expressed by the animals. In both cases the drugs decrease reactivity to the threatening situation, thus moving the animal to a level of defensiveness where approach to the aversive stimulus is more likely to occur. The non-selective 5-HT_2 antagonist ritanserin failed to show the same anxiolytic profile. Chronic administration of the tricyclic antidepressant imipramine caused anxiolytic effects similar to that of diazepam and chlordiazepoxide, but the panicolytic benzodiazepine alprazolam, administered acutely, had no effect. The full pharmacological validation of this test awaits further analysis since neither the effect of anxiogenic drugs nor the selectivity of the model have been explored.

The Light/Dark Transition Model

The light/dark transition model was elaborated by Crawley and Goodwin (Crawley and Goodwin, 1980; Crawley, 1985), based on the exploratory behaviour of mice in a two-compartment box, where one chamber is brightly lit and the other dark. In such conditions, mice and rats have a clear preference for the dark side of the box and the number of transitions made by them between the two compartments and the time spent in the bright side have been used as indices of anxiety. Over the years several procedural modifications have been introduced in order to improve the feasibility of the test or its pharmacological predictability. These modifications include, among others, the introduction of a connecting tunnel between the two compartments (De Angelis, 1992), changes in the dimensions or spatial disposition of the compartments (Imaizumi, Miyazaki and Onodera, 1994; Shimada *et al.*, 1995), use of fully automated data collection (Onaivi and Martin, 1989; Young and Johnson, 1991b; Hascoët and Bourin, 1998) and analyses of additional behavioural indices of anxiety and locomotor activity (Chaouloff, Durand and Mormede, 1997; Smythe *et al.*, 1998; Hascoët and Bourin, 1998).

When interpreting the pharmacological results so far obtained with the light/dark transition model, one should keep in mind that what poses as a simple procedural modification may change the way animals respond to the aversive challenge. As discussed before, the type of defensive response determines the effect of anxiety-modulating drugs in the test. For instance, increasing the illumination of the bright compartment may decrease the amount of risk assessment made by the animal in the transition area between the two compartments of the apparatus. Although this alteration may not necessarily lead to changes in the absolute amount of time spent in the two compartments, it may interfere with the expression of an important set of GAD-related behaviours. Moreover, when comparing results of different studies it is important to notice that in this test, as in other anxiety models, uneven levels of basal anxiety are expressed by different strains of animals (Crawley and Davis, 1982; Hascoët and Bourin, 1998; Griebel *et al.*, 2000). Baseline anxiety also changes along the animal's life span (Hascoët, Colombel and Bourin, 1999). Discrepancies among studies on these and others parameters may be responsible for many of the reported conflicting drug effects found in the literature.

Despite the above caveats, drug results with anti-anxiety agents are remarkably consistent, using either the original protocol or modified versions of the test. Thus, reported results show that acute injection of benzodiazepines (e.g., diazepam, chlordiazepoxide and alprazolam) or buspirone-like drugs have anxiolytic effects in both mice and rats (Crawley, 1985; Pich and Samanin, 1989; Young and Johnson, 1991a; Shimada *et al.*, 1995; Sanchez, 1996; Griebel, Sanger and Perrault, 1996b; Hascoët and Bourin, 1998). Overall, the time spent in the bright compartment seems to be more reliable for assessing anxiety relief than the number of transitions (Hascoët and Bourin, 1998).

However, conflicting results (anxiolytic, anxiogenic or lack of effect) have been obtained with acute injection of the antidepressants imipramine (Pich and Samanin, 1989; Onaivi and Martin, 1989; Young and Johnson, 1991a; De Angelis, 1996), fluoxetine (Kshama *et al.*, 1990; De Angelis, 1996; Sanchez and Maier, 1997; Artaiz, Zazpe and Del Rio, 1998) and citalopram (Griebel *et al.*, 1994; Sanchez and Maier, 1997). Other SSRIs, such as paroxetine, sertraline and fluvoxamine, were ineffective in rats (Sanchez and Maier, 1997), but paroxetine exerted an anxiolytic effect in mice (Hascoët, Bourin and Nic Dhonnchadha, 2000). However, as none of these drugs has been evaluated after chronic administration, the pharmacological profile of the test is incomplete.

Some drugs known to increase anxiety in man and in rodents, such as mCPP and pentylenetetrazol (PTZ), also have an anxiogenic effect in the light/dark transition model (Onaivi and Martin, 1989; Griebel *et al.*, 1991; Bilkei-Gorzo, Gyertyan and Levay, 1998). However, other anxiogenic drugs, such as yohimbine and FG-7142, have shown either an anxiogenic or no effect in the test (Pich and Samanin, 1989; Singh *et al.*, 1991; Shimada *et al.*, 1995; Hascoët and Bourin, 1998; Bilkei-Gorzo, Gyertyan and Levay, 1998).

The selectivity of the light/dark transition model for anxiety-modulating drugs has been supported by findings that the neuroleptics, chlorpromazine, piperone, haloperidol and clozapine (Young and Johnson, 1991a; Costall and Naylor, 1995; Bilkei-Gorzo, Gyertyan and Levay, 1998) as well as the psychostimulant amphetamine (Young and Johnson, 1991a; Bilkei-Gorzo, Gyertyan and Levay, 1998) did not alter the classical anxiety indices. However, in the case of amphetamine and of another psychostimulant drug caffeine, evidences of anxiogenic effects are also reported (Onaivi and Martin, 1989; Imaizumi, Miyazaki and Onodera, 1994; Hascoët and Bourin, 1998).

Fear-Potentiated Startle

The fear-potentiated startle paradigm has been successfully used for studying the neurobiological basis of fear/anxiety conditioning,

mostly by Davis and coworkers (Davis, 1986; Davis *et al.*, 1993; Walker *et al.*, 1997). This model, first described by Brown, Kalish and Faber (1951), is based on principles of Pavlovian classical conditioning and its performance involves two distinctive steps. Initially, the animals are trained to associate a neutral stimulus (the to-be-conditioned stimulus), generally a light, with an aversive stimulus such as an electric foot-shock. After training, the animals are submitted to an intense sound and the amplitude of their startle reflex to this unconditioned aversive stimulus is measured, either in the presence or in the absence of the conditioned stimulus (light). It has been shown that the amplitude of the acoustic startle reflex is higher when the eliciting acoustic stimulus occurs in the presence of the light. This potentiation of the startle response can be found even one month after the training session. Further evidence shows that maximum potentiated startle in the test session is achieved at the time after light onset which was followed by foot-shock presentation in training, that is, when shock is most expected. These results strengthen the view of fear-potentiated startle as a model of anticipatory anxiety (Davis *et al.*, 1993). Considering the above definition of fear, given by Gray and McNaughton (2000), a more appropriate denomination for this test would be 'conditioned anxiety-potentiated startle'. However, for sake of clarity the original name is preserved.

The effects of different classes of psychoactive drugs in rats submitted to the fear-potentiated startle are reviewed by Davis *et al.* (1993) and Hijzen *et al.* (1995).

A selective anxiolytic effect in this test is characterized by attenuation of fear-potentiated startle with no change in the baseline level of startle, that is, the startle response obtained in the absence of the conditioned stimulus. Decreases in both measures may indicate non-specific impairment of motor control. Overall, benzodiazepines, including alprazolam, as well as buspirone-like drugs decrease fear-potentiated startle, often without any change in baseline startle. However, it is interesting to notice that in some cases buspirone and ipsapirone, a similar 5-HT_{1A} agonist, *increase* baseline startle response, in addition to decreasing potentiated startle (Kehne, Cassella and Davis, 1988). These results suggest that these drugs may have differential effects on conditioned and unconditioned responses. In fact, the mechanisms by which 5-HT_{1A} drugs exert their effects seem to be complex, as attested by evidence showing that 5-HT_{1A} antagonists also have anxiolytic effects in this test (Joordens, Hijzen and Olivier, 1998).

As observed with acute injection of the antidepressants amitriptyline (Hijzen *et al.*, 1995) and fluvoxamine (Joordens *et al.*, 1996), administration of imipramine either acutely or chronically (3 weeks) did not affect either fear-potentiated or baseline startle. The lack of imipramine effect has led to the view that this test dissociates anticipatory anxiety from panic (Davis *et al.*, 1993). However, the effects of SSRIs, such as fluoxetine and citalopram, in the test are still unknown.

As expected, the anxiogenic drug yohimbine enhanced potentiated startle, but mCPP had no such effect. The selectivity of the test for anxiety-modulating drugs has been obscured by false positive results obtained with haloperidol (Hijzen *et al.*, 1995) and with the α_2-adrenergic agonist clonidine (Davis *et al.*, 1993). As in the light/dark transition model, amphetamine has been shown to be either anxiogenic (Borowski and Kokkinidis, 1998) or without effect (Hijzen *et al.*, 1995).

Defensive Burying

The conditioned defensive burying test was originally developed by Pinel and Treit (1978, 1979) and explores rodent's species-typical behaviour of burying objects that represent or are associated with aversive stimulation. In the original description of the test, the authors reported that rats shocked once by a probe mounted on the wall of the test box returned to the probe and buried it with bedding material from the floor of the box. They also showed that this behaviour was still observed even when the shock had been delivered 20 days before testing, supporting the role of aversive learning in this paradigm (for further details on the behavioural validation of this test see Treit (1985)). Later on, it was observed that anxiolytic drugs, like diazepam, chlordiazepoxide and pentobarbital, decreased the time spent by animals spraying and pushing bedding material towards the shock source in a way comparable to their relative potencies in clinical settings (Treit, Pinel and Fibiger, 1981). Other psychoactive drugs as picrotoxin, PTZ, d-amphetamine and morphine, injected in a single dose, were ineffective.

However, with the growing use of the test in other laboratories, conflicting results emerged (see Craft, Howard and Pollard, 1988). Soon it became clear that procedural changes, such as intensity and number of shocks delivered (Treit and Fundytus, 1988; Tsuda, Ida and Tanaka, 1988; Treit, 1990), previous habituation to the test box (Treit and Fundytus, 1988) as well as the animal's strain (Pare, 1992) and age (Lopes-Rubalcava, Fernandez-Guasti and Urba-Holmgren, 1996), may influence drug effects. Furthermore, by measuring only the time spent by rats burying the probe it was not possible to clearly separate the anxiolytic effect of the drug from its non-specific effects on general motor activity, due to sedation and/or ataxia. The latter problem still remains as one of the main drawbacks of the model. Nevertheless, the introduction of other indices of anxiety, such as the latency to initiate the defensive burying (Beardsley *et al.*, 1990) and the frequency of contact with a constant electrified probe (Treit and Fundytus, 1988), offered some gains in predictability of drug response. Yet the use of concurrent behavioural tasks for checking animals general activity, such as the rotarod or the arena, has been adopted to discriminate confounding drug effects (Sakamoto *et al.*, 1998; Fernandez-Guasti and Lopez-Rubalcava, 1998).

The conditioned defensive burying test consistently detects acute anxiolytic effects of benzodiazepines and buspirone-like drugs in both rats and mice (Treit, 1990; Fernandez-Guasti, Hong and Lopez-Rubalcava, 1992). Chronic treatment with the antidepressants imipramine, pargyline and desipramine had no effect on rats' burying behaviour (Beardsley *et al.*, 1990).

The pharmacological predictability of the test has been questioned because of failure in consistently detecting anxiogenic drug effects. Neither PTZ nor picrotoxin (Treit and coworkers, 1981, 1987, 1990) enhance burying behaviour and FG-7142, in fact, decreased its expression (Rohmer, Di Scalar and Sandner, 1990). Conflicting results have been obtained with yohimbine, since this drug is reported to be either anxiolytic (Tanaka *et al.*, 2000) or anxiogenic (Tsuda, Ida and Tanaka, 1988; Lopez-Rubalcava and Fernandez-Guasti, 1994) and even to have no effect (Treit, 1990). The selectivity of the test has also been undermined. Whereas the neuroleptic haloperidol had no effect (Sakamoto *et al.*, 1998), as would be expected, other agents ineffective on GAD, such as chlorpromazine (Treit, Pinel and Fibiger, 1981), clonidine and prazosin (Lopez-Rubalcava and Fernandez-Guasti, 1994) behaved as anxiolytics in the animal test (however, in the case of chlorpromazine, lack of effect on anxiety indices was observed with a modified conditioning protocol (Treit, 1990)).

MODELS OF PANIC DISORDER

The available models of panic disorder (PD) are based on two complementary hypotheses. The first considers panic to be related

to the flight response induced by proximal threat (Blanchard and Blanchard, 1988), as discussed in the introduction to this paper. The second hypothesis suggests that panic attacks are due to activation of the medial hypothalamus and/or DPAG (Gentil, 1988; Graeff, 1988, 1991; Deakin and Graeff, 1991), which are brain structures that integrate escape and flight responses, respectively (Table XIX-1.1). The former hypothesis gave origin to the fear/defence test battery (Blanchard, Flannelly and Blanchard, 1986), the mouse defence test battery (Griebel, Blanchard and Blanchard, 1996a) and the one-way escape task in the elevated T-maze (Graeff, Viana and Tomaz, 1993). The second proposal led to animal models that use either electrical (Jenck, Moreau and Martin, 1995; Schenberg *et al.*, 2001) or chemical stimulation (Beckett *et al.*, 1992b) of the DPAG, as well as chemical stimulation of the dorso-medial hypothalamus (DMH) (Shekhar, 1994).

The experimental evidence implicating the DAPG in panic has been extensively discussed elsewhere (Graeff, 1988, 1991; Deakin and Graeff, 1991; Graeff *et al.*, 1996b). One of the main arguments in favour of this view is that electrical or chemical (by excitatory amino acids or anti-GABA agents) stimulation of the DPAG evokes abrupt and clumsy flight responses in experimental animals, reminiscent of a panic attack (Graeff, 1988, 1991). Similar results have been reported with infusion of $GABA_A$ antagonists into the DMH (Shekhar and Di Micco, 1987), although escape induced by $GABA_A$ antagonists or synthesis inhibitors injected into this brain area is more co-ordinated and well oriented toward environmental goals than that obtained in the DPAG (Schmitt *et al.*, 1986). Another argument is that electrical stimulation of the DPAG of patients undergoing neurosurgery produces neurovegetative changes and feelings of terror that are characteristic of clinical panic attacks (Nashold, Wilson and Slaughter, 1974; Amano *et al.*, 1978). Moreover, a PET study carried out by Reiman *et al.* (1989) showed that the midbrain tectum, which includes the DPAG was activated during lactate-induced panic attack.

Blanchard and colleagues (2001) have recently reviewed several of above models of PD. The following description is in large measure based on their account. We have added the version of DPAG electrical stimulation developed by Schenberg's group and the chemical stimulation of the DPAG model introduced by Marsden and coworkers. Because the mouse/defence test battery and the elevated T-maze address both to GAD and PD, they will be analysed separately.

Fear/Defence Test Battery

The fear/defence test battery measures defensive behaviours of wild rats (*Rattus rattus*), more specifically, freezing, flight, defensive sonic vocalization and defensive attack to an approaching/contacting predator, a human subject (Blanchard and coworkers, 1986, 1993). It has been shown that these responses are more evident in wild than in laboratory rats (Blanchard, Flannelly and Blanchard, 1986). Overall, benzodiazepines (diazepam, chlordiazepoxide and midazolam) and buspirone-like drugs did not affect the expression of freezing, avoidance and more importantly, flight responses, during confrontation with the predator. The lack of effect of these GAD-effective drugs on these defensive responses has supported analogies between the test and PD. These compounds do decrease defensive threat/attack reactions towards the predator (Blanchard *et al.*, 1993, 1997, 1998), but the reliability of these behaviours as indices of anxiety has been questioned (Blanchard *et al.*, 1997).

The complexity of this test, derived mainly from the use of wild animals, limits its widespread use in psychopharmacological analysis. This fact can be attested by the lack of studies on the effects in this test of panicolytic drugs such as alprazolam and antidepressants, or panicogens such as yohimbine, in animals submitted to this test.

Electrical Stimulation of the Dorsal Periaqueductal Grey

In the version of this model utilized by Jenck and coworkers, rats are trained to switch off DPAG electrical stimulation by jumping into the opposite compartment of a shuttle-box. Several drugs that affect PD were tested to assess predictive validity. As expected, the anti-panic agents alprazolam and clonazepam impaired switch-off behaviour dose-dependently, while the panicogenic agents caffeine, yohimbine and flesinoxan and CCKb agonists facilitated escape from DPAG electrical stimulation. Nevertheless, several results seem to be inconsistent with the hypothesis that DPAG stimulation models PD, such as the anti-aversive action of the panicogenic mCPP, and of single administration of the SSRIs fluvoxamine and fluoxetine. The latter drugs are known to aggravate PD in the initial phase of drug treatment (Saran and Halaris, 1989; Den Boer and Westenberg, 1990). In addition, there were false positive results, since the antipsychotic agent haloperidol and the CCK_B antagonist L365,260 had anti-aversive effects in the animal test, but do not improve PD (for primary references, see Blanchard, Griebel and Blanchard, 2001).

In the version of DPAG electrical stimulation developed by Schenberg and coworkers (Schenberg *et al.*, 1990; Sudré *et al.*, 1993), the rat is placed inside an arena and the intensity of the electrical current is gradually increased. In this way, a sequence of behavioural and neurovegetative changes is elicited, namely immobility, running, jumping, exophthalmus, micturition and defecation. Pharmacological analysis indicates that running and jumping seem to be correlated with panic. Thus, the threshold current intensity for eliciting these responses was markedly increased by chronic, but not acute, administration of the panicolytic drugs clomipramine and fluoxetine. Long-term administration of the anxiolytics diazepam and buspirone as well as of the selective NA reuptake inhibitor maprotiline was ineffective. Moreover, the panicogenic drug PTZ decreased running threshold (Schenberg *et al.*, 2001; Vargas and Schenberg, 2001). The latter study also showed that plasma levels of ACTH were not affected by DPAG electrical stimulation that induced panic-like behaviour. This finding further supports this test as a model of PD, in relation to claims that the hypothalamus–hypophysis–adrenal axis is not activated during panic attacks (Liebowitz *et al.*, 1985).

Chemical Stimulation of the Dorsal Periaqueductal Grey

In this model microinjection of the excitatory amino acid d,l-homocysteic acid (DLH) is made in both rostral and caudal DPAG, causing explosive motor behaviour characteristic of proximal defence. This behaviour is quantified in terms of response duration, arena revolutions and number of defensive jumps made by the rats inside an arena (Beckett, Marsden and Marshal, 1992a). In the automated version of the test, distance travelled is recorded with a computer-driven automated tracking system (Beckett and Marsden, 1995). As reported with electrical stimulation (Nogueira and Graeff, 1995), pre-treatment with 5-HT_{1A} agonists directly injected into the DPAG markedly attenuated the defence reaction elicited by DLH (Beckett *et al.*, 1992b; Beckett and Marsden, 1997). Conversely, systemic injection of the 5-HT_{1A} agonist 8-OH-DPAT enhanced the DLH effect (Beckett and Marsden, 1997), probably because stimulation of autosomic receptors in the dorsal raphe nucleus decreases inhibitory 5-HT input to the DPAG. Also, intra-DPAG injection of the 5-HT_2-receptor agonist mCPP intensified the defence reaction induced by DLH (Beckett, Marsden and Marshal, 1992a). Chronic (3 weeks), but not short-term (2–3 days) treatment with imipramine enhanced the 5-HT_{1A}-mediated inhibition of flight induced by intra-DPAG injection of DLH (Mongeau and Marsden, 1997a). The latter result supports the suggestion that the therapeutic effect of antidepressants on PD is due to

enhancement of 5-HT inhibition of DPAG neurones commanding flight (Graeff, 1991). Finally, intracerebroventricular injection of the panicogen CCK-4 prolonged the tachycardia induced by intra-DPAG DLH, but failed to affect the flight response. The latter was potentiated by systemically injected butoxycarbonyl-CCK-4, indicating a peripheral site of action (Mongeau and Marsden, 1997b). Although only one panicolytic and one panicogenic drug have been used so far to validate this model, the above results testify to its importance for the study of the neurobiology of PD.

Chemical Stimulation of the Dorso-Medial Hypothalamus

Infusion of the $GABA_A$ receptor blockers bicuculline and picrotoxin into the DMH induces oriented escape response accompanied by hyperventilation and increases in arterial blood pressure and heart rate, reminiscent of a panic attack (Shekhar and Di Micco, 1987). The effects of bicuculline infusion have been abolished following chronic treatment with either imipramine or clonazepam (Shekhar, 1994). The same drug regimen is clinically effective on PD (Nutt, 1991). Also as expected, the panicogenic agents yohimbine and fenfluramine enhanced the behavioural and physiological effects of bicuculline in the DMH (Keim and Sekhar, 1999). However, because a comparative study has shown that escape from the hypothalamus is controlled by environmental stimuli whereas that from the DPAG is undirected (Schmitt *et al.*, 1986), the DMH model is less akin to panic attacks.

COMBINED MODELS OF GENERALIZED ANXIETY AND PANIC DISORDERS

The two animal models analysed below are intended to assess behaviours related to both GAD and PD in the same animal.

The Mouse Defence Test Battery

Based on their ethoexperimental analysis of defence (see the introduction of this paper), Blanchards' group elaborated a test battery to measure both risk assessment, related to GAD and predator-induced flight, related to PD. In the mouse defence test battery (MDTB) a mouse is placed inside an oval runway and a deeply anaesthetized rat, held by the experimenter's hand, is approached at a fixed speed. When the rat–mouse distance reaches about 1 m, the mouse generally flees, running in the alley until the threat is out of sight. This defence reaction is taken as an index related to panic (Griebel, Blanchard and Blanchard, 1996a). During the predator's approach, mice also express risk-assessment behaviour consisting of an abrupt movement arrest often followed by orientation toward the approaching rat. Risk-assessment behaviour (approach to/withdrawal from the rat) is also evidenced when the predator–prey interaction is carried out in a straight alley. These risk-assessment responses are taken as indexes related to GAD. The pharmacological profile of the MDTB evidences its high predictive validity. Thus, flight was reduced by two high-potency benzodiazepines that are clinically effective on PD, namely alprazolam and clonazepam, as well as by chronic administration of several antidepressants, including imipramine, fluoxetine, moclobemide and phenelzine. Whereas either acute or chronic alprazolam had no appreciable effect on risk-assessment responses, chronic administration of imipramine and fluoxetine decreased the expression of these behaviours. The results obtained with these two antidepressants are consonant with their anxiolytic effects on GAD symptoms (Nutt, 1991; Rickels *et al.*, 1993). In addition, panicogenic agents, such as yohimbine, flumazenil and cocaine, as well as therapeutic drugs like imipramine and fluoxetine, that nonetheless aggravate PD in the initial phase of treatment, facilitated flight in the MDTB test when injected acutely. Finally, drugs clinically ineffective on PD, such as the benzodiazepine anxiolytic chlordiazepoxide, the $5\text{-}HT_{1A}$ agonist buspirone and the $5\text{-}HT_2$ antagonist miaserin, did not affect the same flight response, but consistently reduced the GAD-related risk-assessment behaviours (for primary references, see Blanchard, Griebel and Blanchard (2001).

The Elevated T-maze

The elevated T-maze (ETM) is derived from a hypothesis about the role of 5-HT in GAD and PD. In an attempt to conciliate conflicting results on the role of 5-HT in anxiety, Deakin and Graeff (1991) have suggested that conditioned anxiety is related to GAD while unconditioned fear is associated to PD. The former would be facilitated whereas the latter would be inhibited by 5-HT released from fibres originated in the dorsal raphe nucleus (DRN). As a consequence, the ETM was intended to separate conditioned anxiety from unconditioned fear (Graeff, Viana and Tomaz, 1993; Viana, Tomaz and Graeff, 1994). The apparatus is derived from the elevated X- or plus-maze (Handley and Mithani, 1984; Pellow and File, 1986) by sealing the entrance to one of its enclosed arms. As a result, the ETM consists of three arms of equal dimension (50×12 cm) elevated 50 cm from the floor. One of these arms is enclosed by lateral walls (40 cm high) and stands perpendicular to the two opposite open arms. In the experimental session, the rat performs two consecutive tasks, inhibitory avoidance and one-way escape, supposed to represent conditioned anxiety and unconditioned fear, respectively. When placed at the end of the enclosed arm, the rat does not see the open arms until it pokes its head beyond the walls of the closed arm. Being on the open arm seems to be an aversive experience, since rats have an innate fear of height and openness (Treit, Menard and Royan, 1993). This would allow the animal to learn inhibitory avoidance if repeatedly placed inside the enclosed arm to explore the maze. On the other hand, when the rat is placed at the end of one of the open arms it can move towards the closed arm, presumably performing an escape response.

The ETM may also be viewed as a way to circumvent the ambiguities of the elevated plus-maze (see the introduction of this paper). According to Handley *et al.* (1993), the elevated plus-maze is a mixed model, leading to inconsistencies in drug response. To circumvent this shortcoming, systematic recording of behavioural items has been added. Some of these items are selectively affected by non-benzodiazepine anxiolytics, thus improving the predictive value of the test (e.g., Rodgers and Johnson, 1995). However, this strategy is time consuming and strips the elevated plus-maze of its main advantage, which is simplicity. Also, critical behavioural items, such as risk assessment may be more easily observed in contexts different from the elevated plus-maze. An alternative to ethological analysis is the separation of functional categories of behaviour through experimental analysis, which is accomplished by the ETM.

A series of experiments has been performed to validate the ETM behaviourally (Zangrossi and Graeff, 1997). The results obtained showed that restraining the animals at the end of the enclosed arm for 30 s did not change the first (baseline) withdrawal latency, indicating that rats are not escaping from the experimenter's hand. In addition, rats trained in a T-maze with the three arms enclosed did not show the usual increase in withdrawal latency along three consecutive trials. Therefore, open arm experience seems to be critical for inhibitory avoidance learning. The same experiment also showed that the latency to leave the open arm did not undergo habituation over five consecutive trials, evidencing the aversive motivation of this response.

For pharmacological validation, drugs that are known to either decrease or increase clinical anxiety have been used (Graeff *et al.*, 1998). Table XIX-1.2 summarizes the results obtained. As may be seen, the anxiolytics diazepam, buspirone, ipsapirone and ritanserin impaired inhibitory avoidance while leaving one-way escape unchanged. This selective effect on avoidance correlates with the clinical effectiveness of these drugs on GAD, as opposed to their inefficacy on PD (Nutt, 1991). It is worth mentioning that several additional drugs that had anxiolytic-like effects in other animal models of anxiety also selectively impaired avoidance in the ETM (Graeff, Ferreira Netto and Zangrossi, 1998). However, since these drugs have not been assayed in humans, clinical correlation cannot be assessed.

Also as predicted by Deakin and Graeff's hypothesis, the anxiogenic agents yohimbine, TFPP and mCPP facilitated inhibitory avoidance in the ETM. However, the two last drugs attenuated escape, a result that contrasts with clinical reports on aggravation of PD by the same drugs. It may be argued that in these clinical studies anticipatory anxiety rather than panic was enhanced, since mCPP also enhances anxiety in normal subjects (Charney *et al.*, 1987). Moreover, Deakin and Graeff (1991) have further hypothesized that the brain mechanisms that underlie anxiety and panic interact, the former exerting inhibitory influence on the latter. Thus, drugs that facilitate avoidance could indirectly decrease escape. Against this argument, however, stand the results obtained with yohimbine, which facilitated avoidance without impairing escape in the ETM. Another caveat is that in these studies rats had not been pre-exposed to the open arm before the test (see later).

Tricyclic antidepressants usually aggravate anxiety earlier in treatment, the therapeutic effect appearing only following several days of repeated administration (Johnson, Lydiard and Ballenger, 1995). This correlates with the anxiogenic-like effect of clomipramine and imipramine on inhibitory avoidance following single injection (Table XIX-1.2). More important, an anxiolytic effect of imipramine on avoidance developed after chronic administration, and escape from the open arm was simultaneously impaired, which correlates well with the clinical effectiveness of this drug regime on both GAD and PD (Rickels *et al.*, 1993; Johnson, Lydiard and Ballenger, 1995). Nevertheless, the interpretation of the drug effects on escape is not as straightforward as on avoidance. As expected, this behaviour was unaffected by acute clomipramine. Yet, it was impaired by a single injection of imipramine. This inconsistency reminds one of the above-discussed differences between yohimbine, on one hand, and mCPP and TFPP, on the other.

Table XIX-1.2 Effect of clinically assayed drugs on the tasks performed in the elevated T-maze

Drug class	Compound	Avoidance	Escape
Anxiolytic			
	Diazepam	−	0
	Buspirone	−	0
	Ipsapirone	−	0
	Ritanserin	−	0
Anxiogenic			
	Yohimbine	+	0
	MCPP	+	−
	TFMPP	+	−
Antidepressant			
	Clomipramine	+	0
	Imipramine	+	$-^{p,2}$
	Imipramine[c]	−	$-^{p,2}$
5-HT releaser			
	d-Fenfluramine	+	−

Key: + Facilitation; − impairment; 0 no change; [c]chronic administration; [P]pre-exposure to the open arm; [2]two trials.

In addition, there was what appears to be a critical methodological difference, namely the rats used in the imipramine study had been pre-exposed to the open arm for 30 min before the test, and one additional measure of escape latency was taken. This procedure had two consequences: (1) the first escape response became significantly faster and (2) the anti-escape effect of chronic imipramine became statistically significant (Teixeira, Zangrossi and Graeff, 2000). Further comparisons with intracerebral drug injection led to the conclusion that pre-exposure to the open arm renders the escape task more sensitive and reliable to the effect of different brain manipulations. This is probably due to the habituation of behavioural reactions to novelty (exploration, behavioural inhibition), which are likely to interfere with one-way escape.

The 5-HT releaser and uptake inhibitor fenfluramine has been reported to induce attacks in panic patients (Targum and Marshall, 1989). Thus, the impairment of escape that was found with this drug (Table XIX-1.2) was quite unexpected. Nevertheless, Targum and Marshall (1989) themselves point out that, unlike the sudden surge of natural panic attacks, fenfluramine induced a slow wave of anxiety, more like an enhancement of anticipatory anxiety. Accordingly, d-fenfluramine has been shown to facilitate inhibitory avoidance in the ETM (Table XIX-1.2). Results obtained with two human models of anxiety seem to correlate with the opposite effects of d-fenfluramine on the two tasks of the ETM. Thus, the same drug tended to enhance conditioning of skin conductance responses in healthy volunteers, whereas markedly reducing the rise in anxiety induced by simulation of public speaking, a model of anxiety that has been associated with panic (Hetem *et al.*, 1996). Furthermore, a recent study showed that pre-treatment with d-fenfluramine enhanced anticipatory anxiety in PD patients following CO_2 inhalation. In contrast, the intensity of the CO_2-induced panic attack was decreased by the same drug treatment (Mortimore and Anderson, 2000). From these results, d-fenfluramine is expected to have anti-panic properties. Indeed, an open study (Solyom, 1994) as well as a case history (Hetem, 1995) provide suggestive clinical evidence supporting this view. Unfortunately, clinical investigation had to be interrupted because d-fenfluramine was withdrawn from the market due to cardiovascular untoward effects (Curzon and Gibson, 1999). In any case, the results obtained are enough to show that what at first seemed to be a major flaw of the ETM model turned out to strengthen the predictive value of escape in regard to PD.

Taking into account the above discussion on models of GAD, the drug profile of ETM avoidance comes as no surprise. Indeed, inhibitory avoidance of the open arm of the ETM is not unlike classical conflict tests. However, the present version has the advantages of not involving hunger or thirst, which are increased by some anxiolytic drugs (e.g., Berridge and Pecina, 1995). Moreover, the unconditioned aversive stimulus is neither painful nor artificial, like electric foot-shock. Another point that deserves some comment is the similarity of the motor performance in both tasks performed by the rat in the ETM. This serves as a control for non-specific drug effects on motor activity, particularly when the withdrawal latencies for the enclosed arm and the open arm are changed to opposite directions by the treatment. However, whenever the latencies are similarly increased or decreased, there is need for independent assessment of motor effects. Measuring motor activity inside an arena fulfils this requirement (Teixeira, Zangrossi and Graeff, 2000), albeit adding further complexity to the model.

The results of experiments with intracerebral drug injection (Graeff, Viana and Mora 1996a, 1997) and of a recent c-Fos study (Silveira *et al.*, 2001) indicate that the neural substrate of inhibitory avoidance is different from that of one-way escape.

MODELS OF SPECIFIC PHOBIAS

Specific phobias have been attributed to traumatic learning experiences. The classical conditioning of animal phobia in a boy by Watson and Rayner (1920) typifies this approach. Nevertheless, reports of traumatic experiences are lacking in most histories of phobic patients (Barlow, 1988). In addition, despite current dangers in civilized societies including, for example, electricity plugs or approaching cars, practically no exaggerated fears for such objects occur in the clinic. Instead, the most frequent phobic objects belong to certain categories that are constant through history and among different cultures, such as heights (acrophobia), closed environments (claustrophobia), the sight of blood (erythrophobia) or poisonous animals. This led to the alternative view that specific phobias are an exaggeration of ancestral fears, evoked by objects or situations that threatened survival of our hominid ancestors (Marks 1987; Marks and Nesse, 1994). A place for learning is kept in these theories of specific phobia, but learning prepared by evolution, such as in the experiments carried out by Ohman, Erixon and Loftberg (1975) showing that aversive conditioning to images of spiders was far easier and stronger than to pictures of flowers.

From this theoretical perspective, animal models of specific phobia should explore defence reaction against species-specific fears. Examples are the primate fear reaction to snakes (Mineka, 1985) and rats' avoidance response to cat odour (Zangrossi and File, 1992a). Whereas both tests have been used for analysing the behavioural characteristics of fear reactions and aspects of fear habituation/extinction, only the latter has been employed in pharmacological investigations.

The cat odour test developed by Zangrossi and File (1992a, 1992b) is based on the studies of Blanchards' group on the A/DTB discussed above. In this test, rats are exposed to a cloth impregnated with cat odour while in their single home-cage. These rats spend a greater amount of time hiding underneath the shelter formed by the water and food compartment of the home-cage and make fewer contacts with the cloth, compared to animals exposed to a neutral odour cloth. The benzodiazepine chlordiazepoxide has only a weak effect on rats' avoidance reactions; $5\,mg\,kg^{-1}$ of the drug increased the time spent in contact with the cloth, but did not decreased the time spent sheltering. On the other hand, this drug was fully effective in counteracting the anxiogenic effect of cat odour detected by the elevated plus-maze and social interaction tests (Zangrossi and File, 1992b). Acute or chronic (21 days) treatment with other anxiety-modulating drugs, such as buspirone, imipramine and the MAO inhibitor phenelzine, also failed to consistently decrease rats avoidance response to cat odour (File, 1995). Based on evidence showing that human-specific phobias are resistant to treatment with the drugs currently used to alleviate anxiety disorders (Marks, 1987), Zangrossi and File (1992b) have suggested that rats' avoidance to cat odour reflects the development of a phobic-like state. However, the generality of this proposal has been recently questioned by reported results showing that under a different test condition (use of a specific test-cage, different strain of rats and way of presenting the cat odour), benzodiazepine drugs can inhibit the avoidance response to cat odour (Dielenberg, Arnold and McGregor, 1999; McGregor and Dielenberg, 1999). Therefore, more studies are necessary to characterize this test as a model of specific phobia.

Although the homology argument is less strong, the suggestion has also been made that second exposure to the elevated plus-maze generates an emotional state that is akin to phobia (File *et al.*, 1993; File, 1993, 1995). This idea comes from evidence showing that benzodiazepines and barbiturates have either a weak or no anxiolytic effect in rats or mice previously exposed to the elevated plus-maze (File, 1990, 1993). This contrasts with the marked anxiolytic effect of these compounds on the first trial (see Rodgers, 1994). This phenomenon, called one-trial tolerance (OTT), has been observed even when the second trial is performed 2 weeks after the first, and is independent of drug state (either drugged or non-drugged) in the first trial. These results suggest that in the first experience with the plus-maze the animals are acquiring a different anxiety state that is insensitive to benzodiazepines. This idea is supported by findings showing that in a factorial analysis study, plus-maze scores of anxiety taken on the first and the second trials load on different factors. Interestingly, increasing the total time of testing on both trials from the usual 5 to 10 mins, abolishes the phenomenon of OTT. This result has been interpreted in terms of fear extinction after prolonged exposure to the aversive stimuli generated by the elevated plus-maze (File *et al.*, 1993). Based on knowledge that specific phobias respond favourably to exposure to the feared object/situation (Marks, 1987; Barlow, 1988), File *et al.* (1993) proposed that the trial second (trial-two) in the elevated plus-maze represents phobia. This trial-two phobic state would be different from that generated by exposure to cat odour in the sense that, whereas the former is acquired by a learning process, the latter is innate. Recent studies have explored the neural substrate involved in trial-two fear conditioning, revealing the participation of the basolateral amygdala (File, Gonzalez and Gallant, 1998) and dorso-medial hypothalamus (File, Gonzalez and Gallant, 1999) in this process. Further studies have shown that benzodiazepines and buspirone-like drugs exert differential effects on plus-maze trial-one and trial-two anxiety scores after microinjection in brain areas as the DRN (Gonzalez and File, 1997), dorsal hippocampus and lateral septum (Cheeta, Kenny and File, 2000).

MODELS OF OBSESSIVE–COMPULSIVE DISORDER

Clinical observation has shown that hand-washing and checking whether windows or doors are locked are the prevailing compulsive rituals. Rapoport (1991) suggested that these rituals may be related to animal behavioural routines of self-grooming and checking territorial borders, respectively. The adaptive function of grooming is to protect the animal against diseases caused by micro-organisms or parasites, while checking prevents invasion of the territory needed for reproduction and feeding by animals of the same or alien species. Neuroethological research has implicated the striatum in the control of these behavioural routines. Thus, an indirect support to Rapoport's ideas came from functional neuro-imaging studies showing hyperactivation of the nucleus caudatum of symptomatic obsessive–compulsive disorder (OCD) patients, which faded following successful treatment with either fluoxetine or cognitive behavioural therapy (Baxter *et al.*, 1996).

Acral Lick Dermatitis

Based on the preceding argument, Rapoport has looked for repetitive behaviours in animals that resemble compulsive symptoms, and found acral lick dermatitis in dogs the most appealing. Since OCD is responsive to clomipramine (Zohar *et al.*, 1988), similar treatment for the dog's disease was suggested, and indeed was effective (Goldberger and Rapoport, 1991). On this basis, acral lick dermatitis became a candidate for an animal model of OCD (Rapoport, Ryland and Kriete, 1992). The efficacy of clomipramine on canine compulsive disorder was confirmed by a double-blind, placebo-controlled study (Hewson *et al.*, 1998). Like human OCD, acral lick dermatitis is improved by SSRIs, such as fluoxetine (Wynchank and Berk, 1998) and citalopram (Stein *et al.*, 1998). However, acral lick dermatitis is not a true experimental model, in the sense that the conditions cannot be reproduced at will in the laboratory, but rather

an animal analogue of OCD. Also, the use of a large and expensive subject such as dogs, and particularly sick dogs, detracts from its feasibility.

Adjunctive Behaviour Models

Some attempts to model OCD in laboratory animals have been made. Earlier operant behaviour studies have shown that when rats are given food reinforcement under fixed interval schedules, several collateral (adjunctive) behaviours are generated during the post-reinforcement period, among which are compulsive drinking or polydipsia (Falk, 1961). Woods and coworkers (1993) have shown that schedule-induced polydipsia was attenuated by chronic administration of the anti-obsessional drugs clomipramine, fluoxetine and fluvoxamine, and not affected by desipramine, haloperidol and diazepam, all of which are ineffective on OCD. These results qualify schedule-induced polydipsia as a potential animal model of OCD.

Another possible model of OCD is food-restriction-induced hyperactivity. Rats fed for 90 min per day lose some weight and then stabilize. If given access to a running wheel, these animals run excessively, loose weight, and often die. A pharmacological study has shown that chronic administration of fluoxetine decreased running and weight loss. Conversely, depleting 5-HT stores with para-chlorophenylalanine potentiated the effects of food restriction. Imipramine, which is ineffective on OCD, was also devoid of any action in this model (Altemus *et al.*, 1996).

Other Potential Models

Further repetitive behaviours that have been considered as potential models of OCD are the spontaneous alternation of food-deprived rats in a T-maze (Yadin, Friedman and Bridger, 1991) and marble burying in mice (Ichimaru, Egawa and Sawa, 1995). Both were responsive to SSRIs.

Pharmacological models for OCD have also been suggested. One of those is chronic treatment with the dopamine agonist quinpirole, inducing stereotyped behaviour in rats interpreted as compulsive checking. This would provide face validity to the model. This behaviour is attenuated by clomipramine, and its mechanism seems to involve sensitization of dopaminergic neurotransmission in the striatum, implicating this neurotransmitter in OCD (Szechtman, Culver and Eilam, 1999).

There is high comorbidity between OCD and Tourette's syndrome (see, e.g., Frankel *et al.*, 1986). From this background, a transgenic mouse model of comorbid Tourette's syndrome and OCD was created by expressing a neuropotentiating cholera toxin transgene in a subset of dopamine D_1 receptor-expressing neurones thought to enhance cortical and amygdalar output to the striatum. These animals manifest episodes of perseveration and repetitive behaviour, as in OCD, and repeated climbing/leaping and tics, as in Tourette's syndrome (McGrath *et al.*, 2000). Although of interest for the study of the role of glutamate in these conditions, this model lacks pharmacological validation.

CONCLUDING REMARKS

In the preceding sections several potential models of specific anxiety disorder were analysed. These tests have been selected mainly according to the criterion of construct validity or homology. For that, a theoretical stance based on the evolutionary paradigm has been taken.

Since present-day psychiatric classification is based on phenomenology, it may not be a suitable basis for establishing the neurobiological correlates of anxiety disorders. As pointed out by Gray and McNaughton (2000), genetic studies indicate that there is no specific inheritance for diagnostic categories, because what is inheritable is a general vulnerability for anxiety disorders. In this regard, dimensional approaches of personality may qualify as an alternative approach to provide better psychological correlates of biological markers.

Table XIX-1.3 presents an attempt to evaluate comparatively the models of GAD and PD, which have been more systematically explored than the models for other diagnostic categories. From the traditional criteria of model validation, the criterion of analogy was omitted, as it makes little sense within the comparative approach adopted here. In addition, the criterion of feasibility was added, because it often determines the extent to which a given animal model will be used.

Drug response remains the cornerstone for empirical validation, but under the precedence of homology the correlation between drug response in the model and in the clinic is viewed as a test for hypotheses about the model and the disorder rather than a separate criterion. Nevertheless, some critical remarks are due, concerning the interpretation of pharmacological correlation. With regard to models of GAD, non-benzodiazepine agents, such as buspirone and ritanserin, have anxiolytic-like effects after single administration in several models of GAD. However, these drugs improve clinical anxiety only after weeks of continuous administration (Nutt, 1991; Johnson, Lydiard and Ballenger, 1995). Therefore, the question of whether the effects in the animal models reflect reduced anxiety or merely behavioural disinhibition (Thiébot, Bizot and Soubrié, 1991) remains. Against the latter view are the results showing that buspirone-like drugs decrease probe burying as well as attenuate potentiated startle (see earlier). In these tests, the anxiolytic effect is expressed as decreased, rather than increased, motor activity. Yet, the case of potentiated startle is weakened by the demonstrated correlation between the intensity of startle potentiation and the degree of freezing (Leaton and Borszcz, 1985). Thus, it may still be argued that anxiolytic drugs reduce potentiated startle because they decrease behavioural inhibition.

Another instance is the effect of chronically administered antidepressants in models of GAD. In some cases a lack of effect of these treatments is suggested to support the specificity of the model. However, clinical studies have shown that prolonged administration of these drugs improves GAD, in addition to

Table XIX-1.3 Comparison among animal models of GAD and PD

Disorder	Animal model	Homology	Predictability	Feasibility
GAD				
	Light–dark transition	+++	+++	+++
	ETM avoidance	+++	+++	+++
	ADTB	+++	+++	++
	MDTB	+++	+++	++
	Potentiated startle	++	++	+
	Shock-probe burying	++	++	++
PD				
	FDTB	+++	++	+
	MDTB	+++	+++	++
	ETM escape	+++	++	+++
	DPAG electrical stimulation	+++	++	+
	DPAG chemical stimulation	+++	+	+
	DMH chemical stimulation	++	+	+

Key: ETM, elevated T-maze; ADTM, anxiety defence test battery; MDTB, mouse defence test battery; FDTB, fear/anxiety test battery; DPAG, dorsal periaqueductal grey matter; DMH, dorso-medial hypothalamus.

other anxiety disorders (Nutt, 1991; Rickels *et al.*, 1993). In the same way, psychostimulants, such as caffeine, cocaine and amphetamine, are often ineffective in GAD models, and this is taken as evidence for their drug selectivity. Yet, human studies show that these drugs may increase anxiety (Mitchell, Laurent and de Wit, 1996; Nehlig, Daval and Debry, 1992). Such instances undoubtedly blur the pharmacological validation of GAD models.

Similar restrictions can be made with respect to panic models. All the animal models of PD analysed show a reduction of the panic index after treatment with at least one clinically effective antipanic agent. This is indeed a necessary condition for a test to be classified as a model of panic disorder. Yet, it is not sufficient, since the same drug treatments also alleviate GAD (Nutt, 1991; Rickels *et al.*, 1993). Another criterion is the increase of the behavioural index of panic by drugs that induce panic attacks. Once more the interpretation of the results obtained is not straightforward. Some of these agents are specific, in the sense that they induce panic attacks only in panic patients. Among these are sodium lactate and inhalation of CO_2. Others, like cocaine, mCPP, yohimbine, caffeine and d-fenfluramine, induce anxiety in normal subjects as well, although panic patients may be particularly sensitive to these drugs. Therefore, it is difficult to ascertain whether the latter drugs induce a true panic attack or otherwise enhance anticipatory anxiety (Bourin, Baker and Bradwejn, 1998).

The above caveats expose the fragility of the current animal models. Only the capacity for detecting new, clinically useful drugs or behavioural therapies will ultimately validate any animal test, and warrant the approach advocated here of developing animal models theoretically oriented to specific anxiety disorders.

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XIX-2

Aminergic Transmitter Systems

Neil McNaughton

INTRODUCTION

A fundamental goal of biological psychiatry should be the separation of symptoms from syndromes. It is clear, for example, that 'headache' is not an appropriate diagnostic category. You cannot use it to define the treatment of the underlying disorder giving rise to the symptom. Nor is the regular decrease in headache symptoms produced by aspirin or paracetamol evidence for common aetiology of the causes of headache or for an 'analgesic insufficiency' as the primary cause of headache.

This problem is particularly acute with mood disorders. In the case of depression, which at first blush seems more coherent as an entity than the spectrum of 'anxiety disorders',

> 'in the DSM-III the "choice principle" was introduced. For the diagnosis of a particular syndrome the presence of X out of a list of Y symptoms suffices, no matter which ones. Hence the various disorders that are distinguished are symptomatologically ill defined. In fact, many different syndromes carry the same designation: major depression and dysthymia.... The utility of the ... discrete diagnostic entities characterized by multiple criteria ... is questionable. Our studies at least have failed to validate them.... Another fundamental shortcoming ... is the lack of an aetiological axis [since] with today's methodologies, one can arrive at an aetiological hypothesis with no less reliability as one can regarding the presence or absence and severity of particular psychopathological symptoms.... Another [problem] is that of [classification in the presence of] comorbidity ... For the diagnosis of major depression, psychotic and organic conditions have to be ruled out. The diagnosis of generalized anxiety disorder cannot be made in the presence of a mood disorder.
>
> This principle is not applicable in biological psychiatry. One can and should not simply discart [*sic*] the possibility that a biological variable observed in a psychotic condition is linked to a concurrent depression, or one found in depression is in fact related to an anxiety disorder....
>
> For [these] reasons I consider these concepts unsuited for [mood disorder] research, particularly biological research, requiring as it does well-defined and assessable diagnostic concepts.'
>
> Van Praag (1995, p. 270)

The problem of comorbidity may be even more acute in the diagnosis of anxiety disorders. For example, panic and obsession, *as disorders*, may well have no necessary connection to anxiety as such. Rather, each may be an indirect cause of, essentially 'normal', anxiety when they occur (as unexpected, uncontrollable and bizarre events) in someone with a pre-existing sensitivity to threatening events. Conversely, both panic and obsession can occur as a simple, non-pathological, consequence of high levels of anxiety. Even this anxiety need not be pathological — consider the obsessive behaviour of a parent with a young and extremely mobile child at an airport.

Syndrome identification faces two additional problems. First, is a lack of connection between cause and treatment. A purely cognitively mediated post-traumatic stress disorder can nonetheless result in brain damage and require pharmacological treatment. Panic attacks resulting from a neural disturbance may be best treated psychologically — resolving the anxiety-related problems engendered by the panic attacks without completely eliminating the latter (Franklin, 1990). Other exclusion criteria, such as the exclusion by 'DSM-III-R [of] persons with socially phobic symptoms secondary to axis III conditions ... [such as] disfiguring or disabling conditions [can lack] empirical basis.... [and cause] a patient group [to be] overlooked with regard to treatment options' (Oberlander, Schneier and Liebowitz, 1994).

A final problem for diagnosis is that the grouping of 'anxiety disorders' as a single class in DSM-III, DSM-IIR and DSM-IV seems inappropriate from the point of view of pharmacology or preclinical analysis of brain systems. These suggest that the different symptomatologies and therapeutic responses of patients presenting with 'anxiety disorders' reflects a confusion of diagnostic categories. Diagnoses should, but currently do not, reflect the primary brain systems that are dysfunctional in any particular case. Symptomatology must often reflect the brain systems, whether functional or dysfunctional, that have extremes of activity. For both these reasons, brains systems will be the focus of the next section.

The most significant aminergic neurotransmitter systems for anxiety and the conventional grouping of anxiety disorders are the monoamines serotonin and noradrenaline. As discussed in the section entitled 'The Neuropsychology of the aminergic systems' basic research implicates them as modulators of the entire defense system with a clear contribution to anxiety — in the sense that this can be distinguished from fear (Blanchard *et al.*, 1988, 1991, 1997). Drugs that manipulate them have therapeutic effects that usually span several of the different types of anxiety disorder (den Boer *et al.*, Chapter XIX-13). Finally, disturbances of their metabolism appear to play a significant role in the generation of anxiety disorders (see below). A weaker case can, also, be made for dopamine. The preclinical review below will also include consideration of acetylcholine because, although not a monoamine, it shares many of the anatomical and functional features of the monoamines.

The primary conclusion is that the success or failure of treatment with a particular drug is likely to reflect the secondary impact of alteration in monoamines on primary brain systems more often than an original disturbance of monoamines was the primary basis of the symptomatology.

THE NEUROPSYCHOLOGY OF ANXIETY

The view of the neuropsychology of anxiety disorders in this chapter is justified at great length in Gray and McNaughton

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(Gray and McNaughton, 2000) and in its appendices available at http://www.oup.co.uk/academic/medicine/medical_updates/neuropsych_anxiety/ (see also Mohanty and Heller, Chapter XVIII-7).

The most important point to note is that, for each anxiety disorder or symptom recognized by the DSM and ICD classifications, there appears to be a distinct pharmacological specificity (Table XIX-2.1). This specificity is demonstrated by dissociations produced by particular drugs (e.g., buspirone affects anxiety and depression but not panic) and can be obscured in the clinical setting by the fact that a number of drugs can have concurrent but distinct effects on different 'anxiety disorders'. Indeed, clinicians will tend to prefer less specific drugs as a means of protecting against incorrect diagnosis.

Together with much other data (Gray and McNaughton, 2000), this allows us to allocate each symptom and/or syndrome to a specific portion of a hierarchically organized defense system (Figure XIX-2.1). However, this allocation does not allow us to assign a particular symptomatology to pathology in a particular area and so use it to define a particular syndrome. For example, a primary panic disorder (arising, say, from paroxysmal activity in the periaqueductal gray) could result in panic attacks. Given the effects of arousal on panic, full blown attacks are more likely to occur in a public place. Any particular place occasioning the first attack will condition anticipatory anxiety and so an increased probability of panic. The resultant increased general arousal would increase the probability of panic in other locations. Thus conditioning would produce agoraphobia as a symptom not a cause. But this would be likely to be diagnosed as agoraphobia proper. Panic attacks would then be both a primary cause of anxiety *and* a secondary consequence. Conversely, a primary anxiety disorder (conceivably presenting as agoraphobia) could result in panic attacks as a symptom of extreme anxiety (Goisman *et al.*, 1995; Marks, 1987). Indeed, a pure primary 'panic disorder' might not be diagnosed as such unless it was accompanied by additional symptoms resulting from its interaction with a neurotic personality and its resultant production of symptoms of anxiety (Holt, 1990; Gray and McNaughton, 2000).

Table XIX-2.1 Various classes of drugs effective in treating neurotic disorders and their relative effects on different neurotic syndromes and the extent to which they share classical anxiolytic side effects (muscle relaxant; anticonvulsant, sedative, addictive). Exceptional effects of individual members of a class are ignored (e.g., the antidepressant and panicolytic actions of benzodiazepines such as alprazolam). It should be noted that antidepressant monoamine oxidase inhibitors (MAOI), in particular phenelzine, are unlike novel anxiolytics (novel) such as buspirone and tricyclic drugs such as imipramine (IMI) that have separate anxiolytic and antidepressant action. They treat depression but also appear particularly effective in treating atypical depression (in which many symptoms overlap anxiety disorders but are resistant to anxiolytic drugs). They have not been reported to be effective in generalized anxiety

	Class	Novel	IMI	CMI	MAOI	SSRI
Phobia	0	?	?	?	?	?
Generalized Anxiety	–	–	–	–	0?	–
Panic Attacks	0	0	–	—	–	–
Obsessions/Compulsions	0	?	(–)	—	(–)	—
Atypical Depression	0	?	(–)	?	–	?
Unipolar Depression	0	–	–	–	–	–
Classical Anxiolytic Side Effects	=	#	0	0	0	(#)

Class, classical anxiolytics such as benzodiazepines, barbiturates and meprobamate; CMI, Clomipramine; 0, no effect; –, reduction; —, extensive reduction; +, increase; ( ), small or discrepant effects; =, same as classical anxiolytics; #, opposite to classical anxiolytics. Adapted from Gray and McNaughton (2000), see also den Boer *et al.* Chapter XIX-13.

THE NEUROPSYCHOLOGY OF THE AMINERGIC SYSTEMS[1]

The general form of the anatomy of the different aminergic systems is very similar (see also previous chapter). This is shown in cartoon form in Figure XIX-2.2. For example, serotonergic cell bodies are located in the raphe nuclei of the brainstem. From there, they send projections forward making collateral connections with many structures before innervating the cortex fairly diffusely. 'Early studies that used older tracing techniques reported exceedingly few [descending] projections from the dorsal raphe (DR) to the brainstem. . . . [However, there are] moderate to dense projections from the DR [to] pontomesencephalic central gray, mesencephalic reticular nucleus pontis oralis, nucleus pontis caudalis, locus coeruleus, laterodorsal tegmental nucleus, and raphe nuclei, including the central linear nucleus, median raphe nucleus, and raphe pontis' (Vertes and Kocsis, 1994, p. 340). Of particular interest in relation to anxiety disorders, there is serotonergic input to the periaqueductal gray, the hypothalamus, the amygdala, the septo-hippocampal system and the frontal and cingulate cortex (see e.g., Handley, 1995, Fig. 1). As can be seen from Figure XIX-2.1, these structures essentially represent the entirety of the defense system and collectively are involved in the entire spectrum of clinical anxiety disorder.

This anatomy suggests that the aminergic systems are modulatory of other structures in a very general sense. They originate in very small, often caudally placed, nuclei and then innervate huge areas of the forebrain via multiple collaterals. Their fibres, as a group, follow three basic paths (Gaykema *et al.*, 1990; Jacobs and Azmitia, 1992) which 'appear to have remained remarkably stable across phylogeny' (Jacobs and Azmitia, 1992, p. 179). The most ventral innervation of the telencephalon usually involves fibres that send collaterals into the amygdala *en passage* to the temporal portion of the hippocampus. Next there are fibres that pass through the septal area in the medial forebrain bundle and then run in the fornix/fimbria, just under the corpus callosum (the light grey band in Figure XIX-2.2) to innervate the septal portion of the hippocampus. The third path is taken by fibres primarily destined for the cortex. They pass over the corpus callosum peeling off progressively from the cingulum bundle to innervate the neocortex. At its most caudal extent the cingulum bundle provides some fibres that innervate the septal parts of the hippocampus, retrosplenially (Azmitia and Segal, 1978). The dopaminergic system is the most rostral and least diffuse of these systems.

In the case of the noradrenergic system conventional synapses are formed only in the dentate gyrus (Koda, Schulman and Bloom, 1978) and the lack of specialization of the bulk of terminals suggests release of noradrenaline is more neurohormonal than as a neurotransmitter (Descarries, Watkins and Lapierre, 1977; Shimizu *et al.*, 1979).

Nonetheless, there appears to be differentiation of cells even in the locus coeruleus with respect to targets (McNaughton and Mason, 1980). For the other aminergic systems there are discrete components, originating in different nuclei that then have both distinct and overlapping projections compared to other aminergic nuclei.

The amine systems are very similar in anatomy to the ascending cholinergic system. Their similarity to it in function will also be discussed below and, although this does not appear particularly significant for clinical pathology, the amine systems and cholinergic systems have extensive reciprocal interactions with each other. The cholinergic system has a basically similar anatomy to the amine systems but there is better evidence that its connections to the

[1] This section (except for 'Dopaminergic Systems') is a summary of the relevant portions of Appendix 10 of Gray and McNaughton (2000). Many of the ideas are either those of J.A. Gray or arose in discussion with him. Where no reference is given for a statement it can be found at http://www.oup.co.uk/academic/medicine/medical_updates/neuropsych_anxiety/app10/.

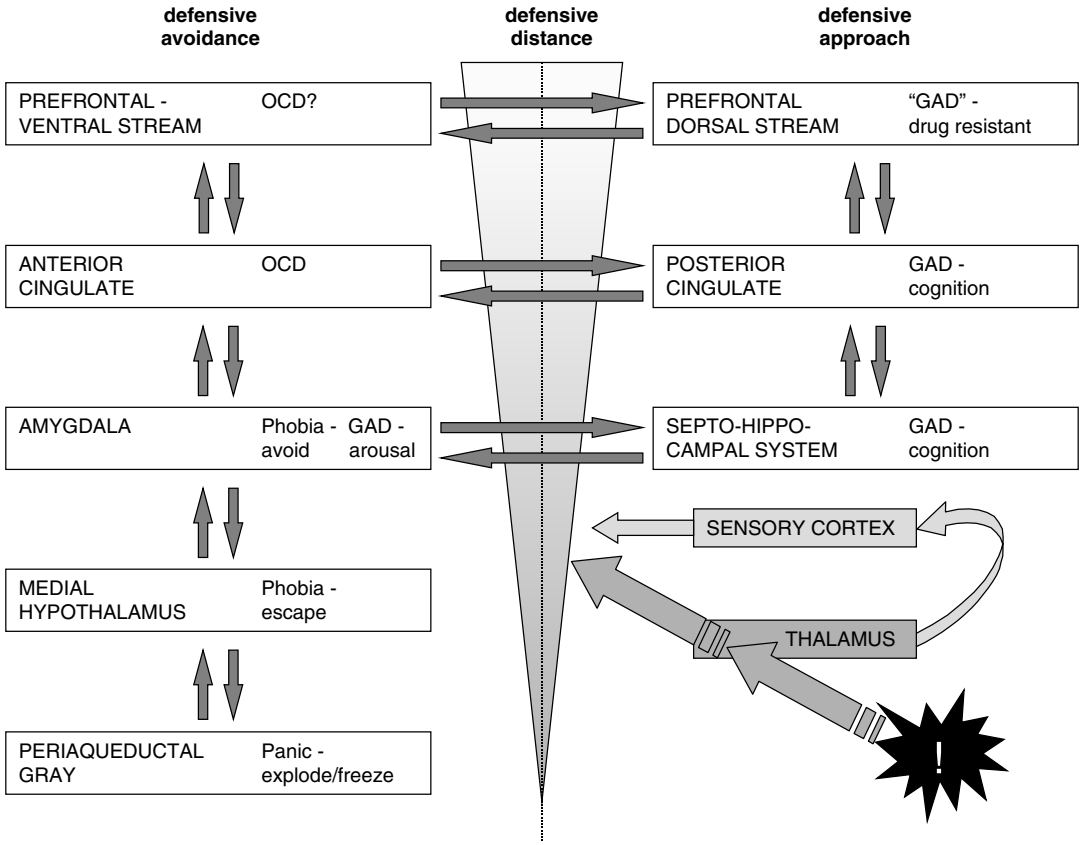

Figure XIX-2.1 A two-dimensional view of the neural systems controlling defense and their relation to neurotic disorders. There is a hierarchy of reciprocally connected neural systems (Graeff, 1994) in which control of behaviour is exerted by the lowest levels when danger is most immediate with control passing to progressively higher levels as the danger becomes less immediate (see also Table XIX-2.3, Figure XIX-2.3). This reflects a dimension of 'defensive distance' (Blanchard and Blanchard, 1989, 1990). In addition there is an essentially orthogonal dimension of defensive direction (Gray and McNaughton, 2000). Avoidance of a dangerous situation and approach into a dangerous situation are controlled by distinct sets of structures. The perception of defensive distance and direction depends on processing by sensory systems that can also be viewed as hierarchical. Signals of danger (!) activate a 'quick and dirty' route through the thalamus that can initiate immediate action if a crude assessment of the situation suggests this is necessary and also activate, in parallel, slower and more sophisticated cortical processing that can confirm or cancel the action or inaction resulting from the crude assessment (LeDoux, 1994, 1998). Figure adapted from Gray and McNaughton (2000), Figures 1.8 and 11.1

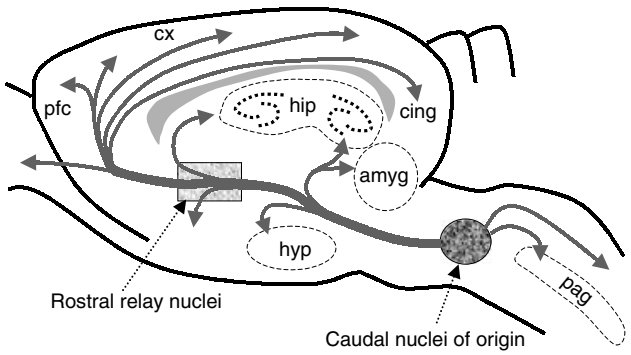

Figure XIX-2.2 A schematic overview of the general anatomy of the aminergic and cholinergic systems. In general, nuclei of origin are relatively caudal and then may relay more rostrally. There tend to be few cells with many collaterals providing diffuse innervation of much of the forebrain. There appears to be some topographic anatomical organization of the systems but relatively little functional differentiation between the parts. The dopamine system is the most rostral, most differentiated, and least diffuse. The cholinergic system originates most caudally, appears functionally least differentiated (see text), and is most diffuse. For the detailed anatomy of each system, see Gray and McNaughton (2000) Figures 6.5, 6.6 and 6.7

forebrain are highly topographically organized (Gaykema *et al.*, 1990, 1992; Zaborszky *et al.*, 1997). The cholinergic system is different from the amine systems in having the most caudally placed nuclei of origin and, possibly as a consequence, having extensive relay nuclei in the basal forebrain. However, functionally, the superficially more differentiated cholinergic nuclei and pathways appear to be part of a coherent, diffuse system originating in the pons (McNaughton *et al.*, 1997, 2001; Swain-Campbell and McNaughton, 2001).

For all the aminergic systems, and the cholinergic system, there is evidence both for a general modulatory role and some capacity of the systems to select subsets of targets of that modulation. The preclinical data on each of these systems is summarized below in alphabetical order followed by an overview of them as contributing to a common general modulation of higher systems. This preclinical summary provides a skeleton for the body of interpretation of the clinically oriented data in the section entitled 'Aminergic involvement in clinical anxiety'.

Cholinergic Systems

Neither anti- nor pro-cholinergic drugs are generally used clinically as anxiolytics. However, systemic or specific intracranial

anticholinergic dysfunctions have not only similar behavioural effects to noradrenergic and serotonergic dysfunction, but also similar neural effects. They all change the signal-to-noise ratio in targets such as the hippocampus and amygdala. Yet, in man, the anticholinergics are amnestic rather than anxiolytic; and some pro-cholinergic drugs may even be anxiolytic (see Brioni *et al.*, 1994; Garvey *et al.*, 1994).

The similar behavioural profiles of nominally 'anxiolytic' and nominally 'amnestic' treatments may reflect a partial overlap in functions. Benzodiazepines block the release of acetylcholine onto cortical targets and this could underlie some but not all of their behavioural effects (Anglade *et al.*, 1994; Sarter and Bruno, 1994). Anticholinergic drugs, by contrast, probably do not produce their amnestic effects via release of endogenous benzodiazepine ligands and there are definite cases where cholinergic dysfunction produces neural effects quite unlike those of anxiolytics (Gray and McNaughton, 2000, Appendix 10).

There are few reports of the effects of specific cholinergic lesions on behaviour. The effects of systemic anticholinergic (usually antimuscarinic) drugs have been well studied (Bignami, 1976; Aigner, 1995) and a superficial resemblance to the behavioural profile of anxiolytic drugs can be seen in the general release of behavioural inhibition they produce (Carlton, 1969). However, they differ from anxiolytics in that they impair some learning tasks in which there is no behavioural inhibition and, consistent with this, acetylcholine is released when simple learning is occurring (Orsetti, Casamenti and Pepeu, 1996).

> 'While there is little doubt that manipulation of cholinergic function can rather specifically alter offensive behaviour in a variety of species, evidence also supports a role for central muscarinic receptors in defensive responding. . . . Antimuscarinics have . . . been reported to inhibit shock-induced defensive fighting in rats and mice . . . Under more naturalistic test conditions . . . scopolamine reduced fear reactions in laboratory rats confronted with a cat . . . reduced fear was indicated by consummatory behaviour in the presence of the cat, more approaches to the cat enclosure and less freezing'.
>
> Rodgers *et al.* (1990, p. 575)

More detailed ethological analysis of the role of anticholinergics in fear and anxiety, respectively suggests they do not alter 'avoidance, freezing, defensive threat or attack in wild *Rattus rattus* confronted by the experimenter and other threat-related stimuli . . . During cat exposure, however, [they] . . . increased the amount of time spent in the vicinity of the cat, increased scanning and rearing, and reduced grooming behaviour. Although reliable, the latter effects were not pronounced' (Rodgers *et al.*, 1990, p. 575). Rather than a genuinely anxiolytic effect this suggests 'a situation- and response-dependent alteration in mechanisms of selective attention' (op. cit. p. 581). However, even in the 'ethological' form of the elevated-plus-maze test of anxiety anticholinergics are anxiogenic (Rodgers and Cole, 1995). Likewise, anticholinergics can reduce fear conditioning to a tone while leaving conditioning to context intact. Hippocampal lesions have the opposite effect, while amygdala lesions affect both (Young, Bohenek and Fanselow, 1995).

Overall then, while the cholinergic system may influence anxiety in a variety of ways these appear to be both anxiolytic and anxiogenic depending on other factors. Like peripheral injections of adrenaline or the partial pressure of carbon dioxide, then, it appears to be able to influence central systems controlling anxiety without being a fundamental component of such systems.

Dopaminergic Systems

There are two main dopaminergic systems innervating the forebrain that only partially conform to the picture of Figure XIX-2.2. 'A dorsal tier of dopamine neurons receive input from the ventral (limbic-related) striatum and from the amygdala and [largely as the mesolimbic system] project widely throughout cortex. A more ventrally located group of dopamine cells receives input from both the limbic and association areas of striatum and projects widely [largely as the nigro-striatal system] throughout the striatum, including the sensorimotor region. Through these projections the limbic system has an enormous influence on dopamine output and can therefore affect the emotional and motivational "colouring" of a wide range of behaviours' (Haber and Fudge, 1997). We will focus here on the more ventrally coursing mesolimbic system and, in particular, its projections to prefrontal cortex (PFC). While strongly implicated in schizophrenia, this input to prefrontal cortex appears also to be involved in anxiety and depression.

The initial component of the ventral system is the dopaminergic input from the ventral tegmental area (VTA) to the nucleus accumbens (or more fully nucleus accumbens septi, NAS). This system is presented in texts as being a 'reward pathway'. However,

> 'Stress consistently has been found to activate peripheral and central catecholamine systems. Dopamine turnover in the prefrontal cortex is especially sensitive to stress produced by relatively mild footshock, conditioned fear or exposure to a novel cage. . . . electrolytic lesions of the central nucleus of the amygdala significantly attenuated the increase in DA turnover in the prefrontal cortex normally seen after mild stress. . . . Dopamine neurons in the ventral tegmental area receive direct input from the central nucleus of the amygdala . . . Stress may activate . . . the central nucleus of the amygdala. In turn this could activate dopamine cells in the ventral tegmental area that project to prefrontal cortex and thereby increase dopamine turnover in this brain area.'
>
> Davis *et al.* (1994)

We will deal later with the peculiarity that stress increases PFC dopamine by double the amount of NAS dopamine (which in turn is about double the amount of striatal dopamine release) in contrast to the fact that 'the noradrenergic projections to forebrain appear to respond to stress in a uniform manner' (Abercrombie *et al.*, 1989). What is needed at this point is

> 'a unifying interpretation that can account for the functions of NAS DA in a variety of behavioural contexts: (1) its role in appetitive behavioural arousal, (2) its role as a facilitator as well as an induced of reward processes, and (3) its presently undefined role in aversive contexts. . . . NAS DA [appears to] facilitate flexible approach responses. . . . Fixed instrumental approach responses (habits) . . . involve the nigro-striatal system more. . . . NAS DA [operates] in two stages: unconditioned behavioural invigoration effects and incentive learning effects. (1) When organisms are presented with salient stimuli (e.g., novel stimuli and incentive stimuli), NAS DA is released and invigorates flexible approach responses. (2) When proximal exteroceptive receptors are stimulated by unconditioned stimuli, NAS DA is released and enables stimulus representations to acquire incentive properties with specific environmental context [*sic*]. . . . Both conditioned and unconditioned aversive stimuli stimulate DA release in the NAS [because] NAS DA invigorates *approach responses* toward 'safety'. Moreover, NAS DA modulates incentive properties of the environment so that organisms emit *approach responses* toward 'safety' (i.e. avoidance responses) when animals later encounter similar environmental contexts.'
>
> Ikemoto and Panksepp (1999) [my italics]

In this view, dopamine release is not a sign of aversion even in aversive paradigms. Indeed 'brief aversive foot shock delivered for the first time does not increase NAS DA, but NAS DA levels increase more and more as, . . . with succeeding trials, animals develop more effective approach to safety strategies' (op. cit.). The increase in dopamine release during avoidance learning to

some extent parallels that of corticosterone — which ceases to be released as avoidance becomes perfect, and so shock is no longer delivered (Coover, Ursin and Levine, 1973). However, with repeated inescapable stress, dopamine is not released during the stress but only after it. In this, 'the activation of the mesolimbic dopaminergic system induced by aversive stimuli adapts to repeated experiences differently from that produced by pleasurable events' (Imperato *et al.*, 1992). It also contrasts with both noradrenaline and corticosterone. Like dopamine, they are not released by shock initially. But unlike dopamine they continue to be released in response to conditioned fear (Brady, 1975a, 1975b).

Thus, in aversive paradigms the release of dopamine appears to equate with 'relieving non-punishment'. It is released by the omission of an expected punisher. Such omission can been equated functionally with reward (Gray, 1975) since stimuli associated with either will elicit approach and are functionally antagonistic to aversive events. In this view the 'anxiolytic' effects of D2 agonists (Bartoszyk, 1998) may reflect the antagonistic relationship between punishers and rewards when these are associated — an antagonism that is not affected by conventional anxiolytic drugs (McNaughton and Gray, 1983). Conversely, desensitization of these receptors by, for example, repeated methamphetamine administration could have the inverse effect (Tsuchiya, Inoue and Koyama, 1996).

The peculiarities of the PFC dopaminergic input remain to be dealt with. As noted above this often shows a greater release of dopamine to aversive stimuli than does the NAS. More surprisingly, unstressed rats can show a DA increase in PFC to both appetitive and aversive stimulation at times when the NAS shows an increase to appetitive but a decrease to aversive stimuli (Di Chiara, Loddo and Tanda, 1999). After chronic mild stress, the responses to appetitive stimuli decreased and to aversive stimuli increased in both areas — with the NAS response to aversive stimuli reversing to the increase commonly observed.

> 'We have proposed that NAS shell DA is involved in associative learning and in the acquisition of motivation, whereas PFC DA is involved in its motor expression. . . . [It is through] phasic DA transmission in the NAS shell . . . [that] stimuli are attributed a motivational value that reflects the biological value of the stimulus . . . [hence] D_1 antagonists . . . impair the acquisition of conditioned place preference and place aversion. . . . This hypothesis interprets the symptoms of anhedonia, lack of interest, and loss of self esteem that are typical of depression as the result of blunting of appetitive motivation; it interprets feelings of worthlessness and guilt . . . as the result of enhancement of aversive motivation. . . . [These] changes in DA responsiveness are [likely to be] secondary to a primary dysfunction of tonic noradrenergic transmission. . . . The relative ineffectiveness of dopamine agonists as antidepressants [is because] increase of tonic DA transmission by a DA agonist would not correct the dysfunction in phasic DA responsiveness that takes place in depression.'
>
> Di Chiara *et al.* (1999)

Thus, at least part of the distinctive dopamine release in PFC would be due to increased noradrenaline release acting on dopaminergic terminals presynaptically. Increased noradrenaline release in PFC increases dopamine levels and decreased noradrenaline release decreases dopamine levels, suggesting a tonic modulation (Gresch *et al.*, 1995). Further, an initial brief experience of stress can release noradrenaline without dopamine or serotonin, with the latter released in addition on a second experience (Jordan *et al.*, 1994). Release of noradrenaline may then produce sensitization of dopaminergic and serotonergic terminals. However, part could also be due to selective activation of a small pool of VTA and NAS cells projecting only to PFC, as occurs with purely cognitively mediated stress in the absence of concurrent release of noradrenaline or serotonin (Kaneyuki *et al.*, 1991). These authors even suggest that inhibition of VTA DA neurons may be 'implicated in the specific anxiolytic action of benzodiazepines'. Certainly, very large doses of benzodiazepines reduce stress-induced dopamine release in PFC (Feenstra, Botterblom and Van Uum, 1995) and, like the anxiolytic effects of benzodiazepines, this does not show tolerance (Hegarty and Vogel, 1995). But chronic stress eliminates the effect (Finlay, Zigmond and Abercrombie, 1995).

There are three main reasons for rejecting this point of view. First, is the fact that prefrontal lesions are effective in treating anxiety that is resistant to conventional anxiolytic drugs. Anxiolytic action on prefrontal dopamine could be a component of their effects — but prefrontal cortex clearly contains additional anxiety-related mechanisms. Second, rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty found the response was graded to the general intensity rather than valence of stimuli with 'emotional arousal [being] a common denominator'. Interestingly, in this study dopamine release outlasted, e.g., eating by 10–20 min (Feenstra and Botterblom, 1996). Third, data (Espejo, 1997) that suggest that PFC DA 'activation reflects either heightened attention or activation of cognitive processes [that is] a general phenomenon in various adaptive situations'. PFC DA depletion *increased* + maze anxiety while decreasing motility. 'The findings confirm that prefrontocortical dopamine activation is necessary for coping with an anxiogenic challenge, allowing the animal to display adaptive exploratory responses in a fear-inducing environment' (op. cit.).

Thus, for prefrontal dopamine we seem justified in borrowing the conclusion of Ikemoto and Panksepp for NAS dopamine. 'Both conditioned and unconditioned aversive stimuli stimulate DA release [to] invigorate approach responses toward "safety" [and] modulate incentive properties of the environment so that organisms emit approach responses toward "safety" (i.e. avoidance responses) when animals later encounter similar environmental contexts.'

Noradrenergic Systems

The locus coeruleus is the nucleus of origin of ascending noradrenergic fibres that has been most clearly implicated in the control of anxiety. Further, receptors for the benzodiazepine-GABA complex exist in the locus coeruleus and probably mediate some part of the action of classical anxiolytics (Iversen and Schon, 1973). A major output from the locus coeruleus travels in the dorsal noradrenergic bundle (DANB) and follows the general plan of Figure XIX-2.2, reaching frontal cortex, cingulate cortex, pyriform cortex, hippocampal formation, amygdala, thalamus, hypothalamus and basal forebrain. A second output, the ventral noradrenergic bundle travels more ventrally and innervates more ventral structures including an innervation of the hypothalamus and septum that overlaps that of the dorsal bundle (Moore and Bloom, 1979; Owen *et al.*, 1982). The ventral bundle also carries fibres from other noradrenergic nuclei.

The anatomy of the ascending noradrenergic system limits its possible functions. There are only about 1500 cells in the locus coeruleus of the rat and these innervate huge regions of the brain, including the olfactory bulb, much of the neocortex, the hippocampus, septal area and amygdala, some thalamic and hypothalamic nuclei, the geniculate bodies, the cerebellum, and the spinal cord. Although there is some topographic organization, each cell projects to multiple areas (Olson and Fuxe, 1971; Pickel, Krebs and Bloom, 1973). The system cannot, therefore, carry large volumes of data and is likely to be generally modulatory.

Consistent with this, noradrenergic activity increases throughout the brain in response to aversive stimuli (Segal, 1978) and stress, and specifically to increases in corticotropin-releasing hormone (Valentino, Foote and Page, 1993; Smagin, Swiergiel and Dunn, 1995), with a significant part of this being due to outflow from

the locus coeruleus (Corrodi *et al.*, 1971). This increase may be a significant component of stress-induced anxiety since it is blocked by classical anxiolytics. Novel anxiolytics tend to increase locus coeruleus firing (Trulson and Henderson, 1984; Wilkinson *et al.*, 1987) but this appears to be an effect of feedback from presynaptic blockade of noradrenergic terminals by $5HT_{1A}$ agonists. So, all classes of anxiolytic drug appear to block the release of central noradrenaline.

However, novel stimuli and reward can also release noradrenaline. Like dopamine, then, it seems less specifically concerned with aversion than generally related to arousal in the sense of activation of the ascending reticular activating system or the regulation of attention or vigilance (Aston-Jones, Chiang and Alexinsky, 1991).

A role in vigilance would account for changes in noradrenergic output in relation to sleep phases without a role for noradrenaline in the control of sleep itself and for the release of noradrenaline by unconditioned stimuli and 'by stimuli which are not themselves intense or conspicuous, but are salient to the animal by virtue of conditioning' (Aston-Jones *et al.*, 1994, p. 4468). Thus, when the significance of stimuli is changed, noradrenergic cell firing shifts to the new target stimulus (Aston-Jones *et al.*, 1994; see also Rajkowski, Kubiak and Aston-Jones, 1994).

Detailed comparison of the effects of anxiolytic drugs with lesions of the DANB (Table XIX-2.2) shows some interesting parallels and dissociations. Where anxiolytics are without effect so too are DANB lesions. Likewise, where DANB lesions have an effect it is essentially the same as those of anxiolytics drugs, in size as well as nature. However, there are a substantial number of anxiolytic-sensitive tasks where DANB lesions are totally without effect. A review of the electrophysiological evidence suggests that inhibition of release of noradrenaline by anxiolytic drugs (acting at cell bodies in the case of classical anxiolytics and at terminals in the case of novel anxiolytics) accounts for part of the behavioural profile of the anxiolytics. This could include the slight pro-convulsant effect of buspirone and anxiolytic SSRIs (Ferraro *et al.*, 1994; Kokaia *et al.*, 1994).

Table XIX-2.2 Comparison of the common effects of classical and novel anxiolytics (Anx), common effects of lesions of the septum and hippocampus (S = H), lesions of the amygdala (Amyg), lesions of the dorsal ascending noradrenergic bundle (NA), lesions of the ascending serotonergic system (5HT) and systemic blockade of muscarinic cholinergic systems (ACh). Adapted from Gray and McNaughton (2000)

	Anx	S = H	Amyg	NA	5HT	ACh
Simple rewarded learning	0	0	0	0	0	−
Simultaneous spatial discrimination	0	0	?	0	?	?
Defecation	0	0	?	0	?	?
Escape	0	0	−	0	0	?
Frustration	0	0	−	0	?	?
1-way avoid	0	0	−	0	?	0
Aggression	+	−	−	0	−	?
Fixed Interval	−	−	0	0	?	0
Water maze	−	−	0	0	?	−
Reversal	(−)	−	0/−	0	?	?
DRL	−	−	−	0	−	−
Social Interaction	−	−?	−	0	−	?
Passive avoidance	−	−	−	0/−	−	−
Non-spatial Avoidance	+	+	?	0	?	+
2-way avoidance	+	+	−	0/+	?	+
PREE	−	−	0	−	?	?
Rearing	−	−	+	−	−	−
Conditioned freezing	−	−	–	−	?	?
Extinction	−	−	−	−	−/0	−
Successive discrimination	−	−	−	−	0	−
Single alternation	−	?	−	−	?	?
Spontaneous alternation	−	−	−	−	?	−
Conditioned suppression on baseline	−	0	−	−	−	?

A partial involvement of noradrenaline in the effects of anxiolytic drugs and, by implication, the control of anxiety is understandable if it is a quite general arousing system similar in function to the old idea of the reticular activating system (Magoun, 1963). Such arousal, in aversive situations, would normally be termed alarm and even, in some meanings of the word, 'anxiety'. Thus, release of noradrenaline, centrally, is like activation of the sympathetic system peripherally (Aston-Jones, Chiang and Alexinsky, 1991; Van Bockstaele and Aston-Jones, 1995; Haller, Makara and Kruk, 1998).

On the other hand, noradrenergic cells 'decreased tonic discharge . . . during certain high arousal behaviours (grooming and consumption) when attention (vigilance) was low. . . . The most effective and reliable stimuli for eliciting LC responses were those that disrupted behaviour and evoked orienting responses' (Aston-Jones, Chiang and Alexinsky, 1991, pp. 501). So, 'LC cells respond to novelty or change in incoming information, but do not have a sustained response to stimuli, even when these have a high level of biological significance' (Sara, Vankov and Hervé, 1994). Central release of noradrenaline would, then, increase vigilance to external events consistent with the fact that it increases the neural signal-to-noise ratio (Gray & McNaughton, 2000, Appendix 10).

The effect of noradrenaline is not limited to stimulus processing. Its release also increases the vigour of ongoing behaviour (Aston-Jones *et al.*, 1994), another echo of the effects of dopamine. The noradrenergic system, then, appears important for general vigilance and reactivity to stimuli (including, of course, aversive stimuli) but does not equate with arousal in the sense that this can be high when external stimuli are being ignored.

Of particular relevance to clinical anxiety and its relation to depression is the specific case of such effects of noradrenaline on aggressive behaviour.

> 'Hormonal catecholamines (adrenaline and noradrenaline) appear to be involved in metabolic preparations for the prospective fight; the sympathetic system ensures appropriate cardiovascular reaction, while the CNS noradrenergic system prepares the animal for the prospective fight. Indirect CNS effects include: the shift of attention towards socially relevant stimuli; the enhancement of olfaction. . . ; the decrease in pain sensitivity; and the enhancement of memory (an aggressive encounter is very relevant to the future of the animal). . . . [However, while] a slight activation of the central noradrenergic systems stimulates aggression, a strong activation decreases fight readiness. This biphasic effect may allow the animal to engage or to avoid the conflict, depending on the strength of social challenge.'
>
> Haller, Makara and Kruk (1998)

Serotonergic Systems

Central serotonin has previously been postulated to be involved in anxiety (Iversen, 1984) and in specifically anxiolytic action (Moon *et al.*, 1994) but also appears implicated in many other psychiatric disorders (Swerdlow, 1995; Boulenguez *et al.*, 1998; Collier *et al.*, 1996; De Oliveira *et al.*, 1998; Dean *et al.*, 1995; Gobert *et al.*, 2000; Gurevich and Joyce, 1997; Laruelle *et al.*, 1993; Leonard, 1996; Naylor *et al.*, 1996; Sumiyoshi *et al.*, 1996). The serotonergic system conforms to the general plan of Figure XIX-2.2. However, there is noticeable anatomical differentiation within it that is significant for the possible role of different areas in the causes of anxiety. This differentiation cuts across the differentiation of receptor subtypes that is significant for the treatment of anxiety.

> 'The median raphe supplies the dorsal hippocampus and medial septum, the proposed origins of the Behaviour Inhibition System, while the dorsal raphe nucleus innervates the lateral septum and ventral hippocampus, the possible origin of safety signalling. The amygdala, the "head nucleus" of the Defence System, is almost entirely supplied by the dorsal raphe nucleus.'
>
> Handley (1995, p. 108–109)

This differentiation is not complete, as there are minor contributions of, e.g., the dorsal raphe to the septo-hippocampal system. In particular, the dorsal raphe sends projections (which may well be collaterals of the same source cells and hence carry the same information} to the periaqueductal gray (5HT2/1C receptors); ... the amygdala (5HT1A receptors), the ventral striatum (5HT1D receptors), the hippocampus (5HT1A receptors), and the frontal cortex (5HT2 receptors). It can therefore have widespread effects on the defense system.

The dorsal raphe does not appear totally committed to defense, however, since it seems 'to play a role in modulating circadian rhythms ... [and] these modulations may be, in part, mediated by the [direct] retinal projection to the periaqueductal gray and serotonin neurons in the dorsal raphe nucleus' (Shen and Semba, 1994, p. 166; see also review by Morin, 1994). Although there are fewer data on central serotonergic lesions than noradrenergic there are enough to reinforce this conclusion. Inspection of Table XIX-2.2, shows that to a large extent, like noradrenergic lesions, serotonergic lesions have no effect when anxiolytics have an effect and that when serotonergic lesions have an effect so do anxiolytics. Also, as with noradrenergic lesions, there are many cases where anxiolytics have an effect but serotonergic lesions do not. Strikingly, these blanks in the serotonergic and noradrenergic profiles appear to complement each other so that the effects of anxiolytics could, perhaps, be accounted for by concurrent suppression of central noradrenergic and serotonergic function. If this is true then neither system can, by itself, be the basis for anxiety.

Table XIX-2.3 Levels of threat processing and related levels of neural integration. Adapted by Gray and McNaughton (2000) from Graeff (1994)

Level of processing	Behaviour	Neural substrate
Potential danger (to approach)	Risk assessment and Behavioural Inhibition	Posterior Cingulate Septo-Hippocampal System
Potential danger (to avoid)	Avoidance	Anterior Cingulate Amygdala
Distal danger	Escape Inhibition of Aggression	Medial Hypothalamus
Proximal danger	Freezing Flight Fight	Periaqueductal grey

Graeff (1993) (see also Graeff *et al.*, 1996; Graeff and Zangrossi, Chapter XIX-1) provides a detailed analysis of the role of serotonin in the defense system. His starting point is a unidimensional hierarchical view of threat processing which in a slightly modified form (Table XIX-2.3) is the foundation on which the two-dimensional Figure XIX-2.1 was constructed and a further dimension of avoidability could also be added (Figure XIX-2.4). Figure XIX-2.3 shows his picture of how serotonin (5HT) interacts with the lower levels of the defense system.

The key point is that the periaqueductal gray, medial hypothalamus and amygdala will often be activated together by threatening stimuli. But, explosive undirected escape or aggression (periaqueductal gray) is inappropriate if avoidance (amygdala) or directed escape (medial hypothalamus) is possible. As indicated in

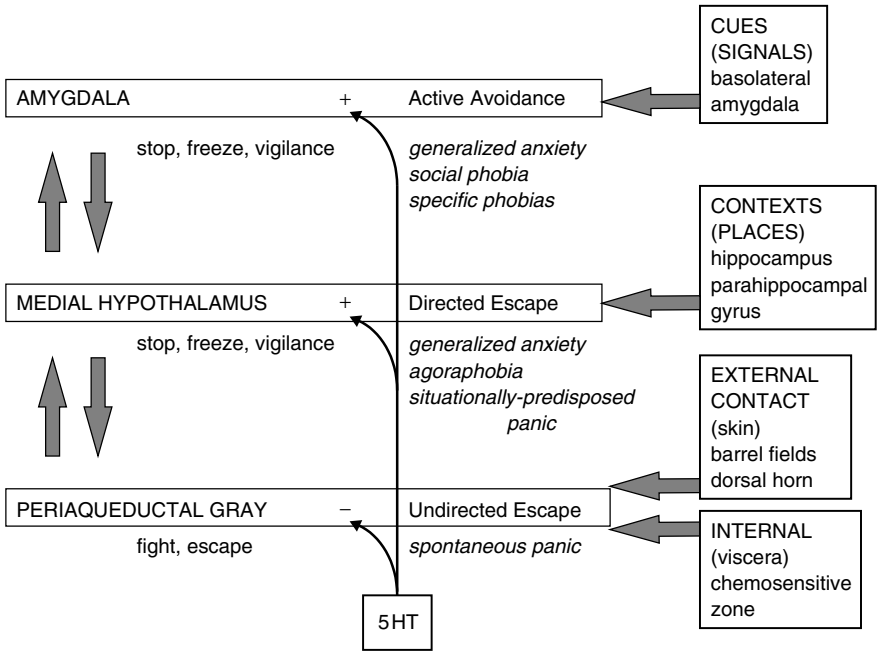

Figure XIX-2.3 The role of serotonin (5HT) in the control of anxiety as proposed by Deakin and Graeff (1991). The opposing effects of input to PAG, compared to structures higher in the defense hierarchy, provides an explanation of the paradoxical effects of some treatments and for phenomena such as panic associated with relaxation or sleep. Note that Deakin and Graeff localize generalized anxiety to the amygdala and hypothalamus and view the hippocampus as a purely sensory structure. This is essentially the same as the view of LeDoux (1994) but differs from Figure XIX-2.1. However, these differences of detail do not have major consequences for the predictions to be derived from the Deakin and Graeff model or for the general principles of operation they attribute to the serotonin system

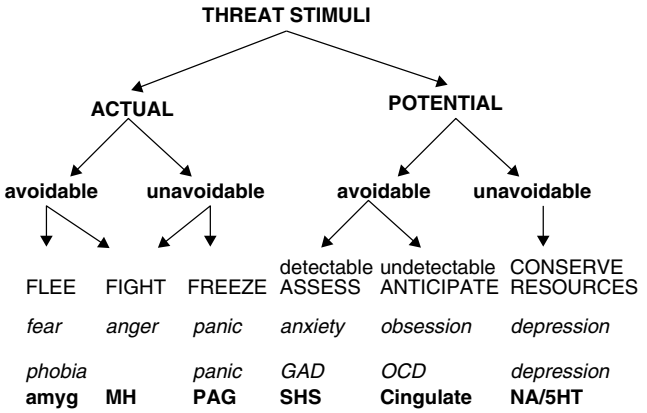

Figure XIX-2.4 The functional differentiation of threat stimuli and its likely relation to neural systems. The stimuli are categorized into different functional classes in the top three rows. The specific functional output required by each class is shown in the fourth row (capitals) and its relation to nominal emotions in the fifth row (small italics). It should be noted that these emotions are presumed to be usually functionally adaptive. The related psychological disorders are given in the sixth row (large italics) and the principle neural system involved in pathology or symptomatology as well as normal function is indicated in the last row. Amyg, amygdala; MH, medial hypothalamus; SHS, septo-hippocampal system; NA/5HT changes in the balance between the monoamine systems. From Gray and McNaughton (2000), Figure 11.2

the figure, (see also Deakin and Graeff, 1991) serotonin shifts the balance between the structures. It 'facilitates defensive behaviour elicited by potential or distal danger signals ... by acting on the amygdala, but, in the periventricular system, inhibits the expression of fight/flight responses that are adaptive only when the threat stimulus is proximal to the animal' (Graeff, 1993). In humans also, 'measures that are believed to represent conditioned anxiety were increased by D-fenfluramine, ... a drug that seems to release 5HT selectively from terminals of the dorsal raphe, ... whereas those thought to reflect unconditioned fear were attenuated' (Graeff, Viana and Mora, 1997). This idea of antagonism between anxiety and panic (at least at low levels of anxiety) also accounts for such paradoxical phenomena as relaxation-related panic attacks (Graeff, 1994).

A similar differential effect, coupled to receptor- or channel-linked polymorphisms may underlie the occasional paradoxical effects of SSRIs. These are usually beneficial in patients with panic. However, fluoxetine can occasionally exacerbate pre-existing panic and Altshuler (Altshuler, 1994) reported two cases where fluoxetine induced panic *de novo* that continued as non-remitting spontaneous panic attacks (see discussion of kindling below) in two patients with no prior history of panic. He noted that 'in the two cases presented, the timing of the onset of the panic attacks (within 10 days of medication) is certainly earlier than the time of onset of the [usual therapeutic] action'. Thus, variation in differential effects across the defense system could result from different rates of receptor adaptation and/or sensitization in different parts of the defense system to serotonin released from collaterals of the same neurons onto different receptor subtypes.

The release of serotonin (and the firing of raphe cells) seems to prime tonic or repetitive motor circuits while suppressing phasic, orienting, startle, and related circuits. This priming also has major effects on circuits (primarily in the septo-hippocampal system) whose business is to *inhibit* ongoing, tonic motor circuits. Thus, the firing of serotonin cells will often be functionally silent; and the effects of serotonin on behavioural inhibition, for example, will become functionally evident only when other conditions are fulfilled. One way to look at this is to view the serotonin signal as increasing 'motor attention', an effect that will have obvious behavioural consequences only when an event occurs to interrupt the motor programme.

Despite their involvement in the processing of threat, then, serotonin systems (like dopamine and noradrenaline) cannot be seen as threat promoting or threat inhibiting as such.

The situation is rendered even more complex by the effects of drugs active at different serotonin receptors. The receptor subtypes cut across the distinct neural systems we have been discussing so far and so select out only part of the action of serotonin with respect to each system while having effects general to all systems. This problem is particularly acute with $5HT_{1A}$ receptors. These are the principal targets of novel anxiolytics such as buspirone and the SSRIs. The drugs act as serotonin agonists in the sense of acting on the $5HT_{1A}$ receptor in the same way as serotonin but, in a number of areas, these receptors are autoreceptors involved in inhibitory feedback and so have an inhibitory effect on overall serotonergic output. Agonists at other serotonin receptors tend to have opposite effects to $5HT_{1A}$ agonists (Aprison and Ferster, 1961; Graeff and Schoenfeld, 1970; Stein, Wise and Berger, 1973). An important point for understanding possible genetic differences in vulnerability to anxiety disorders is that each receptor is a potential target of genetic modulation or deletion and that disorder may result as much from a disruption of normal balance between components of the serotonin system as from loss of a specific receptor-mediated action. A brief summary of the receptors is given in Table XIX-2.4.

Table XIX-2.4 Serotonin (5HT) receptor subtypes. For reviews see Griebel (1995), Saxena (1995), Zifa and Fillion (1992), Wilkinson and Dourish (1991), Uphouse (1997) and Meneses (1999)

5HT-1A	Anxiolytic at low doses. Action both at autoreceptors (dorsal and median raphe) and presynaptically (septum, hippocampus, amygdala). This results in a combination of functional 5HT-agonist-like and functional 5HT-antagonist-like actions.
5HT-1B	Occur in rodents but not humans.
5HT-1C	Now reclassified as part of the 5HT2 family, see 5HT2C.
5HT-1D	Likely to be a homologue of 5HT-1B. High density in the basal ganglia, lower in the hippocampus, neocortex and raphe nuclei. Function unknown. Peripherally involved in vasodilation. Antagonists useful in migraine.
5HT-2A	May be separate receptor or a separate state of the 5HT2B (q.v.).
5HT-2B	(and 5HT-2A) are distinct from 5HT2C. Highest density in the hippocampal formation, frontal cortex, cingulate cortex, nucleus accumbens, hypothalamus and mammillary bodies. Moderate density in the basal ganglia. The mixed 5HT2/1C antagonist, ritanserin, improves generalised anxiety disorder but worsens panic (see Graeff, 1993; see also Figure XIX-2.3 and Griebel, 1995, p. 374). 5HT2 receptors are also involved in the regulation of sleep, temperature and some aspects of motor control.
5HT-2C	(originally 5HT-1C). Highest density in the choroid plexus. Function unknown (but see Zifa and Fillion, 1992, p. 432).
5HT3	Highest density in the area postrema. Low, but detectable levels in septum, hippocampal formation, frontal cortex, cingulate cortex, nucleus accumbens, amygdala, thalamus and hypothalamus. Antagonists initially suggested to be anxiolytic but main current regular use is as anti-emetics (Saxena, 1995).
5HT-4	Agonists have effects on gastric motility.
5HT5+	for 5HT-*n* with $n > 4$ there is virtually no current evidence of function but they may be involved in learning and memory, possibly via modulation of cholinergic function (Meneses, 1999).

Neural sites are not identifiable with specific receptor subtypes. 5-HT_{1A}, 5-HT_2, and 5-HT_3 occur in the septo-hippocampal system, in the amygdala (5-HT_{1A}, 5-HT_3); in the frontal and cingulate cortex and in the nucleus accumbens, and hypothalamus (5-HT_2, 5-HT_3). Except for 5-HT_{1A} and 5-HT_3, most types are found in the basal ganglia. It seems unlikely that specific ligands will have specific, coherent functions (see also Griebel, 1995).

Under normal conditions, indeed, different levels and patterns of activation of relatively diffuse serotonergic projections could have fairly specific effects on particular areas. They could also affect particular functions within areas through the differences among receptor subtypes. The receptors can differ in their affinity (allowing, e.g., opposite effects to different concentrations of transmitter); in their transduction pathways (targeting different second messenger systems or rates of change); in their amount of desensitization/downregulation (allowing differential changes in response to acute as opposed to chronic serotonin release); and in their reaction to the cellular environment, including interaction with other serotonin receptors (Uphouse, 1997).

Like the cholinergic, dopaminergic and noradrenergic system, then, the serotonergic system is in a position to modulate the function of limbic structures and to influence the processing of threatening stimuli and the motor output resulting from them. Of all the systems we have considered so far it has the closest involvement in fear and anxiety. However, also like the noradrenergic system, there is no reason to see the serotonergic system as having a function specifically dedicated to defense as such — although specific receptor ligands could, in principle, have much more selective targets. Conversely, some selective ligands may have more influence on learning and memory than anxiety (Meneses, 1999).

Interactions of the Systems

The neuroanatomy and primary physiological effect (an increase in signal-to-noise ratio) of the systems we have considered is very similar. They may, then, each discharge an essentially similar function, but triggered by different environmental conditions.

In many cases the critical conditions may partially overlap and the systems act synergistically as with spatial learning (Decker and Gallagher, 1987). In some, there is evidence that noradrenaline produces its effects by releasing acetylcholine (Dalmaz, Introini-Collison and McGaugh, 1993) or that acetylcholine acts to increase the release of noradrenaline (Engberg and Hajos, 1994). Likewise, there are synergistic interactions between central serotonergic and cholinergic systems (Riekkinen, Sirvio and Riekkinen, 1990) and many 'histological, electrophysiological, pharmacological and behavioural data suggesting that serotonin is able to modulate central cholinergic function and that this modulation may have, in some respects, cognitive implications' (review by Cassel and Jeltsch, 1995, p. 31). Indeed, one study that recorded from putative cholinergic, noradrenergic and serotonergic neurons concluded 'the rapid cholinergic system controls the general condition of the brain (including sleep and wakefulness), cooperating with the "slow" noradrenergic and serotonergic systems. The three systems, which may interact mutually, may share the function of "the ascending reticular activating system" ' (Koyama, Jodo and Kayama, 1994, p. 1030). We also noted, in the section 'Dopaminergic Systems', interactions between the systems that were often quite local (e.g., presynaptic effects of noradrenaline on dopaminergic cells in PFC).

This is not to say that these systems or their interactions are solely concerned with sudden changes of state of the sort that would characterize normal anxiety in response to ecologically valid threatening stimuli. As is extensively dealt with elsewhere in this book, both serotonin and noradrenaline have important separate involvements in responses to stress, which may underlie the therapeutic effects of their specific re-uptake inhibitors in depression. There may also be important interactions between them in determining the behaviour of depressed people, for example, 'the expression of tyrosine hydroxylase [which controls noradrenaline synthesis] in locus coeruleus may be relevant in the pathophysiology of suicide' (Ordway, Smith and Haycock, 1994, p. 680).

Preclinical Overview of the Aminergic Systems

Combining all of the preclinical data reviewed in this section, we can arrive at a relatively simple picture of the aminergic systems as a whole (and of the similar cholinergic system) — despite the complexities of each of their component parts. Taken together, they appear to function to prime sensory-motor systems for action. At the neural level this represents an increase in signal-to-noise ratio, decreasing the effectiveness of ongoing background processing while increasing the response to any higher level upcoming input. Importantly, their activity, on this view, will only have major functional effects if there is such an upcoming signal to process. Much of the time changes in aminergic or cholinergic activity may be functionally silent.

The systems differ in the circumstances under which they produce such priming. Cholinergic systems act on a short time scale to increase the acuity of the processing of the sensory stimuli (and their associations/'memories') likely to be crucial for upcoming action. Dopaminergic systems act to increase the vigor of current approach responses and increase the probability of future approach responses (including, in both cases, approach to safety). Noradrenergic systems act, like a central 'sympathetic nervous system', to prime systems for upcoming sudden, unexpected action, in particular orienting responses. This priming involves an increase both in acuity of processing and in the vigor of upcoming motor output. Serotonergic systems are most strongly associated with motor output as opposed to sensory processing and prime systems for upcoming actions that cope with common repeated requirements.

To put this another way:

> 'The serotonergic system will be more concerned to prevent the dominant motor programme (e.g., avoidance) from being interrupted by concurrent activation of some other motor programme (e.g., escape); the noradrenergic system will be more concerned to prevent the dominant controlling stimulus from having its control of behaviour interrupted by other concurrent stimuli; and the cholinergic system will be more concerned to prevent the current-to-be-associated stimulus from having its associative connections interrupted by other concurrently activated associations (Vinogradova *et al.*, 1993). These increases in signal-to-noise ratio will have functional effects only if the target structure is processing a signal and if the result of that processing is a functional output.
>
> In achieving these different effects, we can assume that the aminergic systems produce largely similar direct neural effects, but produce their different patterns of response through requiring different adequate stimuli for their activation. We can also assume that the fundamental effect of release of transmitter by the systems is not only similar between them but very simple. The apparent complexities of the effects of drugs and lesions are then attributed to the complexities of the functions of the various target areas.'
>
> Gray and McNaughton (2000, Appendix 10)

AMINERGIC INVOLVEMENT IN CLINICAL ANXIETY

Determining the functional role of aminergic transmitter systems in anxiety disorders is complicated by at least six methodological issues.

First, is the problem of localization of action. This can be exemplified by panic. Related compounds, such as adrenaline

(epinephrine) when present in the blood stream can induce high levels of arousal and hence panic (Veltman *et al.*, 1996, 1998) while the state of platelet adrenergic receptors can influence panic (Gurguis *et al.*, 1999). Likewise, peripheral physiological challenges such as an increased partial pressure of CO_2 can induce panic. Interpretation of the effects of any pharmacological manipulation or state-related biochemical marker on any aspect of any anxiety disorder must therefore allow for the fact that a non-specific peripheral change rather than a specific central change may be the basis of observed effects. Likewise, even if a central action can be demonstrated, it may be as non-specific as a peripheral challenge.

Second, the three monoamine transmitters share a common enzyme that destroys them in the synapse and so renders them ineffective: monoamine oxidase (MAO). Any pharmacological changes (produced by monoamine oxidase inhibitors, MAOI) or effects of genetic differences in MAO-related systems (Deckert *et al.*, 1999) can be due to changes in any or all of the monoamine systems. Of course, MAO itself is a perfectly respectable aetiological candidate — and will be considered separately from the individual monoamines below, as will catechol-0-methyl transferase (COMT) an enzyme that breaks down dopamine and noradrenaline but not serotonin.

Third, dopamine is a precursor to noradrenaline. Treatments that affect dopamine synthesis (e.g., α-methyl-p-tyrosine) will therefore affect noradrenaline synthesis as well. Demonstration of specificity to one or the other transmitter will therefore require additional manipulations (e.g., administration of di-hydroxy-phenylserine that acts as a precursor for noradrenaline but not dopamine).

Fourth, as discussed in the section 'Interactions of the system', there are clear neural interactions between the systems. This is a particular problem in assessing drugs that act presynaptically. Not only may, for example, a $5HT_{1A}$ agonist have the primary effect of releasing noradrenaline but, concurrently, in a different part of the brain it could act to inhibit the release of noradrenaline or one of the other aminergic systems or the cholinergic system.

Fifth, such a presynaptic agonist may, again as exemplified by $5HT_{1A}$, be a functional antagonist of its parent system by acting at autoreceptors. An effect of a nominal agonist (i.e. a compound that has an effect at a receptor like the transmitter) at one receptor may be the equivalent of an antagonist at a different receptor type or of a synthesis blocker. Indeed, where there are high and low affinity receptors, low affinity receptors frequently have an opposite effect to high. The former provide the endogenous ligand with a form of negative feedback that can prevent overdriving of the system.

Sixth, there is a therapeutic lag in the clinical effects of specific serotonergic re-uptake inhibitors (SSRI), specific noradrenergic re-uptake inhibitors, $5HT_{1A}$ binding drugs and even benzodiazepines (e.g., Wheatley, 1990, Figure 14.2). This can lead one to ask whether an abnormality in any system that is involved in these therapeutic approaches to anxiety reflects anxiety disorder itself. They may rather be non-specifically involved as critical components of feedback loops maintaining symptoms.

These problems exist for each symptom or syndrome being studied and are rendered even more problematic by the diagnostic problems of the DSM scheme detailed at the beginning of this chapter. In understanding the role of the aminergic systems in anxiety two simplifying strategies can be used. The first is to base the analysis on the dissection of the anxiety disorders provided by the preclinical data. The second is to focus most attention on acute challenge studies in non-clinical populations (where results are not confounded by pre-existing disorder, but may of course be non-specific) and on genetic analysis of clinical populations (where the detected differences between probands and controls must have existed before the onset of the disorder).

Specific Dopaminergic Involvement

The bulk of attempts to address this question have focussed on changes in plasma or urinary levels of noradrenaline or its metabolites. A review of the data up to 1990 (Norman, Judd and Burrows, 1990) found 'a number of studies are suggestive of a role of catecholamines in the aetiology of anxiety states, particularly panic disorder'. Let us focus on the words 'catecholamines' and 'suggestive'. Why do they not draw specific conclusion about dopamine? Why are their conclusions not definite?

Dopamine presents major problems for attempts to claim specificity. It has, as a major metabolite, noradrenaline and so treatments that alter its synthesis also change noradrenaline. Further, it shares both MAO and COMT with noradrenaline as breakdown enzymes. In essence, this means we must rely on the preclinical data for our assessment of its role in anxiety and anxiety-related disorders. These did not suggest any major involvement. Likewise, genetic polymorphisms of the D4 receptor and dopamine transporter do not appear to be strongly linked to panic (Hamilton *et al.*, 2000).

Even if we ignore specificity to dopamine, why can we not make definite claims about the two catecholamines, lumped together?

> 'Evidence ... has been sought in the measurement of epinephrine, norepinephrine and [its metabolite MHPG] in the periphery.... Interpretation of the findings in relation to changes in brain adrenergic function is, of course, problematic, as indeed is the relationship for other peripheral markers. This question has been addressed for the use of plasma-free MHPG concentrations. In brain "free" or unconjugated MHPG is the major metabolite of norepinephrine and its measurement provides a good estimate of turnover. Evidence from animal studies suggest [*sic*] a direct correlation between MHPG concentrations in noradrenergic-containing brain regions and CSF and plasma in untreated animals or in those treated with drugs that increase or decrease activity. A similar situation pertains in humans where positive correlations between CSF and plasma MHPG concentrations have been observed. Furthermore, the effects of administration of some drugs on plasma MHPG in humans are consistent with their effects on brain MHPG in primates. [However,] the contribution of brain MHPG to plasma concentrations has been estimated as [only] 20–65%. [But], despite [these] significant contributions from outside the brain, is thought to be under central control. [Unfortunately,] numerous other factors such as motor activity, temperature, exercises, sexual arousal, mental tasks requiring concentration and diet may all influence catecholaminergic function.
>
> [Thus, there can be] a highly significant correlation between change in anxiety score and change in urinary MHPG output, but urinary MHPG excretion at baseline and state anxiety score did not covary significantly. Baseline anxiety cannot be used to predict baseline MHPG and vice versa.... Urinary MHPG does not appear to be a useful marker to study comparison with normal control groups. On the other hand within subject designs (i.e. pre- and post-treatment) may be more useful for assessing individual changes of adrenergic function in anxiety disorders.'
>
> Norman, Judd and Burrows (1990, pp. 226–228)

An important negative conclusion, here, is that there is no evidence for a general baseline increase in catecholamines as a cause of excessive anxiety responses. Much more likely, given the preclinical data, is that anxiety-induced arousal results in changes in catecholaminergic output as a non-specific symptom.

Attempts to alter dopamine in normal subjects have been no more conclusive.

> 'Decreased dopaminergic function can be elicited experimentally using low doses of apomorphine, which are thought to act as a presynaptic agonist. However, problems with apomorphine include concern about its specificity for the presynaptic rather than postsynaptic receptor, and side effects.... Repeated administration of α-methyl-p-tyrosine [reduces noradrenaline and dopamine and]

> produces primarily fatigue and sedation [while] administration of the catecholamine ... precursor, tyrosine, attenuates mood lowering and cognitive deficits associated with stressful environmental challenges.... Reducing catecholamine neurotransmission by means of acute phenylalanine/tyrosine depletion ... were similar to those reported to occur in response to other used to decrease catecholamine neurotransmission in humans ... [It] lowered mood, energy, and calmness, but only the mood-lowering effect ... after an aversive psychological challenge (public speaking and mental arithmetic) ... was statistically significant.'
>
> Leyton *et al.* (2000, pp. 52–59)

These effects, then, could have been due to changes in noradrenaline as well as dopamine and appeared to relate more to depression than anxiety. It should also be noted that they are in the opposite direction to the effects that would be predicted if release of catecholamines by stress were producing anxiety but are in the same direction as would be predicted from the therapeutic effects of noradrenergic re-uptake blockers and monoamine oxidase inhibitors.

Specific Noradrenergic Involvement

The evidence of the previous section is as relevant, or irrelevant, to assessment of central noradrenaline function as it was to dopamine. In this section we will consider studies that have attempted to exclude dopamine when assessing the effects of noradrenaline. Noradrenaline has a much longer and closer association with anxiety than dopamine.

> 'In humans electrical stimulation in the region of the locus ceruleus [*sic*] has been reported to produce feelings of fear and imminent death. Coupled with the anxiolytic effects in humans of drugs that decrease locus ceruleus noradrenergic activity and the anxiogenic properties of drugs that increase it, Redmond and Huang suggest[ed] a role for this nucleus in human anxiety states.'
>
> Norman, Judd and Burrows (1990, p. 224)

Our preclinical analysis of the noradrenergic system should make us wary of accepting this suggestion too restrictively, especially in its more specific form that noradrenergic systems are critical for panic disorder. Clearly a pathological increase in 'arousal' could engender anxiety — but only in the non-specific way of other disturbances of the body. However, all anxiolytic drugs so far tested do appear to reduce the outflow of forebrain noradrenaline (although as noted above, buspirone increases rather than decreases locus coeruleus firing as a result of its presynaptic inhibition of outflow). But the same drugs alter other systems, particularly serotonin, in the same way and specific noradrenergic lesions appear to be only partially anxiolytic (Table XIX-2.2). A hard-line equation of noradrenaline release with anxiety does not square with these facts. Equally, as emphasized at the beginning of this chapter a role for a compound in the amelioration of symptoms of a state does not necessarily implicate the systems on which the compound acts in the aetiology of the disorder giving rise to that state.

We are left with the looser question of whether disorders of the noradrenergic system can induce, or increase the likelihood, of anxiety disorders. Certainly, drugs like yohimbine, that increase locus coeruleus firing, can increase panic attacks. But, as emphasised in Section 2 panic and anxiety should be kept distinct and 'the list of "successful" and presumably "biological" challenges has grown quite long over the past few years. It now includes lactate infusion, carbon dioxide inhalation, and hyperventilation as well as oral or intravenous yohimbine, isoproterenol, (nor)epinephrine, caffeine, beta-carboline ligands ... and GABA.... [However,] the effects of [such] panic challenges can be strongly altered by manipulating expectancy experimentally' (Margraf and Ehlers, 1990). This is reminiscent of the older work on the effects of adrenaline challenge in normal subjects. 'The differences in the results of [different] workers are explicable in retrospect by the presence or absence of anxiety-provoking environmental cues. In a neutral setting, the injection of epinephrine (adrenaline) produced no emotional changes, "cold emotion", or "as if" emotion.... In an anxiety-provoking setting, intentionally or accidentally created by the experimenters, subjects showed more evidence of anxiety and in a minority of cases panic attacks were elicited' (Lader and Tyrer, 1975). These data all suggest that increased central or peripheral noradrenaline, like peripheral adrenaline, may increase the effect of existing external or (in the case of panic) internal anxiogenic stimuli but are not anxiety provoking in and of themselves. In this we echo the conclusion of our preclinical analysis that central noradrenaline increase vigilance to and vigor of response to stimuli and the conclusion is nonetheless consistent with the view that these effects need not be limited to aversive stimuli. A final point to note, here, is that apparently selective effects of drugs like yohimbine on patients with panic disorder as compared to controls — which can occur with startle as well as panic (Morgan, III *et al.*, 1995) — may reflect no more than a pre-existing heightened sensitivity to stimuli in such patients. Certainly, carbon dioxide inhalation produces more panic in persons with panic disorder than it does in controls (Gorman *et al.*, 1994).

Surprisingly, there is better evidence for the involvement of noradrenaline in depression, and even in the effects of serotonergic antidepressants, than there is for its involvement in anxiety. For example, in depressed patients treated with tricyclic, SSRI or MAOI antidepressants, plasma MHPG (the metabolite of noradrenaline) was reduced as were both anxiety and depression. But the change in MHPG correlated with the change in depression and not the change in anxiety (Karege *et al.*, 1993). Plasma noradrenaline is also high in depressives, independent of the type of depression, and its level has no relation to the level of anxiety (Kelly and Cooper, 1998). Likewise, in post-traumatic stress disorder there appears to be a general change in the 5HT transporter, which is accompanied by $\alpha 2$ adrenergic receptor changes only in those cases with major depression. Conversely, $\alpha 2$ density prior to treatment of panic disorder predicts the efficacy of that treatment without being changed by it. There is, then, no evidence for $\alpha 2$ involvement in the production of anxiety but 'increased ... density and abnormal coupling may represent an adaptive mechanism ... in Panic Disorder' (Gurguis *et al.*, 1999). Nor is there good evidence that polymorphism of the receptor genes relates to differences in panic (Ohara *et al.*, 2000).

Finally, social phobia (arguably a disorder involving anxiety if not caused by it) in the absence of depression does not appear to be accompanied by peripheral signs of catecholaminergic abnormality (Stein, Asmundson and Chartier, 1994; Stein, Walker and Forde, 1994).

Overall, then, we can be happy with the idea that noradrenergic systems can modulate anxiety. However, it seems clear that increased noradrenergic outflow, as such, need not be linked to anxiety. High levels accompany anxiety states in some cases and can exacerbate panic, but increased outflow is also a common action of all anxiolytic drugs. One way of resolving this apparent paradox (consistent with the fact that anxiolytic drugs, including buspirone, do not alter panic) is to suggest that noradrenaline can increase the probability of panic (as can many other agents) while contributing to decreases in anxiety as such. For noradrenaline, this is speculative. However, we noted earlier Graeff's model of the interaction of serotonin with the defence system where (see Figure XIX-2.3) he postulated an opposite effect on the periaqueductal gray (reducing spontaneous panic) to that on higher levels of the system (increasing avoidance).

Specific Serotonergic Involvement

Serotonergic systems have achieved prominence because of their role in antidepressant action. However, it is clear that they are also involved in specifically anxiolytic action. Disentangling causes and effects is difficult here. But it has been suggested that for some types of depression, the depression is secondary to changes in anxiety and aggression that are in turn secondary to 5HT dysfunction. Specifically, a deficient serotonin system would decrease coping ability and hence increase vulnerability to stress. The stress would activate anxiety and aggression and failure to cope with these would precipitate a depressive episode. Importantly, 'the two subgroups — the one in which anxiety/aggression heralds the depression and the other in which anxiety/aggression are first to respond to antidepressants — coincide to a large extent' (Van Praag, 1996).

'A [single] serotonergic deficit, interacting with different pathophysiological mechanisms, could underlie a component of the vulnerability to [and] recurrence or exacerbation of ... [progressing] panic anxiety disorder, [post-traumatic stress disorder (PTSD)] and OCD.... [In particular, it could underlie the] decreasing well-interval between episodes, and a transition from episodes that were precipitated by losses and related psychosocial stressors to those that occurred more spontaneously' via a kindling-like phenomenon (Post and Weiss, 1998) (Kindling is the gradual development and then increase in severity of seizures to repeated electrical stimulation that does not initially generate seizures). Consistent with this, $5HT_{1A}$ agonists decrease kindling and $5HT_{2A}$ agonists increase it, in line with the relation of these receptors to anxiolytic (and antidepressant) action (Post and Weiss, 1998).

> 'Remarkably, in the restraint stress paradigm, antidepressants with activity at either noradrenergic or serotonergic synapses are sufficient to oppose and block some of the effects of stress on neurotrophic factor gene expression.... Whereas the benefit of not having recurrent depressive episodes based on adequate pharmacoprophylaxis is substantial in itself, it is also possible that antidepressants could impact the course of illness, both by removing the sensitizing effect of episodes on subsequent vulnerability to recurrence and by ameliorating the potential acute and concomitant long-term impact of the stressor.... [Serotonergic vulnerability] could be set either by an inherited genetic mechanism or by an experience-dependent, stressor-mediated impact on gene expression ... or by both mechanisms in combination, providing a "double hit" like that required in the development of some cancers.'
>
> Post and Weiss (1998)

The link with kindling may be even tighter than this. Partial electrical kindling of the limbic system in animals results in increased anxiety-like behaviour that has been explicitly suggested as a model of PTSD and there are many other links between kindling and anxiety in preclinical data (Adamec, 1997).

The idea that serotonin-related changes in depression may be at least in part secondary to changes in anxiety is consistent with results from healthy human subjects subjected to a psychological challenge. Relative to the effects of acute lowering of noradrenaline, acute lowering of serotonin had about twice as big an effect on anxious mood as on depressed mood. Of particular interest relative to van Praag's hypothesis (above) is the fact that, unlike noradrenaline depletion, serotonin depletion increased hostility (Leyton *et al.*, 2000) and that no change was observed in mood in the absence of psychological challenge (making a speech). A similar lack of effect in the absence of challenge has been observed in remitted panic disorder patients (Goddard *et al.*, 1994) and in numerous other studies in healthy subjects (Klaassen *et al.*, 1998). In healthy human subjects submitted to a carbon dioxide challenge serotonin depletion appears to produce a highly variable increase in reported anxiety and produces a significant increase in neurovegetative symptoms of panic (Klaassen *et al.*, 1998). These results are also consistent with van Praag's hypothesis that serotonin deficiency creates a vulnerability to anxiety that is only expressed in response to challenge. However, worsening of symptoms has been observed in remitted OCD patients (Barr *et al.*, 1994 — cited by Goddard *et al.*, 1994).

While a general depletion of 5HT may produce general effects, as with treatment, receptor-specific challenge can produce more specific effects — and even opposite effects. Thus, a $5HT_{2c}$ agonist, while being panicogenic like many other non-specific agents, can increase symptoms in OCD patients in a way that other panicogenic agents do not (Bagdy, 1998). This selectivity mirrors the fact that anti-obsessional agents tend to be panicolytic, but not necessarily vice versa. Likewise 'patients with OCD who also had a family history of OCD had significantly higher whole blood 5-HT levels than either patients with OCD without a family history of OCD or normal control subjects (Hanna *et al.*, 1991)' (Cook, Jr. *et al.*, 1994). The idea that there is a subgroup in which a high (rather than low) level of serotonin (in the blood not necessarily in the brain) is linked to OCD might seem to be supported by the fact that those parents of autistic children who have a high whole blood serotonin have a tendency to higher OCD scores than those with normal whole blood serotonin. However, in their case, hyperserotonemia is much more clearly related to higher depression scores than OCD scores (Cook, Jr. *et al.*, 1994). Unfortunately, Cook *et al.*, did not measure anxiety as opposed to obsession. It is possible, then, that the link with depression was mediated by a change in anxiety.

Support both for this idea, and for a link to central as opposed to peripheral serotonin is provided by studies on blood platelets. These have a serotonin uptake mechanism that appears similar to the central one in that it is blocked by SSRIs. Genetic or environmental changes in this transporter system are likely to occur similarly in platelets and in the brain and are likely to be one of the factors controlling whole blood serotonin levels. Assessment of the serotonin re-uptake mechanism via 'paroxetine binding was negatively correlated with both state and trait anxiety, as well as with depressive and overall PTSD symptoms. However,... [it did not differ] as a function of comorbid psychiatric diagnoses including major depression, other anxiety disorders, and substance abuse' (Fichtner *et al.*, 1995). 'Arora *et al.* (1993) [also] reported ... a negative correlation ... with state-dependent anxiety score. Unlike patients with PTSD, no significant alterations in platelet ... binding ... could be found in major depression. These negative findings in major depression are in agreement with our results that there were no significant differences in ... binding ... between PTSD patients with and without major depression' (Maes *et al.*, 1999).

It might seem peculiar that, on the one hand, a $5HT_{2c}$ agonist or increased blood serotonin levels or decreased serotonin transporter levels should be positively related to neurotic disorder while, on the other hand, challenges that deplete serotonin should increase symptoms related to those same disorders. The difficulty of interpreting such studies (and one of the reasons for this chapter having an extensive preclinical review) is highlighted by the fact that the 'hyperserotonemia' reported in whole blood may be due to 'increased platelet 5-HT uptake.... [such] increased 5-HT uptake may predispose ... to depression' (Cook, Jr. *et al.*, 1994). That is, increased measured whole blood serotonin, if it is inside platelets, would correspond to increased serotonin inside presynaptic terminals and so *decreased* serotonin in the synaptic cleft and so decreased postsynaptic effects. Similarly with the $5HT_{2c}$ agonist, the effects could be the opposite of systemic changes in serotonin if the net effect of the systemic increase on the specific terminals of interest was a decrease of release either through presynaptic or autoreceptors. With the serotonin transporter 'binding correlates highly with tissue 5-HT content' (Fichtner *et al.*,

1995), i.e. the more 5-HT there is around the more transporters there are to transport it. So, while high levels of transporter, all other things being equal, should lead to lower levels of serotonin in the cleft it is likely that high measured levels of transporter indicate that all other things are not equal. It is a higher functionality of the serotonin system that is likely to have produced a feedback increase in number of transporters.

Despite the strong link between the platelet serotonin transporter and anxiety in PTSD, it does not seem to be a basis for anxiety disorder in general. Both social phobics and panic disorder patients have normal binding (Stein *et al.*, 1995). This could be because these are distinct entities from anxiety proper (in which GAD patients would be expected to have low binding) or it may be that transporter changes are related more to aetiology than symptomatology — and are a marker of the means of acquisition of PTSD, or simply of the levels of stress previously experienced.

A causal role for the transporter itself (rather than a role as a marker) is suggested by the linkage of polymorphisms in its genes with anxiety (Evans *et al.*, 1997; Ohara *et al.*, 1998a, 1998b; Katsuragi *et al.*, 1999; Melke *et al.*, 2001). There may be a similar causal role for genetic differences in serotonin synthesis (Du, Bakish and Hrdina, 2001) and these could, of course, combine with transporter differences to produce synergistic (or antagonistic) effects. However, as emphasized earlier, genetic control of the serotonin system is likely to reflect increased vulnerability to stressors rather than a direct and specific promotion of PTSD in general or anxiety in particular.

Given the possible opposite functional effects of different receptor subtypes it is of interest that, as with ' "knock-out" mice lacking the gene that regulates the expression of the 5-HT_{1A} receptor [which] display greater anxiety ... in the open-field and the elevated-plus-maze tests, ... [with] regional 5-HT_{1A} [in] ... healthy volunteers ... there was a significant negative correlation between 5-HT_{1A} binding potential and anxiety in [all of] four regions: the dorsolateral prefrontal cortex, anterior cingulate cortex, parietal cortex, and occipital cortex' (Tauscher *et al.*, 2001).

Aminergic Interactions

There has been much less analysis of the possible interactions of the amine systems at the clinical than preclinical level. Given the difficulty of connecting a particular measure of aminergic activity with functional effect for a single amine (previous sections), we cannot expect too much from studies of more than one amine at a time. The critical feature of such studies will be to partial out the effects of the different systems. For example, we already concluded that anxiety, but not depression, in PTSD is linked to the serotonin transporter. There was also evidence that the serotonin transporter was not linked to depression in cases of depressive disorder as opposed to PTSD. And yet depression itself is a major feature of PTSD and can be treated with specific serotonin agents. Interestingly, in PTSD the presence of major depression is associated with a down regulation of $\alpha 2$ receptors. Decreased serotonergic traffic, then (whether as a result of acute or chronic trauma or genetic polymorphism), could increase the risk of anxiety and/or aggression to stressful events. Combination of this with a decrease in noradrenergic presynaptic autoinhibitory receptors could result in depression, which would also be characterized by increased autonomic activity (Maes *et al.*, 1999). The effectiveness of SSRIs in such cases could then be due to the elimination of one of two necessary or, at least, synergistic changes. If specific noradrenergic re-uptake inhibitors are proved to be effective in PTSD patients with depression, this could result from overcoming decreased autoinhibitory function.

A similar interactive relationship is suggested in generalized anxiety disorder by the pattern of plasma metabolites. Urinary output of serotonergic and noradrenergic metabolites is positively correlated in GAD patients, with 50% common shared variance (Garvey *et al.*, 1995a, 1995b). Apparently the same patient group (Garvey *et al.*, 1995a, 1995b), although there is no cross reference between the papers, showed a weaker[2] relationship (tension being the best with 25% variance accounted for) between a multiple regression function including both these measures and a subset of, mostly somatic, items in the Hamilton Anxiety Scale. The critical point is that the partial correlations of the serotonergic and noradrenergic measures were opposite in sign from each other both within and across items. As with PTSD data, and with the preclinical data, this suggests a complex partially synergistic interaction between the two monoamines in determining anxiety and/or its symptoms and comorbidities.

Conversely, there are 'recent studies of depressed patients where the NE and 5HT neurotransmitter systems have been suggested to produce distinguishable contributions to therapeutic efficacy. ... Dietary depletion of 5-HT pre-cursors leads to clinical relapse in depressed patients who have been successfully treated with SSRIs, but not those treated with NE selective uptake inhibitors, and that blockade of NE synthesis has complementary effects' (Detke, Rickels and Lucki, 1995, p. 71).

Involvement of Monoaminoxidase and COMT

Synergy between the aminergic systems and differences in the balances between different parts of the defense system would account for the wide variations in treatment sensitivity across symptomatologically similar patients. They would also account for the cases where specific noradrenergic agents appear as effective as specific serotonin agents.

The enzyme COMT breaks down both dopamine and noradrenaline and MAO breaks down dopamine, noradrenaline and serotonin. Given synergy between the amines we would expect significant effects on clinical anxiety of disruption of these non-specific amine breakdown systems. It should be noted here that, while tricyclic drugs can be non-specific in their effect on re-uptake systems, genetic polymorphisms are much more likely to be system specific. Polymorphisms affecting the breakdown enzymes will, of course, be more general in their effects. We might, then, expect changes in COMT and MAO to be more significant aetiologically since they would allow synergy. However, for aminergic transmitters, re-uptake is more important for inactivation than breakdown. This said, MAO inhibitors are at least as effective clinically as tricyclic drugs and this appears to remain true for the reversible inhibitors selective for the A form of MAO (MAO-A). These interact much less with tyramine and so are much less toxic (Buller, 1995). Indeed MAO inhibitors can be effective in patients resistant to other antidepressant drugs (Modigh, 1987). They also appear more effective in treating endogenous anxiety than do tricyclic drugs (Sheehan, Ballenger and Jacobson, 1981).

The preclinical data also suggest a potential involvement of MAO-A in anxiety disorders. Mice mutants lacking MAO-A show increased noradrenaline and serotonin in the forebrain (including theoretically important areas such as hippocampus and prefrontal cortex). They show increased fear conditioning to both simple stimuli and environmental context and increased passive avoidance (suggesting effects on both fear and anxiety, see above). However, simple eye blink conditioning is normal suggesting a relatively specific emotional effect (Kim *et al.*, 1997). Interestingly, a polymorphism that would lead to higher levels of MAO activity is found

[2] Some of the weakness in the observed correlations is likely to have been due to compression of the scale scores as a result of the exclusion criteria used rather than reflecting a weak underlying relationship in the normal population.

more in male OCD patients than in either female OCD patients or controls (Camarena *et al.*, 2001).

Polymorphism of genes controlling human COMT can result in as much as a four-fold variation in the activity of the enzyme (Lachman *et al.*, 1996). This might be thought to provide a basis for considerable psychological impact but no marked effects have been reported. It may be significant in OCD but does not appear to be relevant to other anxiety disorders (Ohara *et al.*, 1998a, 1998b).

AMINERGIC INVOLVEMENT IN PERSONALITY

There is a common theme linking the preclinical data, the delayed effects of therapeutic drugs, and the development of disorder later in life linked to polymorphisms present from conception. They all imply that aminergic systems are modulatory of the defense system as a whole. Disorder, then, appears to result from the combination of the presence of long-term general aminergic modulatory risk factors with more specific genetic and environmental factors. 'Modulatory risk factors', in this sense, are best seen as an element of personality.

This is not to imply that personality itself is unmodifiable by events. Even in Australian fire fighters (arguably pre-selected to be relatively insensitive to danger) exposure to a major stressor[3] generated post-traumatic stress disorder in about a third of them and the PTSD appeared to remain chronic (McFarlane, 1989). Thus, rather than being a disorder proper, PTSD may better be thought of as an extreme change in the same personality dimension that controls the risk of anxiety disorder normally (Pitman, Orr and Shalev, 1993). Consistent with this, brief extreme activation of brain defense systems with either anxiogenic chemicals or electrical stimulation can produce increases in reactivity of the defense system that are very long lasting and have been seen as animal models of PTSD (Adamec, 1998a, 1998b, 1998c).

A major contributor to personality, however, is likely to be genes. The tendency to anxiety disorder in current populations has about a 30% contribution from genetic factors and this is true of the tendency to generalised anxiety even when it is comorbid with other conditions, including depression (Kendler *et al.*, 1992a, 1992b, 1992c). This genetic vulnerability appears general to 'neurotic disorders' (including depression) and does not appear selective for individual 'anxiety disorders' (Andrews *et al.*, 1990). With respect to comorbidity, it is interesting that depression and anxiety appear to be the 'result of the same genetic factors. Environmental risk factors that predispose to "pure" GAD episodes may be relatively distinct from those that increase risk for major depression'. By contrast, GAD and panic share only about half of their genetic control, each having a distinct other half (Scherrer *et al.*, 2000). In the case of panic, for example, genetic influences on anxiety, via polymorphisms of aminergic systems, could operate in parallel with panic susceptibility, via polymorphism of CCK systems (Wang *et al.*, 1998a, 1998b). This suggests a tight linking of anxiety with depression but (as argued earlier) a weaker link with panic. The latter is a distinct entity that can be both a cause and a symptom of anxiety, but can occur alone.

The dimensional analysis of personality (Eysenck and Eysenck, 1969; Watson, Clark and Harkness, 1994) suggests that psychiatric patients are simply located near the extreme pole of one or more dimensions of normal personality (Eysenck, 1960). This is analogous to the clustering of disorders such as stroke in those who are at one extreme of the normal distribution of blood pressure. This dimensional approach is becoming increasingly accepted in psychiatry (e.g., Cloninger, Svrakic and Przybecky, 1993). The blood pressure analogy is exact here as personality dimensions reflect liability to disorder not presentation. Prevalence of cases is strongly correlated with mean symptom scores in the normal range, even when diagnosed cases are excluded (Rose and Day, 1990); and there is a high frequency of subthreshold disorders (Goldberg and Huxley, 1992).

In terms of Eysenck's Personality Inventory (Eysenck and Eysenck, 1969; Eysenck and Eysenck, 1976; Costa and McCrae, 1985) those prone to anxiety are neurotic introverts. These people can be viewed as having a 'trait anxiety' that loads about 0.7 on neuroticism and 0.3 on introversion. It should be noted, here, that the risk of specific phobias of items such as snakes (unlike other 'anxiety disorders') is not related to this factor (Marks, 1969) and, consistent with this, such phobias are not relieved by anxiolytic drugs (Fredrikson and Öhman, 1979; Sartory, MacDonald and Gray, 1990). It should also be emphasized that 'trait anxiety' represents a risk for 'state anxiety' and neurotic disorder but is not identical to them. Thus, in the fire fighters mentioned earlier, the traumatic event was required to generate PTSD but, even in this selected population and with this single specific traumatic event, the tendency to neurotic personality predicted the disorder better than the extent of exposure to the event (McFarlane, 1989). Consistent with the heritability of neurotic disorder, the Eysenck dimensions are controlled almost equally by genes and environment (Floderus-Myhred, Pedersen and Rasmuson, 1980).

Factor analysis determines the number of dimensions of personality but not their nature. Although Eysenck's personality inventory is used very extensively it has been argued (Gray, 1970, 1981) that the axes representing the dimensions of neuroticism and extraversion should be rotated resulting in a dimension (0.7 neuroticism, −0.3 extraversion) corresponding to the 'trait anxiety' extracted by others (Cattell, 1965; Taylor, 1953) and an orthogonal dimension of 'impulsivity'. This dimension of 'trait anxiety' is what appears to be changed by the lesions of cingulate and prefrontal cortex used to treat drug-resistant anxiety (Powell, 1979).

There is evidence that 'emotionality' in rats is a homologue of neuroticism or trait anxiety (Broadhurst, 1960; Gray, 1987). It has a major genetic component (Hall, 1951; see review by Gray, 1987) and selective breeding has resulted in the Maudsley Reactive and Maudsley Non-reactive strains of rat.

The two Maudsley strains differ on a huge range of items (for reviews, see Broadhurst, 1975; Blizard, 1981; Gray, 1987). The bulk of these can be interpreted as showing a greater response to fear-provoking stimuli in general rather than to anxiety in particular. Note that this is an increased emotional response rather than an increased sensory response since they do not differ in pain sensitivity (Commissaris *et al.*, 1992) nor in rate of learning as such (Commissaris *et al.*, 1986). They also differ in an animal model of depression (Abel, Altman and Commissaris, 1992; Viglinskaya *et al.*, 1995). and this effect shows a strong linkage between changes in scores on an anxiety test with those on the depression test (Commissaris *et al.*, 1996). Just like 'trait anxiety', then, they appear to influence susceptibility to the full spectrum of neurotic reactions.

I have discussed this issue in such detail because analysis of aminergic systems has been carried out on the Maudsely strains at a depth currently impossible with people with trait anxiety. The Maudsley reactive strain (MR) has an increased response of locus coeruleus neurons to stress (Buda *et al.*, 1994; Blizard and Liang, 1979), need a higher dose of $\alpha 2$ agonist for the same effect and have lower $\alpha 2$ binding in LC, suggesting a less effective autoinhibition[4] (Sara, Devauges and Biegon, 1993). A primary involvement of autoinhibition could well underlie the paradoxical

[3] A bush fire, which comes within the ICD-10 definition of a traumatic situation, i.e. one 'of an exceptionally threatening or catastrophic nature, which is likely to cause pervasive distress in almost anyone (e.g., natural or manmade disaster, combat, serious accident, witnessing the violent death of others, or being a victim of torture, terrorism, rape, or other crime' (World Health Organization, 1992, p. 147).

[4] But note that an opposite pattern appears to be obtained under urethane anaesthesia (Verbanac *et al.*, 1994).

increase in the difference between the two strains on a conflict task after administration of a peripheral noradrenergic neurotoxin (Verbanac *et al.*, 1993). This only reduced central noradrenaline by 30%, whereas over 95% depletion is normally needed for behavioural effects (McNaughton and Mason, 1980) and may have been selectively affecting autoinhibition thus increasing rather than decreasing overall noradrenergic function.

Maudsley reactive rats have also been reported to have higher levels of serotonin in the limbic system (Maas, 1963) and in the brainstem, but the difference in the brainstem does not appear to be a reliable finding (Blizard and Liang, 1979) and, under urethane, neither dorsal raphe discharge rates nor sensitivity of 5HT1A autoreceptors is different (Verbanac *et al.*, 1995). Unfortunately, the sensitivity of presynaptic 5HT1A receptors in the limbic system (on which drugs like buspirone are likely to have their major action) has not been reported. A lack of difference in 5HT levels would be expected if the main difference between the strains, as with healthy humans (Tauscher *et al.*, 2001), is in binding and hence postsynaptic effect of the serotonin released.

Overall, then, we have a picture of polygenetic effects on the efficacy of both the noradrenergic and serotonergic systems that, through synergistic interactions (both within and between the amine systems) contributes to a normal personality factor that can be viewed clinically also as a risk factor for high levels of anxiety and hence anxiety disorder. Additional genetic and particularly specific environmental factors would then increase the risk of, or precipitate, particular disorders. These would then be specific instantiations within a 'trait diathesis common to all anxiety disorders' (Zinbarg and Barlow, 1996; Brown, Chorpita and Barlow, 1998).

CONCLUSIONS

There appears little question that the aminergic systems play a role in adaptive responses to stressors and that their general state of reactivity, set by genes and (often early) experience, contributes to a personality factor of 'trait anxiety' that can be largely equated with neuroticism. However, specific neurotic disorders do not appear to equate with disorder of the aminergic systems as such. They reflect distortions of activity in particular brain centres. The selectivity of therapeutic agents (and receptor-linked genes) then reflects postsynaptic differences in particular terminal areas. The lack of selectivity of trait anxiety for specific disorders reflects changes in synthesis and breakdown that are not only general to many terminal areas but also can be general to several aminergic systems (monoamine oxidase being particularly indiscriminate). The complex effects of kindling, opposite effects of normal and autoinhibitory receptors and direct interactions between terminals of the different amine systems render analysis of the clinical situation difficult. However, there is no reason to suppose that anxiety disorders cannot be viewed as extremes of normal psychological variation or that the component parts of the systems of interest (including their genetic control) cannot be studied, one at a time, in animal models. Highly efficient future treatments are likely if we can understand the different roles of different aspects of monoamine function in anxiety and obtain reliable markers that can reliably indicate specific treatment modalities for specific patients.

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XIX-3

Amino Acid Transmitter Systems

Catherine Belzung, Guy Griebel, Florence Dubois-Carmagnat and Jean Michel Darves-Bornoz

INTRODUCTION

Amino acids correspond to a wide range of compounds, including precursors of catecholamine and serotonin synthesis (tyrosin and tryptophan respectively) as well as neurotransmitter systems such as the excitatory amino acids glutamate and aspartate and the inhibitory amino acids γ-aminobutyric acid (GABA) and glycine. As few studies revealed anxiolytics acting on excitatory amino acids via the N-methyl−D-aspartate (NMDA) complex and/or the metabotropic glutamate (mGlu) receptors, only a short paragraph will be devoted to such agents. Furthermore, data suggesting abnormalities in excitatory amino acid systems in some anxiety disorders will be mentioned. On the other hand, we will focus on anxiolytic drugs acting on GABAergic neurotransmission, as most anxiolytics act via GABA-related mechanisms, first focusing on the mechanisms underlying the anxiolytic action of some anti-anxiety agents such as benzodiazepines (BZs) and second describing the use of such compounds in the clinic, in an attempt to link the pharmacology with neurochemical changes that have been observed in these disorders.

ANXIOLYTIC AGENTS ACTING ON EXCITATORY AMINO ACID NEUROTRANSMISSION

The anti-anxiety-like action of compounds acting on excitatory amino acid neurotransmission has mainly been investigated in pre-clinical studies, using animal models of anxiety. Indeed, because of multiple side effects of such compounds (ataxia, myorelaxation, impairment of learning and memory), such agents cannot be proposed as potential anxiolytic drugs in the clinic.

Glutamate and aspartate bind to two types of receptors: the ionotropic receptors (AMPA, kainite and NMDA receptors) and the metabotropic glutamate receptor. Among the ionotropic receptors, solely the NMDA receptor has been proposed as a potential target for anxiolytic agents in pre-clinical studies.

Ligands of the NMDA Receptor

The NMDA complex consists of various binding sites, including a glutamate recognition site, a polyamine site, a glycine site, a phencyclidine site (channel site) and a Zn^{2+} site. Low doses of the non-competitive NMDA antagonist MK-801 or of the competitive NMDA antagonists AP5, AP7 and CPP elicited anxiolytic behaviour in several animal tests (see Chojnacka-Wójcik and Klodzinska (2001) for a recent review). Similar effects were observed with antagonists (7-CIKYN and 5,7-CIKYN) and partial agonists (HA-966, ACPC and D-cycloserine) of the $Glycine_B$ receptors; however, none of these agents crosses the blood−brain barrier. Finally, discrepant results were obtained with antagonists of the polyamine site (ifenprodil and eliprodil) as these compounds are endowed with anxiolytic properties in some, but not all animal models of anxiety.

Ligands of the Metabotropic Glutamate Receptor

The mGlu receptors are a family of eight receptors designated mGlu1 through mGlu8, which can be divided in three groups based on the similarity of the amino acid sequence, pharmacology and second messenger coupling. The first group, which consists of mGlu1 and mGlu5, is positively coupled with phospholipase C and is sensitive to *trans*-ACPD as well as quisqualate. The second group, which includes mGlu2 and mGlu3, is negatively coupled to adenylate cyclase and is sensitive to *trans*-ACPD but not quisqualate. The third group, consisting of mGlu4, mGlu6, mGlu7 and mGlu8, is negatively coupled to adenylate cyclase and does not respond to *trans*-ACPD and quisqualate but rather binds specific compounds. Antagonists of Group I mGlu receptors such as S-4C3HPG or (S)-4CPG, as well as an antagonist of the mGlu1 (CPCCOEt) or of the mGlu5 (MPEP) elicited anxiolytic effects in pre-clinical models. Furthermore, LY-354740, an agonist of Group II mGlu receptors, displayed anxiolytic activity in several models of anxiety. In fact, as Group II mGlu receptors are localized presynaptically, their receptor agonists may inhibit glutamate release, so that they are parallel to the effects of Group I mGlu receptors antagonists. Finally, regarding Group II mGlu receptors ligands, the issue remains to be clarified as discrepant results have been obtained.

ANXIOLYTIC AGENTS ACTING ON GABAERGIC NEUROTRANSMISSION

Early Anxiolytics Acting via a GABAergic Mechanism

Ethanol

There is evidence suggesting that ethanol, a compound usually termed as alcohol, has been used in prehistoric times. It can therefore be considered as the first anxiolytic compound. Mead, a fermentation product of honey, is considered as the oldest alcoholic beverage; it seems that it existed in the paleolithic age, about 8000 B.C. Alcoholic beverages are known to produce relaxation, elevation of mood, anxiolysis and disinhibition in response to social constraints. At higher doses it induces sedation. Most alcohol users drink occasionally (75% of the population of the USA). However, 15% of users are considered alcoholics. This highlights the great abuse potential of this compound.

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Barbiturates

In 1864, Adolph von Baeyer synthesized barbituric acid (malonylurea). The name of this drug is said to be linked to the presence, on the day of the experiment, of Baeyer in a tavern in which officers were celebrating the Day of St. Barbara, their patron saint. Barbituric acid was devoid of clinical potency but it led to the development of barbiturates after 1903, the date of the synthesis of barbital that became rapidly popular. These compounds rapidly took a dominant place because they facilitated sleep and produced relaxation. However, the bioavailability of barbital was rather low because of the poor solubility in lipids of that drug. Furthermore, it was metabolized slowly so that the effects on drowsiness extended over 36 hours. Consequently, new drugs with short duration of action (amobarbital, pentobarbital, secobarbital) and later, in the 1930s, ultra-short duration of action (hexobarbital, thiopental, methohexital) were introduced into therapeutics. These drugs, termed barbiturates, are widely used as anaesthetics but they also have an excellent efficacy in alleviating anxiety. For example, pentobarbital (Figure XIX-3.1) is effective in most rodent models of anxiety. However, their development in the treatment of anxiety has been spurred by their high abuse potential. This has been shown in animal as well as human studies. Furthermore, it also elicited lethal effects (1500 deaths per year), principally due to accidental poisoning in drug abusers and to suicide.

Carbamates

As in many other cases, the starting point of the use of carbamates in the treatment of anxiety had nothing to do with their action in the central nervous system. In the 1940s, the Wallace Laboratories were developing new antibacterial agents and therefore chemists attempted to improve the potency of phenoxetol, a compound used as disinfectant, by lengthening the carbon chain. When testing the toxicity of that newly synthesized compound, termed mephenesin, they observed that it produced muscle relaxation and a sleeping-like condition in animals. They described this action as 'quieting influence on the demeanor of the animal', an effect that was named in 1946 'tranquillization'. The drug was marketed in 1947 as a short-action muscle relaxant but in 1949 several authors proposed that it may alleviate anxiety. However, mephenesin had several drawbacks, including a very short duration of action. Therefore, researchers attempted to alter the chemical structure of mephenesin to overcome these shortcomings: the result was meprobamate (Figure XIX-3.2), a compound whose duration of action was eight times that of mephenesin. In the early 1950s, Berger demonstrated that meprobamate possessed anxiolytic properties. This was shown in monkeys whose fear was reduced in threatening environmental situations, and in rats, in which meprobamate was effective in disinhibiting behaviour that was suppressed by punishment (Geller–Seifter test). Berger claimed that unlike alcohol or barbiturates, the anxiolytic effects of meprobamate were not associated with impairment of intellectual or physical performance. He explained these effects by an action on 'those specific areas in the brain as the thalamus and the limbic system, that represent the biological substrate of anxiety'. Consequently, between 1950 and 1960, meprobamate was one of the most commonly used drugs for the treatment of anxiety worldwide. Later, however, the image of meprobamate was tarnished by numerous reports of lethal overdoses. Moreover, the anxiolytic effect was accompanied by drowsiness and ataxia.

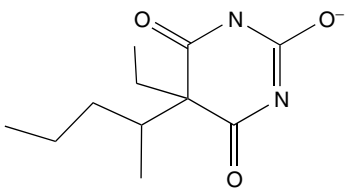

Figure XIX-3.1 The structure of pentobarbital

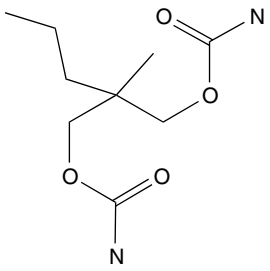

Figure XIX-3.2 The structure of meprobamate

As will be discussed later, these three categories of compounds interfere with GABAergic neurotransmission, which is the principal inhibitory neurotransmitter within the central nervous system.

The Discovery of Benzodiazepines

The Discovery of Chlordiazepoxide

At the end of the 1950s, pharmaceutical companies started to become interested in the field of psychopharmacology. This was related to the commercial success of meprobamate and other psychoactive agents such as chlorpromazine. In this context, a team at Hoffman–La Roche, led by Leo Sternbach, started to study some heptoxdiazines that Sternbach synthesized in the early 1920s when he was a postdoctoral student at the university of Cracow, looking for dyestuffs. In fact, these compounds had no interesting properties as dyes, and no evidence existed in favour of an action of these compounds on the central nervous system. Sternbach decided to study them only because they were unexplored and convenient to test because of their chemical versatility that allowed many transformations. First Sternbach discovered that these compounds were not heptoxdiazines but quinazolone 3-oxides. He then synthesized 40 derivatives and found that all of them, except one that was not tested, were biologically inactive. The last one, labelled RO 5-0690, was disregarded, mainly because of other research priorities. In May 1957, during a cleanup of the laboratory, one of the collaborators of Sternbach found this drug and suggested that it may be tested. Therefore RO 5-0690 was given to the team led by Randall for animal testing. Six tests were used for the screening: a test for sedation and muscle relaxation, a foot-shock test to measure 'taming' effects, another test for muscle relaxation and three tests for anticonvulsant activity. The drug was compared to phenobarbital, chlorpromazine and meprobamate. It was superior to the latter compounds in all tests and therefore Randall announced in 1960 that chlordiazepoxide (the new name of RO 5-0690) may have potent sedative, muscle relaxant, taming and anticonvulsant activity. It was introduced into pharmacotherapy under the tradename 'Librium' (from 'Equilibrium') in 1960, only two-and-a-half years after the first pre-clinical tests. It was then shown that this compound also elicited anti-anxiety effects.

Synthesis of other BZs

A more potent analogue, diazepam, was synthesized in 1961 and, later, 50 other BZs were marketed throughout the world.

Table XIX-3.1 Half-life of major BZs

BZ	Duration of action (h)*
Chlordiazepoxide	55 +/− 35
Diazepam	55 +/− 35
Oxazepam	17 +/− 11
Lorazepam	16 +/− 8
Alprazolam	11 +/− 5

*Including active metabolite

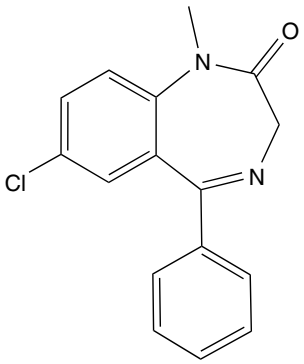

Figure XIX-3.3 Chemical structure of diazepam. The figure shows the core structure made up of the benzene ring (left ring) fused to a diazepine ring (right part, top). The third ring is an aryl substituent ring (bottom, right part)

These new compounds include oxazepam (1965), nitrazepam (1965), clorazepate (marketed in 1968), lorazepam (marketed in 1973), bromazepam (1974), clobazam (1975) and flunitrazepam (1978). The need for new compounds was mainly related to the necessity to develop compounds with a shorter half-life than chlordiazepoxide and diazepam (see Table XIX-3.1 for more details).

BZs are metabolized extensively, generating active metabolites. Some of these metabolites are biotransformed more slowly than the parent compound so that there is no clear relationship between the half-life of a BZ and its duration of action. It should also be noted that BZs display metabolic inter-relationship with common active metabolites. For example, chlordiazepoxide, clorazepate, prazepam, diazepam as well as demozepam are all metabolized in desmethyldiazepam. Some BZs (prazepam, flurazepam) reach the systemic circulation only in the form of active metabolites. All these compounds have a rather close chemical structure. In fact, the name BZ is derived from the benzene ring fused to the diazepine ring (Figure XIX-3.3). They all share common pharmacological properties as they are all sedative–hypnotic, muscle relaxant, anxiolytic and anticonvulsant. Unfortunately, they also display unwanted side effects: they induce anterograde amnesia, tolerance and dependence. However, when compared to the early anxiolytic agents (barbiturates and meprobamate), they displayed a high therapeutic index so that they became rapidly and widely prescribed and very popular, in particular in the treatment of anxiety. In 1972, chlordiazepoxide and diazepam accounted for half of all psychoactive prescriptions in the USA. This led to increasing concerns about their over-use, so that some countries such as the UK introduced a limited prescription list of these compounds in the mid 1980s. However, the prevalence of BZ use is still very high.

Mechanisms of Action

Mechanisms of Action: First Discoveries

The mechanism of action of BZs remained a mystery until a key observation was made in the unravelling of this knot. Indeed, in 1967, electrophysiological studies on the cat spinal cord revealed that diazepam could potentiate the dorsal root potential. The significance of this observation was, however, not realized until the discovery that the dorsal root potential was associated with the activation of local inhibitory neurotransmission using GABA. In fact, the effects of BZs on the dorsal root potential requires an intact GABA system within the spinal cord. It was later observed that the ability of BZs to potentiate GABAergic neurotransmission was a ubiquitous phenomenon, present in many brain areas. This evidence originates not only from electrophysiological studies but also from a biochemical study. The major type of GABA receptor in the brain, termed $GABA_A$ receptor, is associated with an ionophoric postsynaptic Cl^- channel that mediates inhibitory neurotransmission in the brain regulating Cl^- permeability. BZs do not bind to the $GABA_A$ receptor but they potentiate the action of GABA on the $GABA_A$ receptors. Therefore, they require the presence of GABA to express their pharmacological actions.

BZ Receptors

The next milestone in the understanding of the mechanism of action of BZs was the discovery in membrane preparation of high affinity binding sites for [^{3}H]-diazepam that were saturable and stereospecific. This was first reported in 1977 by two independent teams (Braestrup and Squires in Copenhagen, and Möhler and Okada in Basle). One year later, similar binding sites were identified in the human brain, using another ligand, [^{3}H]-flunitrazepam. BZ receptors are located on dendrites, nerve cell bodies and nerve terminals. Autoradiographic studies showed that BZ binding sites are widely distributed within the central nervous system, with the highest concentrations in the cerebral cortex, intermediate concentrations in the limbic system and the cerebellum, and lowest in the pons-medulla and spinal cord (Figure XIX-3.4).

The clinical efficacy of BZs has been attributed to their ability to bind BZ receptors. Indeed, there is a positive correlation between the Ki values for the inhibition of [^{3}H]-diazepam binding by various BZs and their average therapeutic recommended doses (Figure XIX-3.5).

It is important to emphasize that BZs not only bind to BZ receptors. For example, they have also nanomolar affinity for adenosine receptors. However, this is often forgotten so that their psychoactive effects are always attributed to their binding to BZ receptors.

Binding sites are also found in certain peripheral tissues, and represent the so-called mitochondrial BZ binding site. They were first discovered in the kidney, and later they were found in other peripheral tissues such as the adrenal glands and the testes. Such sites also exist in the central nervous system, where they are mainly found on glial cells. These sites are not associated with the $GABA_A$ receptor. The binding site is different from the brain BZ receptor, since it does not recognize all psychoactive BZs (for example clonazepam) and binds some compounds such as the isoquinoline, PK 11195, with nanomolar affinity. They are located subcellularly on the outer membrane of the mitochondria, rather than on the cell membrane. Their function is not well known in all cell types, but it has been suggested that in steroid hormone-producing organs (adrenal glands, testes), they are involved in the transport of cholesterol from the outer to the inner mitochondrial membrane. In the brain, it has been proposed that these receptors may be involved in neurosteroidogenesis.

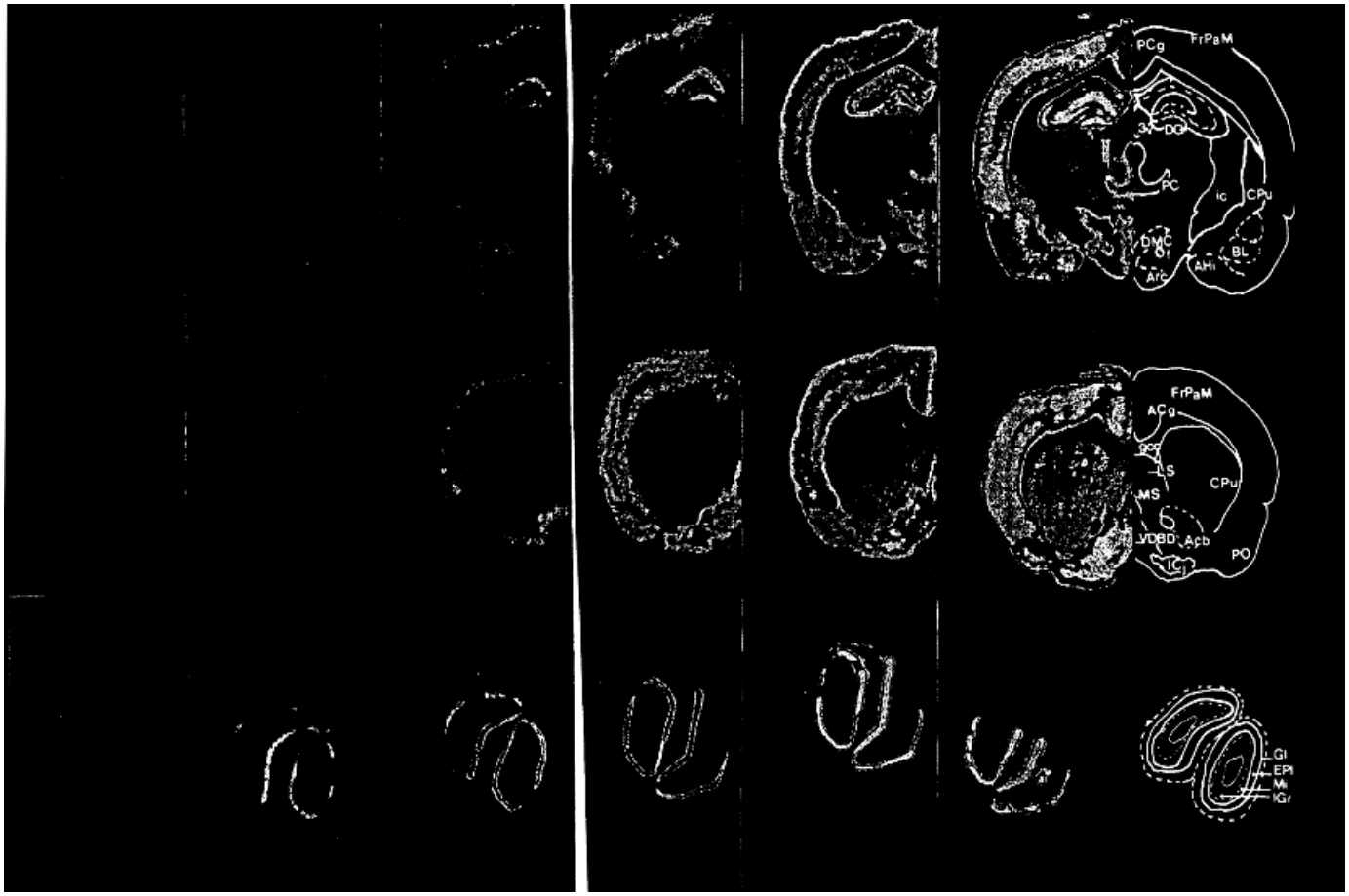

Figure XIX-3.4 Autoradiography of ^{3}H-Flunitrazepam binding sites in the rat brain. PCg: Posterior cingulated cortex; FrPaM: Frontoparietal cortex, motor area; 3v: third ventricle; DG: Dentate Gyrus; PC: Posterior Cingulate; iC: Internal capsule; Cpu: Caudate Putamen; BL: Basolateral amygdaloid nucleus; Ahi: Amygdalohippocampal area; Arc: Arcuate hypothalamus nucleus; f: fornix; Acg: anterior cingulate cortex; gcc: genu corpus callosum; LS: Lateral septum; MS: Medial septum; VDBD: Nucleus Vertical limb, diagonal band, dorsal part; Acb: Accumbens nucleus; PO: Primary olfactory cortex, Icj: Island of Calleja; EPI: external plexiform layer of the olfactory bulb. Reproduced from photostat by permission from Marcel, D., Weissmann-Nanopoulos, D., Mach, E. and Pujol, J.F., 1986. Benzodiazepine binding sites: localization and characterization in the limbic system of the rat brain. *Brain Research Bulletin*, **16**, 573–596

Ligands of BZ Receptors

Up until the early 1980s, it was widely admitted that the chemical structure of BZs was a prerequisite for the binding to the BZ receptor. However, this view was challenged by the discovery that some chemically unrelated drugs (Figure XIX-3.6) such as cyclopyrrolones (zopiclone, suriclone), triazolopyridazines (CL 218,872), phenyl-imidazo-pyridine acetamides (alpidem, zolpidem), quinolines (PK 8165) and pyrazoloquinolines (CGS 8216, CGS 9896) bind to the same site as BZs, sometimes with dissociation constants in the low picomolar range, thus equalling the affinities of the most potent BZs. They act on the BZ receptor in a similar way to BZs.

However, not all compounds that bind with high affinity to the BZ receptor exhibit the same pharmacological profile as BZs. Indeed, compounds have been described that do not have any intrinsic activity when injected alone, but they block the pharmacological action of BZs. These compounds have therefore been termed antagonists of which the first to be identified was flumazenil, also called RO 15-1788 (Figure XIX-3.7). Indeed, this drug has antagonistic properties both *in vitro* and *in vivo*; it is able to block the anxiolytic, anticonvulsant, amnesic, myorelaxant and sedative effects of BZs. Flumazenil is used therapeutically to control BZ anesthesia. Furthermore, other pharmacological agents that bind to the BZ receptor, such as ethyl-β-carboline-3-carboxylate (β-CCE), have been described, but they induce a pharmacological profile opposite to the one induced by BZs. Indeed, they produce anxiogenic, proconvulsant and promnesic effects. They have therefore been termed inverse agonists. Not all inverse agonists have the same potency. For example, compounds such as DMCM and β-CCM (Figure XIX-3.7) are convulsant, while others such as β-CCE or FG 7142 cannot trigger seizures *per se*, but will sensitize animals to the convulsant effects of other pharmacological agents (proconvulsant effects): the first category has been termed full inverse agonists while the second is called partial inverse agonists.

In fact, subsequent studies have revealed compounds that span the complete efficacy spectrum from full agonists to full inverse agonists, including partial agonists such as bretazenil. Antagonists are able to block the effects of agonists and also the effects of inverse agonists or of partial agonists. See Table XIX-3.2

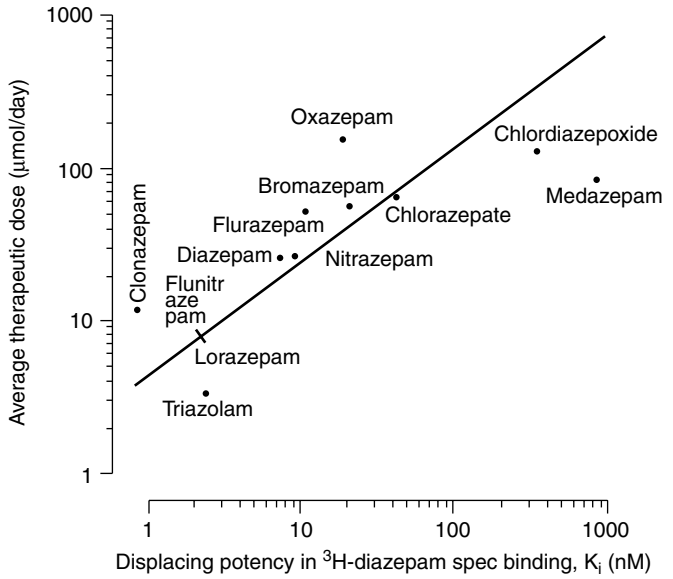

Figure XIX-3.5 Correlation between BZ K_i and mean therapeutical dose recommended. Reproduced by permission from Möhler, H. and Okada, T., 1978. The benzodiazepine receptor in normal and pathological human brain. *British Journal of Psychiatry*, **133**, 261–268

Table XIX-3.2 Properties of the various types of BZ receptor ligands

Different types of ligands	Example of compounds	Pharmacological action
Full agonist	Classical BZ (chlordiazepoxide, diazepam, oxazepam, flunitrazepam, etc.)	Sedative Anxiolytic Anticonvulsant Amnesic Myorelaxant
Partial agonist	Bretazenil	Anxiolytic
Antagonist	Flumazenil	No effect
Partial inverse agonist	β-CCE, FG 7142, RO 15-4513	Anxiogenic Proconvulsant
Inverse agonist	DMCM, β-CCM	Anxiogenic Convulsant Promnesic

A great amount of data exists showing that higher doses of a full agonist are required to produce sedative/myorelaxant effects than to produce anticonvulsant/anxiolytic effects. For example in mice, the dose to induce sedation is twice the minimal effective dose for anxiolytic activity. By combining this approach with *in vivo* binding studies, it is possible to assign a level of receptor occupancy to a given pharmacological effect. This type of analysis demonstrates that for full agonists such as diazepam, between 20 and 40 percent of receptor occupancy is required to produce anxiolytic or anticonvulsant effects. For the same compound, the receptor occupancy must be higher than 60% to elicit sedation or myorelaxant effects (Figure XIX-3.8). This idea has led to the development of partial agonists as anxiolytic agents devoid of sedative side effects. Indeed, partial agonists are not able to activate all the receptors they occupy, so that the dose eliciting sedation may be considerably larger than that inducing anxiolysis (Doble and Martin, 1996).

Endogenous Ligand of BZ Receptors

The presence of BZ receptors has provided some support for the notion that some natural BZ receptor ligands may exist in the central nervous system. Therefore, some research began in the early 1980s that aimed at finding an endogenous ligand for BZ receptors. Such a compound must be present in the organism, bind to the BZ receptors with high affinity and elicit behavioural effects. Diazepam Binding Inhibitor (DBI) is an 86 amino acid peptide that was initially isolated from rat brain on the basis of its ability to displace diazepam from BZ receptors. Splicing of DBI generates several biologically active fragments including the triakontatetraneuropeptide DBI_{17-50} (TTN) and the octadecaneuropeptide DBI_{33-50} (ODN) which are designated by the generic term endozepines. Intracerebroventricular injections of endozepines in rodents elicit anxiogenic effects (Garcia de Mateos-Verchere *et al.*, 1998) and block the anxiolytic action of diazepam. Evidence from *in vitro* and *in vivo* studies indicates that these compounds may act as inverse agonists at the BZ receptor, thus negatively modulating the $GABA_A$ receptor function. Subsequently, it was also observed that endozepines interact with peripheral BZ receptors and stimulate cholesterol transport in the mitochondria, thus participating in the biosynthesis of neurosteroids by brain tissues. It is to be noted here that neurosteroids also modulate the $GABA_A$ receptor function. *In situ* hybridization experiments showed strong DBI mRNA expression in the vicinity of the third ventricle, the hypothalamus and the cerebellum. Long-term isolation in mice, which is a rather stressful procedure in this species, has been shown to induce a decrease in mRNA expression for DBI in the hypothalamus, further suggesting that these peptides may have a biological function related to anxiety and/or stress (Dong *et al.*, 1999).

Interaction with GABA

As mentioned above, BZs potentiate the action of GABA on the $GABA_A$ receptors. Interestingly, the ability of anxiolytic compounds to act on GABAergic neurotransmission is shared by other anti-anxiety agents, such as barbiturates, meprobamate and ethanol. For example, barbiturates such as pentobarbital increase the affinity of $GABA_A$ receptors to GABA and increase the duration of the opening of GABA-activated Cl^- ionophoric channels. Moreover, at high doses, this compound is able to directly open Cl^- channels, even in the absence of GABA. This action is exerted via a specific binding site, termed the barbiturate binding site. As to the mechanism of action of carbamates, recent data suggest that meprobamate may also act at the barbiturate binding site of the $GABA_A$ receptor (Rho, Donevan and Rogawski, 1997). However, the enigma of the mechanism of action of meprobamate is not completely resolved, because meprobamate does not always have the same effects as barbiturates (Haefely *et al.*, 1981). Finally, ethanol is also able to interact with the GABAergic neurotransmission. Indeed, ethanol activates the $GABA_A$ receptor-coupled Cl^- channel, thereby increasing Cl^- conductance and mimicking the action of GABA. Although some effects of ethanol are also mediated via other molecular targets such as the NMDA and the 5-HT_3 receptors, one may suggest that its anxiolytic effects are mediated via $GABA_A$ receptors. Indeed, BZ antagonists such as flumazenil or BZ inverse agonists such as RO 15-4513 are able to block the anxiolytic effects of ethanol in rodents at doses where they do not have any intrinsic activity.

Present knowledge proposes a model in which the $GABA_A$ receptor may in fact be allosterically modulated by compounds binding to at least six different sites (for a review, see Hevers and Lüddens (1998)): the BZ receptors; a binding site for barbiturates; a site for neurosteroids; a site for the convulsant drugs picrotoxine and TBPS; one for flurosemide and one for loreclezole. Binding

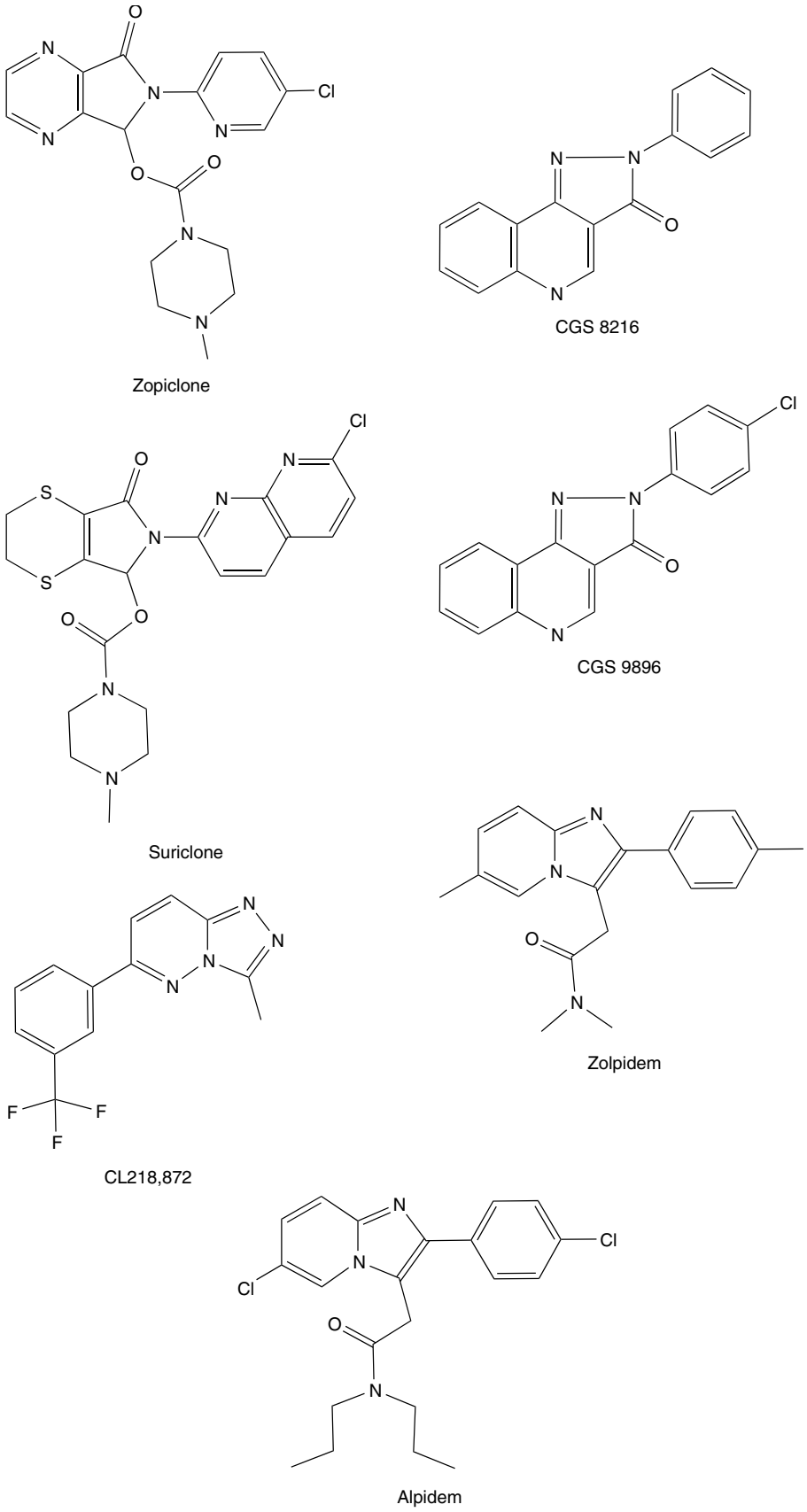

Figure XIX-3.6 Chemical structure of zopiclone, suriclone, CL 218,872, alpidem, zolpidem, CGS 9896 and CGS 8216

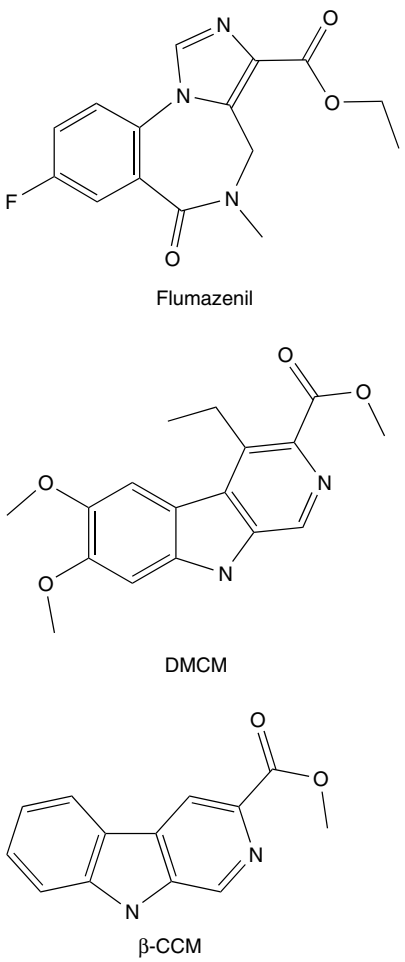

Figure XIX-3.7 Chemical structure of the BZ antagonist flumazenil and of the BZ inverse agonists DMCM and β-CCM

of BZs to the BZ receptors, of barbiturates to the barbiturate binding site, of steroids such as metabolites of progesterone to the neurosteroid site, or of loreclezole to the loreclezole site, are all associated with an anticonvulsant effect underlined by a positive modulation of GABAergic neurotransmission, that is an increase in GABA function. By contrast, binding of picrotoxin to the picrotoxin site induces convulsions, an effect related to the ability of this compound to block GABA-evoked Cl^- conductance. Furosemide, a loop diuretic, inhibits GABA function in some (for example in the cerebellum) but not all neuronal population (for example not in hippocampal neurons) via a mechanism independent of the other allosteric sites.

None of these sites are associated with anxiolytic or anxiogenic effects. As mentioned above, BZs and barbiturates are potent anxiolytics. Antagonists of the picrotoxin site such as etifoxin also induce anxiolysis: this effect is not blocked by flumazenil, thereby indicating that it is not linked to an action at BZ receptors. Some endogenous steroids such as progesterone and its 3α-reduced metabolite produce a dose-dependent anxiolytic response in animal models of anxiety. However, negative modulators of the neurosteroid site, such as pregnenolone sulphate have not been shown to induce the opposite effect in a consistent manner. Finally, some data suggested that loreclezole may induce anxiolytic effects in the rat: these effects are not blocked by flumazenil, suggesting that they are not related to the BZ site. To our knowledge, no data have shown any effect of ligands at the furosemide binding site on anxiety. Moreover, facilitation of GABAergic neurotransmission by the $GABA_A$ receptor agonist THIP or the GABA transaminase inhibitor γ-acetylene GABA (a compound that increases GABA function by inhibiting the degradation enzyme of GABA) does not elicit anxiolytic effects in the rat while drugs that have the opposite action, such as GABA synthesis inhibitors or $GABA_A$ receptor antagonists fail to induce anxiogenic effects (Ågmo *et al.*, 1991).

Molecular Biology of GABAergic Pentamer

The findings mentioned in the precedent chapter can appear contradictory as GABA mimetic drugs are not able to elicit an anxiolytic effect, while the positive modulation of allosteric sites linked to this receptor induce anxiolysis. An explanation of this apparent discrepancy is related to the molecular structure of the $GABA_A$ receptor complex. Indeed, molecular biology has revealed that the GABA receptor is composed of five subunits that co-assemble. Six classes of subunits have been described : $\alpha, \beta, \gamma, \delta, \varepsilon$ and ρ (for a review, see Hevers and Lüddens, 1998). Within each type of subunit, several isoforms are possible: in mammals, these are $\alpha 1-6$, $\beta 1-3$, $\gamma 1-3$, δ, ε and $\rho 1-3$. The $\rho 1-3$ subunits do not seem to co-assemble with α or β subunits within $GABA_A$ receptors and are mainly described in the retina. As to the ε subunit, little information is available because it has only been recently described. The most frequent stochiometric combination

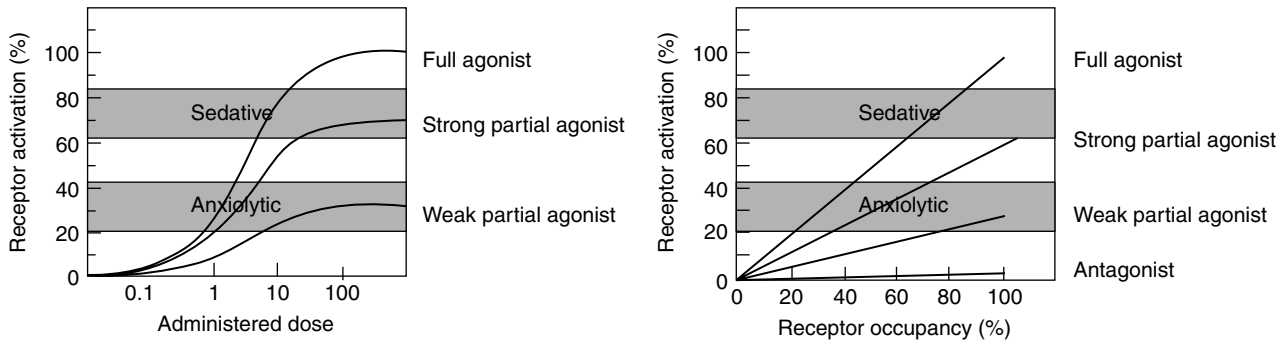

Figure XIX-3.8 Receptor occupancy–activity relationships for full and partial agonists. Left: receptor activation as a function of receptor occupation. Right: receptor activation as a function of dose administered. Reproduced by permission from Doble, A. and Martin, I.L., 1996. The $GABA_A$/Benzodiazepine Receptor as a Target for Psychoactive Drugs. Springer

includes 2 αi, 1 βj and 2 γk as well as 2 αi, 2 βj and 1 γk (with $i = 1–6$, $j = 1–3$ and $k = 1–3$). However, other stoichiometries have been described, such as 3 αi, 1 βj and 1 γk. Thus, the number of possible isoforms of the $GABA_A$ receptor may exceed 100 000. However, not all of the possible isoforms have been described within the central nervous system and approximately 20 isoforms seem to represent the most abundant ones.

Among the $\alpha 1–6$ subunits, the $\alpha 1$ is the most frequent and it has been described in almost all brain areas. It is often co-localized with $\beta 2$ and $\gamma 2$. Clustering is characteristic of GABA-A receptor genes on human chromosomes. In humans, the $\alpha 1$ subunit maps on Chr5q34-q35, in a cluster including the gene for the $\gamma 2$ and the $\alpha 6$ subunit. The human gene for $\alpha 1$ does not appear to have any mutations associated with disease entities. The $\alpha 2$ subunit is mainly localized in the cortex, in limbic areas and in the striatum (where it represents the only α subunit reported). None are found in the cerebellum. It often co-localizes with $\beta 3$ subunits. $\alpha 2$ (with $\alpha 4$ and $\gamma 2$) is expressed at low levels during early development, with a significant increase about one week after birth. However, the biological importance of that is not well understood for the moment. The $\alpha 2$ and the $\beta 1$ subunits map close together Ch4 p13-p12. The human gene for this subunit does not appear to have any mutations associated with anxiety disorders or other diseases. The $\alpha 3$ subunit is mainly localized in the monoaminergic nuclei, particularly on serotoninergic cell bodies in the raphe nuclei, as well as on cholinergic neurons of the basal forebrain. The gene coding for the $\alpha 3$ subunit is located in the central region of chromosome X and has been suggested as a candidate for X-linked manic depression, a psychiatric disease that has some comorbidity with anxiety disorders. The $\alpha 4$ subunit is localized in the hippocampus and the thalamus and the gene encoding for this subunit is located on chromosome 4 in humans. The $\alpha 5$ subunit is located quasi exclusively in the hippocampus and the gene encoding for this subunit is located on human chromosome 15, in the region of the Angelman and Prader–Willi syndromes. In fact, a single paternal allele of this gene is found in cases of Angelman syndrome, and a single maternal allele in cases of Prader–Willi syndrome. However, evidence of abnormal anxiety in these disorders is scarce. Finally, the $\alpha 6$ subunit is located mainly in the cerebellar granule cells and the cochlear nuclei. The gene encoding for this subunit forms a cluster on chromosome 5 with the genes encoding for the $\gamma 2$ and $\alpha 1$ subunits. All these data are summarized in Table XIX-3.3.

Interestingly, the α subunits seem to determine the ability of the BZ receptors to respond to BZ. Indeed, receptors containing $\alpha 4$ or $\alpha 6$ subunits lack the modulation by classical BZs. Classical BZs such as chlordiazepoxide interact indiscriminately with receptors containing $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits, that are termed benzodiazepine-sensitive. These receptors have a histidine at a conserved position ($\alpha 1$-H101, $\alpha 2$-H101, $\alpha 3$-H126 and $\alpha 5$-H105) while the benzodiazepine insensitive receptors containing $\alpha 4$ or $\alpha 6$ subunit have an arginine in the corresponding position. Therefore, diazepam-sensitive receptors can be rendered insensitive by replacing this histidine by an arginine while the regulation of this receptor by GABA is preserved. Recent data show that replacement of histidine by arginine at the 101 position of the gene encoding for the murine $\alpha 1$ subunit gene has rendered the $\alpha 1$-type $GABA_A$ receptors insensitive to diazepam ($\alpha 1$-knock-in mice). These $\alpha 1$-knock-in mice display no overt change in spontaneous behaviour and bred normally but they failed to show the sedative, amnesic and anticonvulsant action of diazepam. By contrast, the anti-anxiety and myorelaxant properties of the BZ are fully retained (Rudolph *et al.*, 1999). A similar point-mutation technique has been used to render $\alpha 2$ and $\alpha 3$ insensitive to BZs. As for the $\alpha 1$-knock-in mice, no obvious modification of basal behaviour was observed. However, pharmacological challenge with diazepam failed to induce anxiolysis in the $\alpha 2$-knock-in mice (Low *et al.*, 2000). No similar modification could be found with the $\alpha 3$-knock-in mice. These observations point to new strategies for drug design, as one can imagine drugs of the future acting specifically on some α-subunit subtypes, that is on BZ receptors within a particular brain area. For example, drug acting in an agonistic way specifically on $\alpha 2$-type $GABA_A$ may elicit an anxiolysis not accompanied by sedation or amnesic effects (see below).

As to the β subunits, they seem to have little influence on the action of BZ receptor ligands. Indeed, *in vitro* studies showed that the type of β isoform did not modify the ability of diazepam to increase GABA-activated Cl^- currents. In fact, they seem to be involved in brain development as they are all expressed in the developing mouse cerebellum, the $\beta 2$ and $\beta 3$ subunits being present at birth, and displaying spatial correspondence with areas of GABAergic synapses. The $\beta 1$ subunit mRNA does not appear until the second week after birth, and may be associated with Bergmann glia or basket cells.

More data suggest that the γ subunits may be involved in the action of BZ. When compared to the $\gamma 2$ isoform, the $\gamma 3$ isoforms displays a marked decrease in affinity for the BZ antagonist flumazenil or the inverse agonist DMCM while replacement of the $\gamma 2$ isoform with a $\gamma 1$ isoform results in an agonistic affinity of DMCM. By using targeted mutation, $\gamma 2$ knock-out mice have been generated ($\gamma 2$ $-/-$). These mice display severe growth retardation, sensorimotor abnormalities and a reduced life span as survival was never superior to 17 days of postnatal life. They are insensitive to BZs, indicating that this subunit is critical for BZ sensitivity. As no behavioural studies could be undertaken in such young mice, features of anxiety-related disorders have been investigated in $\gamma 2$ $+/-$ mice. These mice exhibited a region-specific reduction of BZ receptors. This decrease was more pronounced in some areas of the hippocampus (reduction of 35% in CA1 and 28% in CA3), in the cingulate cortex (-25%), in the frontal cortex (-23%), in the piriform cortex (-25%) and in the lateral septum (-30%) than in other brain areas such as the striatum (-6%), the globus pallidus (-13%) or the amygdala. These mice exhibited enhanced state and trait anxiety that was reversed by diazepam. Furthermore, they displayed a bias for threat cues, resulting in an increased sensitivity

Table XIX-3.3 The properties of different types of α subunits

Type of α subunit	Ability to bind BZ	CNS localization	Human chromosome	Mouse chromosome	Cluster
$\alpha 1$	Yes	All brain areas	Chr 5q34-q35	Chr 11	$\gamma 2$ and $\alpha 6$
$\alpha 2$	Yes	Cortex, limbic areas, striatum	Chr 4p13-p12	Chr 5	$\beta 1$
$\alpha 3$	Yes	Raphe nuclei Basal forebrain	X q28	Central part of X	No
$\alpha 4$	No	Hippocampus, thalamus	Chr 15 p14-q12	Chr 5	No
$\alpha 5$	Yes	Hippocampus	Chr 15q11–q15	Chr 7	$\beta 3$ and $\gamma 3$
$\alpha 6$	No	Cerebellar granule cells, cochlear nuclei	Chr 5q31.1-q35	Chr 11	$\gamma 2$ and $\alpha 1$

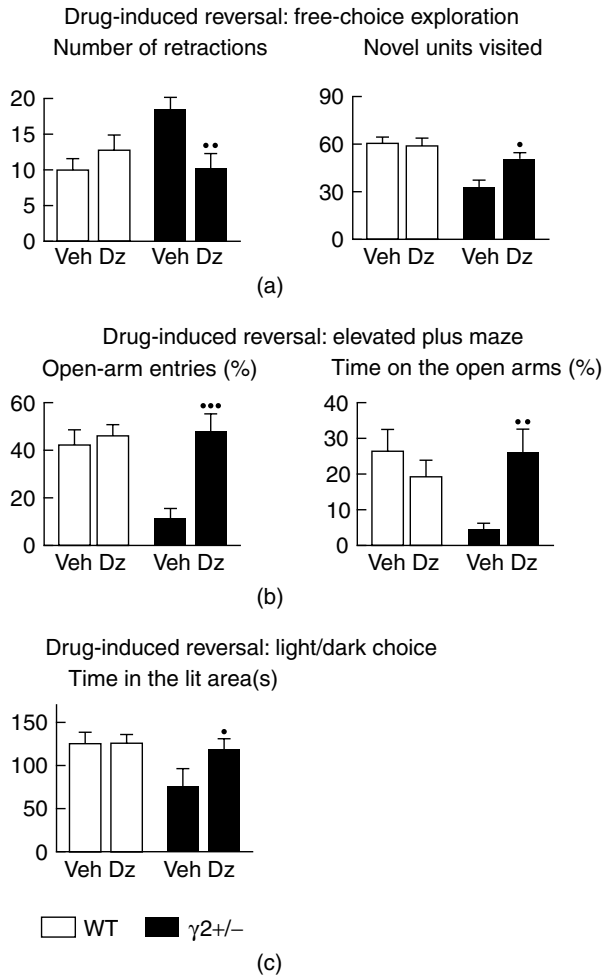

Figure XIX-3.9 Behaviour of wildtype (WT) and $\gamma 2$ +/− mice subjected to three tests of anxiety ((a) the free exploratory test; (b) the elevated plus maze; (c) the light/dark choice test) and treated either with vehicle or diazepam (0.3 mg kg^{-1}, i.p.). Results show that $\gamma 2$ +/− mice display increased anxiety when compared to wildtype mice as they exhibit an increased number of retraction, a decrease in novel units visited, a decrease of entries and time spent on the open arms and a decreased time spent in the lit box. At the dose used, diazepam did not elicit anxiolysis in vehicle-treated WT while it reversed the anxiogenic pattern observed in $\gamma 2$ +/− mice. Reproduced by permission from Crestani, F., Lorez, M., Baer, K., Essrich, C., Benke, D., Laurent, J.P., Belzung, C., Fritschy, J.M., Luscher, B. and Mohler, H., 1999. Decreased $GABA_A$-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nature Neuroscience*, **2**, 833–839

to negative associations (Figure XIX-3.9) (Crestani *et al.*, 1999). Alternate RNA splicing produces two alternative forms of the $\gamma 2$ subunit ($\gamma 2$L and $\gamma 2$S) that are expressed in different brain regions, more $\gamma 2$L being found in the cerebellum, while $\gamma 2$S is in the ascendant in the cerebral cortex. Moreover, $\gamma 2$S is expressed at a more or less constant level throughout brain development, while $\gamma 2$L production increases with maturation. Mice lacking $\gamma 2$L ($\gamma 2$L −/−) display increased sensitivity to BZ agonists associated with a decreased sensitivity to BZ inverse agonists, indicating a possible shift of the BZ receptor from an inverse agonist, leaning towards an agonist preferring configuration (Quinlan, Firestone and Homanics, 2000). However, in that case, one might expect a decrease of anxiety levels in those mice, while the contrary was observed. Surprisingly, $\gamma 2$ over-expression did not elicit any changes in several animal models of anxiety (Wick *et al.*, 2000).

Little information is available concerning the relevance of the δ subunit in the $GABA_A$ receptor in relation to anxiety. A $GABA_A\delta$ null mutant (δ −/−) mouse has been described that did not exhibit any modification of anxiety in animal models. However, the neuroactive steroid ganaxolone was unable to elicit anxiolytic action in the knock-out mice, as it does in wildtype mice suggesting a possible involvement of this subunit in the anti-anxiety-like effect modulators of the neurosteroid site of the $GABA_A$ receptor.

Drugs of the Future Acting at the $GABA_A$ Receptor Subtypes

The search for compounds chemically unrelated to the BZs with more specific therapeutic actions and without their concomitant unwanted effects has led to the development of drugs that selectively bind to a specific $GABA_A$ receptor subtype, display low efficacies at each $GABA_A$ receptor subtype, or combine selective affinity and differential intrinsic activity at these receptors (Griebel, Perrault and Sanger, 2000).

While there are $GABA_A$ receptor ligands claimed in patents or shown to bind selectively for all BZ-sensitive $GABA_A$ receptor subtypes, only compounds selective for the α_1 subtype have been studied extensively. These latter include compounds with greatly varying chemical structures. The most widely studied selective α_1 subtype ligands include the imidazopyridine zolpidem (see Figure XIX-3.6), the β-carboline abecarnil (Figure XIX-3.10) and the pyrazolopyrimidine zaleplon. These compounds are either marketed (zolpidem, zaleplon) or pre-registrated (abecarnil). In animal studies, zolpidem and abecarnil were found to produce sedative activity at much lower doses than those producing ataxia and myorelaxation, and after repeated treatment, did not produce tolerance and physical dependence as was observed with most BZs. However, selective $GABA_A$ α_1 subtype agonists are generally found to display weaker (if any) anxiolytic-like activity in animals than non-selective agents, thereby confirming the findings with α_1 knock-in mice which showed that this subtype is not primarily involved in the anxiolytic effects of $GABA_A$ receptor agonists (Rudolph *et al.*, 1999).

Unlike selective $GABA_A$ α_1 subtype agonists, non-selective $GABA_A$ receptor partial agonists such as bretazenil, imidazenil and Y-23684 (Figure XIX-3.10) were found to display comparable or even greater efficacy in anxiety models than BZs. In addition, they had lower liabilities for sedation and muscle relaxation compared to conventional BZs.

Based on the findings from experiments using mice with point-mutated diazepam-insensitive $GABA_A$ receptor subtypes, that the anxiolytic effects of $GABA_A$ receptor agonists are mediated by the α_2 $GABA_A$ receptor, research for anxioselective compounds acting at the $GABA_A$ receptor subtypes has focused on the development of ligands that display functionally selective agonist activity at the α_2 $GABA_A$ receptor subtype. The recently discovered pyridoindole derivative, SL651498 fulfils this criterion. Although the drug has also high affinities for the α_1 and α_3 subtypes, it displays higher intrinsic efficacy at the α_2 subtype as compared to the other $GABA_A$ receptor subtypes. In animal experiments, SL651498 elicited anxiolytic-like activity qualitatively and quantitatively similar to that of BZs, but unlike these latter, it induced central depressant effects at doses much higher than those producing anxiolytic-like activity. Moreover, in contrast to BZs, SL651498 did not produce tolerance to its anticonvulsant activity or physical dependence, and was much less active than BZs in potentiating the depressant effects of ethanol. The 'anxioselective' profile of SL651498 is in agreement with the idea that $GABA_A$ α_2 subtype plays a major role in regulating anxiety, and suggests that targeting selectively $GABA_A$ receptor subtypes can lead to drugs with increased clinical specificity (Low *et al.*, 2000).

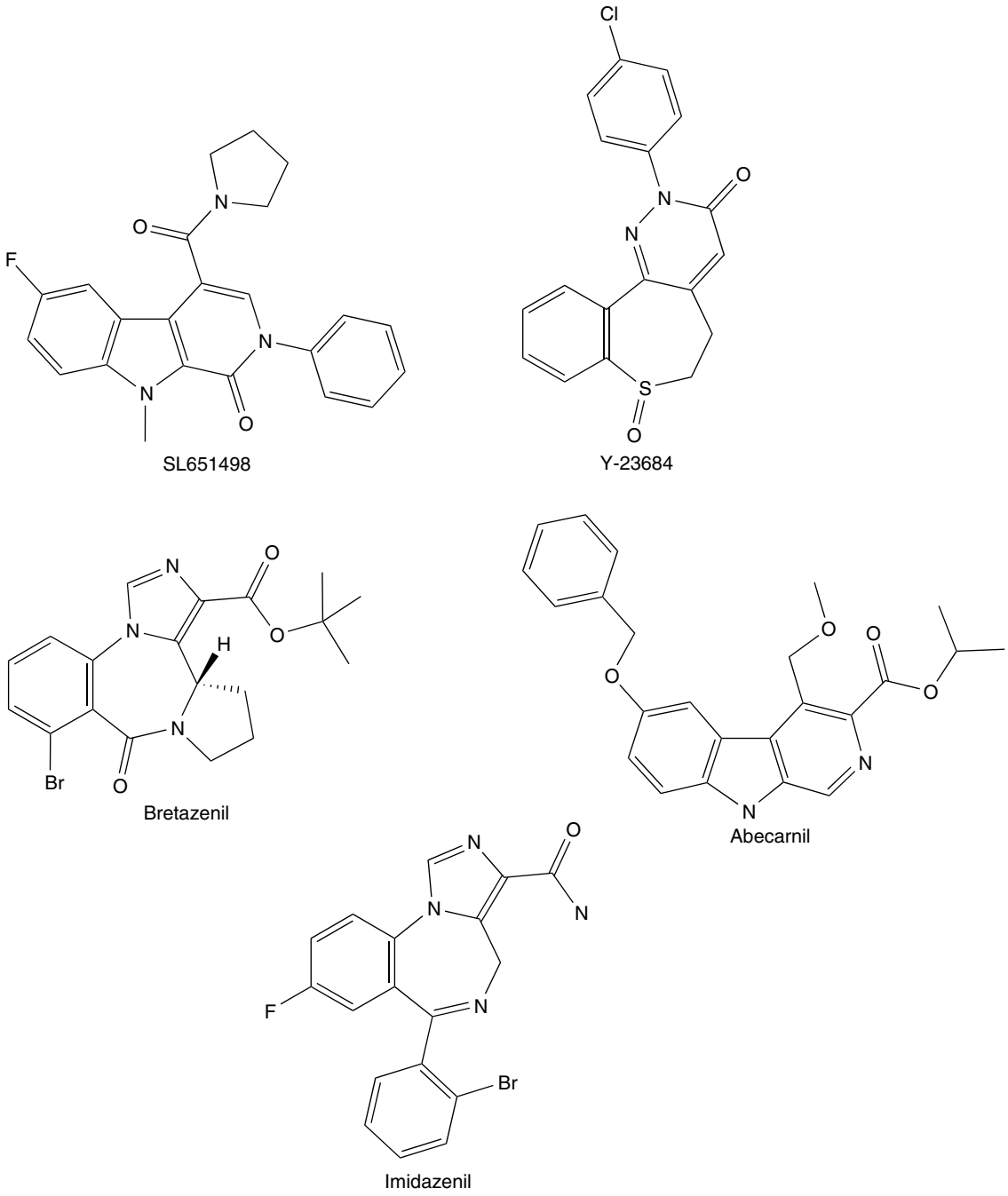

Figure XIX-3.10 Drugs of the future. Chemical structure of the partial agonists bretazenil, imidazenil and Y-23684, of the $\alpha 1$ selective agent abecarnil and of the $\alpha 2$ selective compound SL 651498

AMINO ACID NEUROTRANSMITTER SYSTEM IN ANXIETY DISORDERS

Anxiety Disorders

Anxiety can be considered as an everyday life emotion that ones experiences when subjected to threatening or stressful situations. However, in certain cases anxiety can become excessive, as in anxiety disorders. Anxiety disorders have a lifetime incidence of 16% (Walley, Beebe and Clark, 1994) and it is considered that in any six-month period, 9% of Americans are affected by such an affliction. Anxiety disorders include generalized anxiety disorder (GAD), panic disorder, obsessive–compulsive disorders, phobias and post-traumatic stress disorder (PTSD). In this chapter, we shall not consider dissociative and somatoform disorders as they are studied in further chapters of this book.

Generalized Anxiety Disorder (GAD)

GAD is considered to be a constant state of anxiety, worries occurring for almost any ordinary event. It frequently has other associated disorders accompanied by apprehension, increased tension

and hyperalertness. Even if it is the most common anxiety disorder, (14.80% worldwide incidence), little research has been carried out on investigating the underlying psychobiological features of that affliction. It has been shown that platelet and lymphocyte BZ receptors have low binding in GAD. As for cerebral BZ receptor binding in subjects with GAD, the findings are contradictory. Some have found a decrease (Tiihonen *et al.*, 1997), while others did not see any difference (Abadie *et al.*, 1999).

BZs are largely prescribed in the treatment of GAD (Hoehn-Saric and McLeod, 1991). Patients are generally treated with BZ having long elimination half-life, so that they do not have to take the treatment several times a day to prevent rebound anxiety. The main risk is linked to the ability of BZs to elicit tolerance and to have abuse potential. As patients need long-term medication, there is a high risk to elicit withdrawal symptoms when the treatment is discontinued. It should be noted that these compounds do not only relieve anxiety, but they are also effective in reducing hyperalertness, insomnia, tension and some somatic symptoms. However, one has to keep in mind the fact that some symptoms of GAD, such as tension, somatic modifications or hyperalertness may in fact contribute to the increase in anxiety, as suggested by some emotion theoricians. In that case, neither anxiety nor other symptom of GAD can be suppressed by compounds that are not acting on the somato-visceral perception.

Panic Disorder and Conditioned Fears

Though the delineation of panic disorder as a specific category had heuristic value, it has also left other fruitful conceptions in the dark (Marks, 1987), which are now implicitly coming back when categorizing panic disorder as a fear-conditioned disorder (Gorman *et al.*, 2000). Indeed, in order to understand the onset of panic disorder (characterized by episodic paroxystic anxiety states, phobic fears, and several autonomic- and endocrine-related symptoms) beside heritable aetiological factors, environmental disruption is needed. Several studies have shown an association between disruptions of early attachment to parents and the development of panic disorder (Tweed *et al.*, 1989; Stein *et al.*, 1996).

When the term 'panic attacks' was introduced in the DSM-III in 1980, the prevalent view was that the anxiety disorder characterized by severe spontaneous panic attacks was mainly alleviated by tricyclic antidepressants, while the anxiety disorder corresponding to the absence of such acute crisis (GAD) was treated by BZs. This was the rationale for splitting anxiety neurosis in two different anxiety disorders in the DSM-III. This historical background led to the prejudice that BZs may be ineffective in panic attacks. This view was reinforced by the observation that imipramine was more effective than chlordiazepoxide in panic attacks (McNair and Kahn, 1981). However, the dose of chlordiazepoxide used in that study was rather low. More recently, the contribution of amino acids in panic disorders has been evoked by the demonstration of the efficacy of higher doses of BZs in blocking panic attacks. The prototypical BZs used in the treatment of panic are alprazolam and clonazepam, even if other BZs are also effective agents. Clonazepam has a longer half-life than alprazolam, which allows a decrease in the number of daily administrations and thus avoids interdose rebound anxiety. However, many clinicians continued to claim that BZs had weak antipanic efficacy when compared with antidepressants. This prompted some researchers to compare the potency of alprazolam with imipramine and placebo in a double-bind study (Sheehan and Raj, 1990). Results showed that alprazolam was as effective as imipramine (except for the depressive symptoms that are often co-morbid with panic disorder, which were only treated by imipramine), both compounds being superior to placebo. Alprazolam was also effective in attenuating lactate-induced panic attacks. The common problem is related to the abuse potential observed with BZs. Therefore, these compounds are often prescribed for their short-term effects and then dosage is rapidly tapered and antidepressant treatment initiated. BZ are not the sole treatment of panic attack involving increase in GABA function. Indeed, when patients do not respond to BZ or to antidepressants, a medication with valproic acid can be prescribed. Valproic acid increases GABA function by stimulating the activity of glutamic acid decarboxylase, the enzyme responsible for GABA synthesis, and by inhibiting GABA degradation enzymes such as GABA transaminase. Three studies show that valproic acid was superior to placebo on various components of panic attacks and was able to block lactate-induced panic attacks.

Moreover, psychobiological data have also revealed some abnormalities in GABA neurotransmission in patients with panic disorder, suggesting that pharmacological treatments with BZs or valproic acid may in fact act on these abnormalities. For example, patients with panic disorder have been shown less sensitive to BZs on several psychophysiological measures (Roy-Byrne *et al.*, 1996). Furthermore, pre-clinical findings suggested that a decrease of GABA transmission in a network centred in the amygdala — and involving hippocampus and prefrontal cortex — with projections to midbrain central grey and hypothalamus, leads to paroxystic anxiety responses (LeDoux *et al.*, 1988; Davis, 1992). Finally, intravenous lactate, which induces panic attacks, produces a decrease in circulating plasma GABA levels.

However, most studies of binding at the BZ-$GABA_A$ receptor are contradictory. Indeed, some findings suggest global reduction of binding (Malizia *et al.*, 1998); other findings affirm only local or state-related panic anxiety reduction (Bremner *et al.*, 2000); and further studies found no peculiarity or increase in BZ receptor density in association with up-regulation hypotheses (Brandt *et al.*, 1998; Abadie *et al.*, 1999). The idea that in this disorder the subject has difficulty in protecting himself in a state of stress could bring forward new hypotheses (Kellner and Yehuda, 1999; Strohle *et al.*, 1999).

Obsessive Compulsive Disorder (OCD)

OCD is characterized by repetitive thoughts or behaviour that are felt by the subject and are difficult to prevent. Most of the time the behaviour or thoughts are absurd, and consume so much time that they alter social functioning of the subject.

The psychobiological approach accumulates arguments — including those from lesional models (Laplane *et al.*, 1989) — for abnormalities associated with obsessive and compulsive manifestations in frontocortico-striatal-thalamic networks. Indeed, a hyperactivity of this axis has been shown within orbito-frontal and anterior cingulate cortex, as well as caudate nucleus and thalamus, and a decrease of N-Acetyl-Aspartate (NAA), a putative marker of neuron viability, within striatal areas — which could be sites of primary pathology — and thalamus, a site of integration and relay, especially involved in compulsions (Fitzgerald *et al.*, 2000).

Functional neuroimaging studies in OCD patients during symptom provocation suggests increased glutamatergic activity. Current psychobiological research on OCD puts forward that *basal ganglia* dysfunctions could contribute to the aetiology of the symptoms. Indeed, it has been suggested through functional imaging techniques on subjects with OCD, before and after treatment by Selective Serotonin Reuptake Inhibitors (SSRIs), (substances known for their positive effect on OCD symptomatology) that *caudate nucleus* seems primarily involved in the medication's efficacy on OCD (Baxter, Schwartz and Bergmam, 1992). The interrelations of amino acids such as *γ-aminobutyric acid* (GABA) or *glutamate* with serotonin (5-HT) pathways could be enlightened by some findings. First, the 5-HT_{2a} stimulation on GABA neurons opposes the glutamate action on the striatum, an area known for being rich in neurons

with serotonin synthesis (Chugani *et al.*, 1998). Second, the prefrontal cortex highly innervates the caudate nucleus (Modell *et al.*, 1989) by glutamatergic projections. Third, glutamate can decrease the release of 5-HT in the caudate nucleus in humans (Becquet, Faudon and Hery, 1990) and be at the same time affected in its action by serotonin neurons (Edwards *et al.*, 1996).

As BZs are effective in the treatment of anxiety, their efficacy in OCD has also been evaluated. In fact, BZs are devoid of anti-obsessive–compulsive effect *per se* but they can alleviate the high anxiety or insomnia that are in some case associated with OCD. In this way they can be considered as a symptomatic treatment of OCD. They can also provide a short-term relief for the distress of the patients, before the anti-obsessive–compulsive effects of serotonin reuptake inhibitors can be observed, or before a cognitive therapy can be undertaken. It should be noted that this observation is not true for all BZs. In fact, there is an exception as the BZ clonazepam has been shown significantly more effective as a monotherapy than some antidepressant after 3-week treatment (Hewlett, Vinogradov and Agras, 1992). A proposed explanation as to the superiority of clonazepam over other BZs in the treatment of OCD is related to the fact that this drug may have specific effects on the serotoninergic system.

Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a condition specified by repetition in thoughts, nightmares, physical and mental re-experiencing of the traumatic experience. It is associated with a negative semiology, such as numbing or amnesia, related to the dissociative field. Preclinical data put forward that stress induces cortico-limbic release of glutamate. Clinical observations suggest that N-methyl-D-aspartate antagonists may also induce glutamate release while provoking dissociative-like symptoms. Some authors then hypothesized that hyperglutamatergic states could contribute to acute and chronic consequences of trauma (Chambers *et al.*, 1999).

Among the psychobiological alterations observed in PTSD, the neuroendocrinological peculiarities in the hypothalamic-pituitary-adrenal axis, including cortisol decrease and reactivity alterations are documented the most. Specificities of GABA function have been studied in subjects with PTSD. The failure of the benzodiazepine antagonist flumanezil to produce flashbacks in PTSD suggest a weak role of attention deficit in the chronic disorder. The background of this topic includes the fact that $GABA_A$ receptors inhibit meso-prefrontal dopamine neurons, that BZs and neurosteroids enhance $GABA_A$ inhibition, that altered BZ receptors or a diminution of an endogenous ligand may decrease GABA inhibition and lead to excessive anxiety, and that PTSD may deplete an endogenous ligand and produce receptor alteration.

Morris and his colleagues (Morris *et al.*, 2000) presented an interesting positron emission tomography (PET) study to estimate the binding potential (BP) of $GABA_A$ benzodiazepine receptors *in vivo* using the BZ receptor antagonist radioligand C^{11}-flumanezil, comparing 13 subjects with PTSD and 13 without disorder. B_{max} was the same for subjects both with and without PTSD; K_d was lower in PTSD cases, and the B_p constant of the BZ receptor was higher in PTSD cases, especially in cerebellar, latero-temporal, occipital and prefrontal areas.

BZ medication in PTSD is still controversial. A study has shown that alprazolam induces a slight improvement in anxiety on the Hamilton scale in PTSD patients but there is no superiority of alprazolam over placebo on the PTSD scale. Moreover, alprazolam and clonazepam have positive effects on hyperarousal, as does valproic acid. The GABA transaminase vigabatrin has also been shown to ameliorate the exaggerated startle response that is found in PTSD. These observations suggest that increasing the GABA function may be a treatment of some symptoms of PTSD such as anxiety, increased startle or hypervigilance, rather than a treatment of PTSD *per se*. Furthermore, PTSD is co-morbid with other anxiety disorders such as GAD so that BZ may in fact alleviate some symptoms related to GAD, rather than treat PTSD.

Phobias

The main characteristic of phobias is marked and persistent fear of some specific situations (for example enclosed spaces) or objects/animals (small animals such as mice). Here we may emphasize on a particular type of phobia — social phobia — which is characterized by fear of social situations in which embarrassment may occur, as some arguments suggesting an involvement of the GABAergic system have been proposed. Indeed, compounds potentiating the action of GABA such as benzodiazepines and conventional anticonvulsants have been evaluated as treatments for social phobia. Among the benzodiazepines, clonazepam is the best studied, and showed efficacy in several studies. Among the anticonvulsants, gabapentin and pregabalin, which are analogues of GABA, have been shown to be more effective than placebo in double-blind studies. Furthermore, subjects with social phobia showed abnormalities in peripheral benzodiazepine receptors, which suggests that these receptors may play a role in the pathophysiology of this disorder.

BZ and Anxiety Disorders

With the exception of GAD and, to a lesser extent panic attack, it is noteworthy that BZs or drugs that increase GABA transmission may alleviate some symptoms associated with anxiety disorders (including anxiety, insomnia, tension, startle), rather than treat anxiety disorders *per se*. In these disorders, anxiety is often a symptom subsequent to a core semiology resulting mainly from cognitive activations, for instance 're-experiencing' in PTSD or 'obsession' in OCD, rather than a primary autonomic dysregulation and first rank therapeutic target. This is the reason why the ICD-10 classification of the World Health Organization (WHO) preferred to call the chapter from the DSM-IV 'Somatoform, stress-related and neurotic disorders' rather than 'Anxiety disorders'.

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XIX-4

Peptidergic Transmitter System and Anxiety Disorders

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INTRODUCTION

Since their introduction in the beginning of the 1960s benzodiazepines (BZD) have been mainly used in treating anxiety disorders. With the exception of general anxiety disorder (GAD) the identity of the specific anxiety disorder for which each drug is most effective has been hindered by early clinical trials and psychiatric classification. Few proficient drugs have been launched since, the exception being buspirone a 5-HT_{1A} partial agonist. The recent utilization of 5-HT reuptake inhibitors is mainly in the field of panic disorder, social phobia, post-traumatic stress disorder and possibly GAD. Nevertheless, the treatment of anxiety disorders remains a very active field of research and there are some promising results in animal research with neuroactive peptides in the modulation of anxiety behaviours. This section reviews the literature on the potential effects of cholecystokinin (CCK), corticotrophin-releasing factor (CRF), neuropeptide Y (NPY), substance P (SP) and neurokinin (NK) and natriuretic peptides (NP) in anxiety disorders in various animal and human models.

CHOLECYSTOKININ AND ANXIETY

Cholecystokinin (CCK) is synthesized *de novo* in the brain (Goltermann, Rehfeld and Roigaard-Petersen, 1980). CCK has been shown to induce excitation of central neurons. However, inhibitory postsynaptic effects have been also recorded (Dodd and Kelly, 1981; Mac Vicar, Kerrin and Davison, 1987). CCK receptors have now been classified as CCK1 and CCK2 independent of their localization replacing CCK-A and CCK-B. CCK receptors are widely distributed throughout the CNS, with high densities in the striatum and nucleus accumbens (Hill *et al.*, 1987).

Animal Models

Evidence suggests that CCK is implicated in the regulation of anxiety. The peripheral administration of CCK agonists induces anxiety in various animal species, including mouse, rat, cat and monkey (Bourin *et al.*, 1996; Shlik, Vasar and Bradwejn, 1997). This effect is apparently mediated via CCK-2 receptors (previously named CCK-B) since the selective CCK-2 receptor agonists (CCK-4 and pentagastrin) are effective. Moreover, the anxiogenic action of CCK agonists is reversed by preferential CCK-2 receptor antagonists (L-365,260, Cl-988). It should be noted that CCK is active in ethological, but not in conditioned, models of anxiety. However, several studies were unable to reveal the anxiogenic action of CCK agonists in ethological models either. The anxiolytic action of CCK-2 receptor antagonists is even more doubtful since recent studies did not establish any significant action when CCK-2 receptor antagonists were given as single treatments (Dawson *et al.*, 1995).

Some evidence exists that CCK-induced anxiety may be dependent on the level of pre-experimental stress in animals (Koks *et al.*, in press). Therefore, an attempt was made to reveal the significance of pre-experimental stress on CCK-induced anxiety in the elevated plus-maze. Male Wistar rats were divided into four different groups. Two groups of rats were handled in the experimental room for three consecutive days before the experiment. The other two groups of animals were brought to the experimental room immediately before the beginning of the experiment. The handled and non-handled rats were divided into two groups after the injection of caerulein ($5\,\mu g\,kg^{-1}$), a CCK receptor agonist. Half of the animals were isolated after the injection, whereas the other half were returned to their home cage. The anxiogenic action of caerulein was strongest in the rats brought to the experimental room immediately before the plus-maze exposure and kept isolated after the injection of CCK agonist. By contrast, caerulein did not cause any anxiety in the handling habituated and non-isolated rats. Accordingly, the anxiogenic action of caerulein was dependent on the level of pre-experimental stress in rats. The anxiogenic action of caerulein in the stressed rats was dose-dependently reversed by L-365,260 ($1-100\,mg\,kg^{-1}$), a CCK-B receptor antagonist.

In another study the behavioural and neurochemical effects of long-term treatment with L-365,260, a CCK-B receptor antagonist, were investigated. L-365,260 ($100\,mg\,kg^{-1}$) was administered twice daily for 14 days. The repeated treatment with L-365,260 did not change locomotor activity, but it did reduce the exploratory behaviour of rats in the elevated plus-maze test (Koks *et al.*, in press). The neurochemical studies performed demonstrated that L-365,260 induced a sufficient blockade of CCK-B receptors in the brain. Namely, the density of CCK receptors was increased in the frontal cortex and the serum levels of thyrotrophin were reduced due to the repeated administration of L-365,260. Collectively, it is unlikely that CCK is directly involved in the neural networks mediating anxiety. The anxiogenic effect of CCK is rather mediated via other neurotransmitter systems like CRF, GABA, 5-hydroxytryptamine, noradrenaline and nitric oxide.

Human Studies

The starting point for human studies was in 1984 with the electrophysiological experiment of Bradwejn and de Montigny (1984), which demonstrated that benzodiazepine receptor agonists selectively and specifically antagonized sulphated CCK-8-induced excitation of hippocampal pyramidal neurons in rats. These studies provided evidence that anxiolytic benzodiazepines could antagonize the central action of a neuropeptide and it was proposed that benzodiazepine-mediated antagonism of CCK-induced excitation might be an important mechanism by which benzodiazepines exert

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their clinically relevant action. More importantly, the observation that an anxiolytic drug could block the excitatory action of CCK raised questions about whether CCK might be an endogenous anxiogenic compound.

De Montigny first reported that exogenous CCK-4 produced panic-like attacks in healthy volunteers and that these effects could be attenuated by pretreatment with lorazepam (de Montigny, 1989). Bradwejn and co-workers (1990) administered CCK-4 to patients with a current-point diagnosis of panic disorder using a double-blind placebo-controlled methodology. Bolus injections of CCK-4 (50 mg) precipitated a panic attack, as defined by DSM-III criteria and patient self-report, within one minute following administration in 11 trial patients studied, whereas none of the patients panicked following placebo. CCK-4 treatment elicited an average of 12 symptoms per patient, the most common symptoms being dyspnoea, palpitations/rapid heart, chest pain/discomfort, faintness, dizziness, paresthesia, hot flushes/cold chills, nausea/abdominal distress, anxiety/fear/apprehension and fear of losing control. It has been found that response to CCK-4 reliably differentiates panic disorder patients from healthy controls with no personal or family history of panic attacks. In a double-blind placebo control study, the patients with panic disorder experienced a greater number of symptoms and more intense symptoms following challenge with two doses of CCK-4 (25 and 50 mg) (Bradwejn, Koszycki and Shriqui, 1991). In addition, the incidence of panic attacks was markedly higher in patients than controls following injection of 25 mg (91% vs 17%) and 50 mg (100% vs 47%) of the peptide. Interestingly, the number and intensity of symptoms as well as the symptom profile were remarkably similar in both patients and normal subjects who panicked with the 50 mg dose of CCK-4, suggesting that the enhanced response in patients could not be readily attributed to a tendency to overendorse symptoms. These results are corroborated by studies of Abelson and colleagues (1991) and by van Megen and colleagues (1994) who used pentagastrin, a CCK agonist which incorporates the identical 4-amino acid sequence of CCK-4. Moreover, patients with panic disorder have been shown to have decreased concentrations of CCK-8s in the cerebrospinal fluid relative to control subjects (Lydiard *et al.*, 1992). Concentrations of CCK-8 in lymphocytes were also significantly reduced in patients with panic disorder compared to healthy controls (Brambilla *et al.*, 1993). These findings are also in favour of anomalies in the CCK system due to panic disorder. It is also likely that systemic administration of CCK-4 produces prominent respiratory and cardiovascular alterations in humans through a direct action on the brainstem (Bradwejn *et al.*, 1994).

Antipanic drugs and not other compounds, block the effect of CCK-4 (Bradwejn, 1995). Recently, it was demonstrated that the panic-induced effects of CCK-4 can be antagonized by chronic treatment with imipramine (Bradwejn and Koszycki, 1994). In one study, the pretreatment of patients with the selective CCK-B receptor antagonist L-365,260 dose-dependently blocked CCK-4-induced panic attacks (Bradwejn *et al.*, 1994). In a different study, the action of another CCK-B receptor antagonist Cl-988 was evaluated. There was a significant decrease in sum intensity scores and panic attack frequency following Cl-988 treatment (Bradwejn, 1995). These data apparently support the role of CCK-B receptors in the mediation of panicogenic-like action of CCK-4. Nevertheless, a placebo-controlled trial of L-365,260 did not result in any clinically significant improvement in patients with panic disorder (Kramer *et al.*, 1995). The possible reasons for lack of effect with L-365,260 are not clear, but the poor pharmacokinetic properties of the drug is the most plausible explanation.

A comparison of the neuronal responses to cholecystokinin tetrapeptide between younger and older patients was examined by Flint and colleagues (2000). In both age groups, maximum increase in prolactin Adrenocorticotrophic hormone ACTH and cortisol was significantly greater with CCK-4 than with placebo. Following administration of CCK-4, younger and older groups did not significantly differ in maximum increase in prolactin, ACTH or cortisol; older subjects had a statistically significant smaller increase in Growth Hormone GH compared with younger subjects but the magnitude of the difference was small and of doubtful clinical relevance. Older subjects who had a panic attack had significantly greater elevations of all hormones compared with those who did not panic, and younger panickers had a significantly greater elevation of GH compared with young non-panickers. For the most part, maximum changes in hormonal levels were not correlated with symptom severity, suggesting that other factors may have contributed to the differential effect of panic on the hypothalamic-pituitary-adrenal (HPA) axis.

More recently, the role of the HPA system in panic disorder was investigated (Ströhle, Holsboer and Rupprecht, 2000). Twenty-four patients with panic disorder were given injections of CCK-4 (25 μg). Panic attacks, psychological changes as well as ACTH and cortisol secretion were recorded. Fifteen of the 24 patients experienced a panic attack after CCK-4. ACTH secretion was significantly higher in the patients with CCK-4-induced panic attacks than in those without such attacks. The patients without CCK-4-induced attacks had a brief but less pronounced increase in ACTH concentrations. Cortisol concentrations were not significantly increased after CCK-4 administration. The increased ACTH concentrations suggest that the activation of the HPA system in CCK-4-induced panic attacks plays a physiological role. Corticotrophin-releasing factor (CRF) may be involved in experimentally and perhaps, naturally occurring panic attacks as well (see section on CRF). Several studies have found that the incidence and prevalence of panic disorder decline in later life (Flint, Cook and Rabins, 1996). The reason for this decline is unknown, but it has been hypothesized that age-related changes in brain neurotransmitter function may contribute.

CORTICOTROPHIN-RELEASING FACTOR AND ANXIETY

Corticotrophin-releasing factor (CRF) is a neuropeptide that plays a prominent role in the endocrine, autonomic, behavioural and immune responses to stress, through its action on the major physiological regulator of the HPA axis (Arborelius *et al.*, 2000). In response to stress, CRF released from the paraventricular nucleus (PVN) of the hypothalamus activates CRF receptors on anterior pituitary corticoreceptors, resulting in release of ACTH into the bloodstream. CRF induces various behavioural changes related to adaptation to stress, including food intake suppression, increase in locomotor activity and grooming in familiar environments, induction of aggression and enhancement of arousal.

CRF is widely distributed in the brain, with highest concentrations found in the hypothalamus. Moderate and low levels of CRF are also found in cortical and limbic structures. The amygdala is densely innervated by CRF neurons, which exert excitatory influences on various fear-circuit brain nuclei, including the locus coerulus (Coplan and Lydiard, 1998; Le Doux, 1998).

The effects of CRF are mediated by two specific G-protein receptors called CRF_1 and CRF_2. Tissue distribution analysis showed that CRF_1 receptor expression is most abundant in neocortical, cerebellar and limbic structures, whereas CRF_2 receptor expression is generally localized in subcortical structures, notably in the later septum and various hypothalamic areas (Chalmers, Lovenberg and De Souza, 1995). The CRF_1 receptor displays a high affinity for CRF, while the CRF_2 receptor shows a lower affinity (Grigoriadis, Lovenberg and De Souza, 1996; Radulovic *et al.*, 1999). This anatomical information provides a basis for functional hypotheses related to CRF receptor subtypes, suggesting that CRF may contribute significantly both to behavioural responses to stress and to emotional behaviour itself.

Animal Models

The hypothesis that CRF plays a role in the pathophysiology of anxiety disorders derives mainly from preclinical findings (Arborelius *et al.*, 1999). There are several studies supporting the hypothesis that hypersecretion of CRF is a crucial factor in anxiety disorders (Nemeroff *et al.*, 1984). A number of researchers showed that when CRF was injected into the brain of rats, it produced many of the signs and symptoms seen in patients with anxiety disorders (Arborelius *et al.*, 1999; Koob and Heinrichs, 1999).

A vast literature indicates that intracerebroventricular (i.c.v.) administration of CRF, which presumably increases the concentrations of CRF in the central nervous system, produces physiological and behavioural alterations virtually identical to those obtained in laboratory animals in response to stress, including changes in heart rate and mean arterial pressure, changes in gastrointestinal function, suppression of exploratory behaviour, induction of grooming, reduction of feeding and food intake and disruption of reproductive behaviour. Further actions of centrally administered CRF include the potentiation of acoustic startle responses, the facilitation of fear conditioning and the enhancement of shock-induced freezing and fighting behaviour. These actions of CRF are indicated not to involve the activation of the pituitary-adrenal axis but are mediated by CRF receptors present in the CNS (Griebel, 1999). Micro-injected of CRF directly into the locus coerulus of rats has been found to produce defensive withdrawal responses from a novel environment (Butler *et al.*, 1990) and to reduce drinking behaviour in a brightly illuminated area (Weiss *et al.*, 1994). Similarly, intra-amygdala infusion of CRF has been reported to produce anxiogenic-like behaviour in the open-field test and increase grooming in rats (Liang and Lee, 1988; Lee and Tsai, 1989; Elkabir *et al.*, 1990).

Several peptide and non-peptide CRF receptor antagonists have been studied extensively in experimental models of anxiety. Treatment with antagonists that selectively block CRF_1 receptor action was shown to promote anxiolytic responses in the elevated plus-maze, the light–dark box, the mouse defense test battery and the fear-potentiated startle test (Lundkvist *et al.*, 1996; Schulz *et al.*, 1996; Griebel, Perralut and Sanger, 1998; Okuyama *et al.*, 1999). Alpha-helical CRF, a non-specific CRF receptor antagonist, reduced anxiety-like behaviour in the elevated plus-maze whereas astressin, an antagonist with a higher *in vitro* potency, did not (Brauns *et al.*, 2001). Administration of CP-154,526, a specific CRF_1 receptor antagonist, to rats blocked both the acquisition and expression of conditioned fear, thus suggesting that the CRF_1 receptor might be related to anxiety (Hikichi *et al.*, 2000). The acute administration of CP-154,526, or its analogue antalarmin was demonstrated as blocking CRF- and stress-induced elevations in plasma adrenocorticotrophin and to possess anxiolytic activity in the elevated plus-maze (Lundkvist *et al.*, 1996; Deak *et al.*, 1999), in the light/dark test and the mouse defensive test battery, but not in conflict tests (Griebel, Perralut and Sanger, 1998). After 9 to 10 days of chronic treatment with CP-154,526 ($3.2\,mg\,kg^{-1}$), defensive withdrawal behaviour was also significantly decreased, indicating anxiolytic activity (Arborelius *et al.*, 2000). CP-154,526 was also shown to reduce separation-induced vocalization in rat pups (Kehne *et al.*, 2000).

Antagonism of CRF_2 receptors produces a consistent anxiolytic-like behavioural profile in a number of different animal models. Antisauvagine-30 (anti-SVG-30), a high affinity CRF_2 peptide antagonist was found to produce a dose dependant reduction in the duration of conditioned freezing, increase the level of open-arm exploration and increase the time spent in an open field in rats (Takahashi *et al.*, 2001).

It has been demonstrated that central administration of CRF increases anxiety-like behaviour in rodents and that transgenic mice that overexpress CRF exhibit anxiogenic behaviour. Two recent studies using CRF transgenic mouse lines overexpressing CRF further emphasized the anxiogenic properties of CRF, since these mice exhibited a behavioural state resembling that produced by anxiety (Stenzel-Poore *et al.*, 1996; Koob and Gold, 1997). Studies using CRF_1 receptor knockout mice have revealed that the anxiogenic effects of CRF appears to be mediated by the CRF_1 receptor subtype (Heinrichs *et al.*, 1997; Smith *et al.*, 1998; Timpli *et al.*, 1998). CRF_1 knockout mice show increased anxiolytic-like activity in the elevated plus-maze and a tendency to enter the illuminated region of a light–dark box (Smith *et al.*, 1998; Timpli *et al.*, 1998). Varying results have been observed with CRF_2 knockout mice. Using open-field exploration as a measure, one study reported that CRF_2 knockout mice showed increased centre activity (Kishimoto *et al.*, 2000), another found a decrease in centre scores (Bale *et al.*, 2000) and a third study found no effects of CRF_2 knockout on anxiety behaviour (Coste *et al.*, 2000). In another model of anxiety a decrease in open-arm exploration, suggesting an increase in anxiety was reported (Bale *et al.*, 2000; Kishimoto *et al.*, 2000).

There is considerable evidence that CRF is involved in the anxiogenic and aversive effects of withdrawal from abuse drugs such as cocaine (Basso *et al.*, 1999). Since CRF is suggested to play a critical role in the development of stress and because anxiety is recognized as an important result of cessation of chronic drug administration, it is possible to postulate that brain CRF modulates the neurobiological mechanisms that underlie the anxiety and stress associated with drug withdrawal (Lu, Liu and Ceng, 2001). Lu and colleagues (2001) have suggested that the CRF_1 receptor, but not type 2, mediated the stress-induced reactivation of cocaine-conditioned place preference, as only the CRF_1 antagonist CP-154,526 but not the CRF_2 antagonist, AS-30 was capable of the reactivation.

Suggestions of an inverse relationship between the CRF_1 and CRF_2 receptor systems have been reported using an anxiety model based on adverse early-life experience (Skelton *et al.*, 2000). Thus CRF neuronal systems may be comprised of two separate, but interrelated, subdivisions that can be coordinately and inversely regulated by stress, anxiety or anxiolytic drugs.

Human Studies

Although there is no direct evidence that CRF or CRF receptor ligands may modulate anxiety in humans, clinical data suggesting a role for CRF in anxiety disorders have been accumulating over recent years. Clinical studies have not revealed any consistent changes in cerebrospinal fluid (CSF) CRF concentrations in patients with anxiety disorders; however preclinical findings strongly implicate a role for CRF in the pathophysiology of certain anxiety disorders (Arborelius *et al.*, 1999). CSF levels of CRF have been shown to be elevated in patients suffering from obsessive compulsive disorder (Altemus *et al.*, 1994), post-traumatic stress (Stout, Kilts and Nemeroff, 1995; Holsboer, 1999) but not panic disorder (Jolkkonen *et al.*, 1993). Fossey *et al.* (1996) examined the CSF concentrations of CRF in a group of patients with anxiety disorders and found no difference between four diagnostic categories: panic disorder, generalized anxiety disorder, obsessive compulsive disorder and normal comparison subjects.

The findings that CRF stimulation increases anxiety-related behaviours in a variety of animal models suggest that agents acting at CRF receptors may have therapeutic effects in anxiety- or stress-related disorders. CRF receptor antagonists may represent a new option for pharmacotherapy of stress-related disorders and some of these CRF receptor antagonists are currently undergoing clinical trials to determine their efficacy and tolerability in patients

with anxiety disorders as well as testing of the hypothesis that CRF hypersecretion is responsible for certain features of anxiety disorders.

NEUROPEPTIDE Y AND ANXIETY

Neuropeptide Y (NPY) is widely distributed throughout the peripheral and central nervous system. In the brain high concentrations of NPY are found in most cerebral regions including the cerebral cortex, hippocampus, thalamus and brainstem. However, regarding NPY receptors designated Y1, Y2, Y3, Y4, Y5 and Y6 (Gehlert *et al.*, 2001), the distribution varies. Y1 receptors are essentially present in high density in the cerebral cortex, thalamus and in some parts of the amygdala. Y2 receptors are found in the hippocampus, substantia nigralateralis, hypothalamus and brainstem. The so-called Y3 receptors may only be present in the brainstem but are not well characterized and have not as yet been cloned (Lee and Miller, 1998), and so far their existence remains controversial (Ingenhoven and Beck-Sickinger, 1999). Y4 receptor mRNA is mainly expressed in the peripheral nervous system, in the colon, small intestine and prostate (Michel *et al.*, 1998). Both the human and rat Y5 receptor have been cloned and with its mouse analogue, the Y6 receptor, form the latest members of the neuropeptide Y receptor family. Y5 receptor mRNA has been found in a number of brain regions implicated in the regulation of emotion (Nichol *et al.*, 1999) including hypothalamic nuclei, lateral septum, locus coeruleus and the amygdala.

The presence of NPY receptors in many regions implicated in the regulation of stress and the anxiolysis process provides the hypothesis of the implication of NPY in such mechanisms, leading to studies in animal models of anxiety.

Animals Models

Exogenous neuropeptide Y (NPY) reduces experimental anxiety in a wide range of valid animal models. The i.c.v. administration of NPY induces anxiolytic-like effects in various animal models of anxiety including ethological models such as the elevated plus-maze and social interaction test, models based on fear suppression of behaviour, including non-operant punished drinking, i.e. the Vogel's test (see Griebel (1999) for review), and operant food reinforced paradigm, as well as fear-potentiated startle. Heiling *et al.* (1989) have demonstrated using two rat models of anxiety, namely the Montgomery's conflict and the Vogel's drinking conflict test that NPY (0.4 to 2 nmol i.c.v.) induced anxiolytic-like effects with sedation and ataxia at higher doses. Increased plasma levels of immunoreactive NPY are observed following multiple aversive stimuli such as cold stress and handling (Zukowska-Grojec *et al.*, 1991), hypoxia (Cheng *et al.*, 1992) and emotional stress (Castagne *et al.*, 1987). NPY has an anticonflict effect when micro-injected into the amygdala (Heiling *et al.*, 1993). Micro-injected of NPY into the basolateral nucleus of the amygdala produced an increase in social interaction time in rats (Sajdyk, Vandergriff and Gehlert, 1999). The discovery and use of specific NPY agonists have demonstrated that the Y1 receptors are likely to be involved in the anxiolytic action of NPY (Broqua *et al.*, 1995). In this study the central administration of NPY increased the time spent in the open arms of an elevated plus-maze in the rat. Furthermore the administration of the NPY agonists, NPY_{2-36} and [Leu^{31}, Pro^{34}] NPY was found to increase the percentage of time spent in the open arms of the maze, while the Y2 receptor agonist NYP_{13-36} failed to do so. In the fear-potentiated startle model, a conditioned paradigm which is totally different from the precedent model, NPY, PYY and the agonists NPY_{2-36} or [Leu^{31}, Pro^{34}] NPY had similar effects to those of conventional anxiolytic drugs such as diazepam or even buspirone. Taken together these results are in agreement with the hypothesis that the antianxiety effects of NPY are mediated through Y1 receptors. The use of specific antagonists could help in understanding the involvement of endogenous NPY in the regulation of anxiety in animals. A highly selective, non-peptide NPY1 receptor antagonist, BIBP3226 induced an anxiogenic-like effect in the elevated plus-maze at a dose of 0.5 μg i.c.v. (Kask, Rago and Harro, 1996). This supports the hypothesis that endogenous NPY could reduce anxiety and/or neophobia through Y1 receptors. The anxiolytic-like effect of NPY in the rat social interaction test was antagonized by the specific Y1 receptor antagonist, ((R)-N-[[4-(aminocarbonylaminomethyl)phenyl]methyl]-N2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (Sajdyk, Vandergriff and Gehlert, 1999). In order to confirm the participation of endogenous NPY in regulating anxiety, Wahlestedt *et al.* (1993) synthesized an antisense 18-base-oligodeoxynucleotide on the basis of the sequence of the rat Y1 receptor. The i.c.v. administration of this antisense via chronic implanted guide cannulas (injection of 50 μg, four times over 2 days) produced an anxiogenic-like effect in the rat elevated plus-maze with more than 60% decrease in the activity in open arms, with no difference in overall locomotor activity. After testing, rats were killed and binding was performed on cortical and striatum tissue. The Y1-type binding site was decreased by almost 60%.

On the other hand, BIBP3226-induced anxiogenic effects is counteracted by the administration of 0.5 μg mg^{-1} of the benzodiazepine diazepam (Kask, Rago and Harro, 1996). This suggests that the neuropeptidic Y neurotransmission is connected to the GABAergic system. However, Britton *et al.* (1997) have demonstrated that flumazenil, a benzodiazepine receptor antagonist and picrotoxin, a GABA receptor antagonist, failed to alter NPY-induced punished responding in a rat conflict paradigm. These results confirm that the anxiolytic action of NPY is independent of a direct implication of the GABA/benzodiazepine receptor complex. On the other hand, the hypothesis that the opioid system may interact with NPY, is supported by the fact that naloxone (0.25 to 2 mg kg^{-1}) antagonized the effects of NPY in a conflict test and that central administration of the selective mu opiate antagonist CTAP partially blocked NPY-induced conflict responding (Britton and Southerland, 2001). Furthermore, concerning NPY neurotransmission interaction with other systems, a study reported that the anxiogenic effect of a selective Y1 receptor was prevented by the blockade of CRF receptors, suggesting antagonistic effects of endogenous NPY and CRF in shaping the response to novelty (Kask, Rago and Harro, 1997; Britton *et al.*, 2000).

A recent study, (Kask, Rago and Harro, 1998) reported new data on the mechanism of action of NPY. In fact, NPY has an anticonflict effect when micro-injected into the central nucleus of the amygdala but NPY is also found in other brain structures including the locus coeruleus. Interestingly, the anxiolytic-like effect of NPY in the vicinity of the locus coeruleus may involve the Y2 receptor subtype instead of Y1, as the Y2 agonist NPY_{13-36} was active and the Y1 agonist [Leu^{13}, Pro^{34}] was without effect. One explanation is that NPY transmission in the locus coeruleus modulates noradrenergic transmission via Y2 receptors in response to stress or novelty. Neuropeptide Y_{13-36} was found to be a mixed Y2/Y5 receptor agonist. Taken together these data suggest that the anxiolytic-like effect of NPY is not mediated via the single Y1 receptor subtype and that other receptors such as Y2 and Y5 may be involved. Using the Y5 receptor antagonist CGP71683A, Criscione *et al.* (1998) have shown that high doses of this compound induce conditioned taste aversion suggesting that Y5 receptor antagonism may cause anxiety or sickness behaviour. Recently, Kask *et al.* (2001) have demonstrated that CGP71683A failed to induce anxiogenic-like effects in the elevated plus-maze or decrease social interaction under testing conditions that were appropriate to detect anxiogenic-like effects of the drug. Nevertheless, rats stressed by prior exposure

to an elevated plus-maze test session have shown increased anxiety in the open-field test (Kask *et al.*, 2001). It is suggested that Y1 receptor mediates tonic NPY-induced anxiolysis and that the Y5 receptor contributes to adaptive changes that occur after stress exposure.

A role for endogenous NPY in the control of anxiety and stress is suggested by the fact that acute restraint stress suppresses NPY mRNA and peptide levels, within the cortex and the amygdala (Möller *et al.*, 1997). In the same way, repeated exposure to the same stressor chronically for 10 days leads to behavioural habituation and to an upregulation of amygdala NPY expression. One hypothesis is that endogenous NPY may contribute to the behavioural adaptation of stress. The use of NPY-transgenic (NPYtr) rats in a model of anxiety has provided an important tool in understanding the role of NPY in anxiety. Centrally injected exogenous NPY produced anxiolytic-like effects in the elevated plus-maze; the same effect was found with NPYtr rats only when the elevated plus-maze session was preceded by a stressor (Thorsell *et al.*, 2000). This presumes that neuronal activation may be needed for endogenous release of NPY.

Human Studies

Neuropeptide Y attenuates the action of CRF and other stress-released peptides. Preliminary trials with military personnel undergoing arduous basic training have shown that those individuals with the highest NPY levels tolerated excessive stress better than those with low levels (Morgan *et al.*, 2000). These findings suggest that it could be possible to treat post-traumatic stress disorder (PTSD) with a pharmacological agent promoting higher NPY levels (Friedman, 2000).

SUBSTANCE P AND NEUROKININS

The mammalian Tachykinin TKs are a group of neuropeptides comprised of substance P (SP), Neurokinin A (NK-A), and Neurokinin B (NK-B). These three endogenous peptides bind to G-protein coupled receptors, designated NK1, NK2 and NK3 respectively, to mediate their effects (Regoli, Boudon and Fauchere, 1994). The NK receptors are located in the central nervous system (CNS). NK1 and NK3 are widely distributed in the CNS (septum, striatum, amygdala, periaqueductal grey matter) whereas NK2 is distributed with lower levels in the CNS (Otsuka and Yoshioka, 1993; Maggi, 1995).

Animals Studies

Data suggests that SP may play a physiological role in the modulation of anxiety as this peptide is released after aversive environmental stimuli. The behavioural effects of SP have been investigated in animal models of anxiety (e.g. elevated plus-maze, social interaction). The effect of SP in anxiety models may be dependent on dose and specific brain regions (Griebel, 1999). Indeed, the administration of SP into various brain areas (the lateral ventricles, the region of the nucleus basalis magnocellularis, the bed nucleus of stria terminalis or the basolateral nucleus of the amygdala), produces anxiogenic-like effects in the elevated plus-maze in rodents (rats or mice) (De Lima and Ribeiro, 1996; Jentiens *et al.*, 1996; Teixeira *et al.*, 1996) whereas 1 mg of SP administered in the nucleus basalis magnocellularis showed anxiolytic-like properties in the rat social interaction test (Hasenöhrl *et al.*, 1996; Jentiens *et al.*, 1996).

The effect of NK2 agonists in animal models of anxiety show an anxiogenic-like profile. The endogenous ligand for NK2 receptors (NK-A) or the selective NK2 receptor agonist [β-Ala^8]NK-A-(4-10), a fragment of NK-A have been reported to produce anxiogenic-like effects in the mouse elevated plus-maze (De Lima *et al.*, 1995; Teixeira *et al.*, 1996). Studies are still lacking concerning the effects of NK-B on NK3 receptors, but De Lima *et al.* (1995) showed that senktide (a NK-B analogue) produces an anxiolytic-like effect in the elevated plus-maze, suggesting that NK3 receptors could play a modulatory role in anxiety (Griebel, 1999).

The potential of NK receptor antagonists as a novel treatment for anxiety and depression has been investigated (Kramer *et al.*, 1998). During 6 weeks, in a double-blind placebo-controlled study in patients with major depression, the NK1 antagonist MK 869 (300 mg per day), produced a positive outcome as measured by both the Hamilton depression (HAM-D21) and anxiety (HAM-A) scales.

There is clinical evidence that TK NK1 receptor antagonists may represent an important advance in the search for novel treatments of anxiety (Nutt, 1998). A wide range of chemically diverse SP receptor antagonists have now been synthesized, but vary markedly in their ability to cross the blood–brain barrier. Poor brain penetrant compounds include SR 14033, LY 303870, RPR 100893 and CGP 49823, while good brain penetrant compounds include the piperidines CP 99,994 and GR 203040, the piperidine ether L-733,060, and morpholines such as L-742,694 (Rupniak *et al.*, 1996, 1997; Kramer *et al.*, 1998). L-733,060 also exhibits a long duration of central NK1 receptor binding ($t_{1/2} > 6$ h), compared with the piperidine CP 99,994, making it particularly suitable for chronic administration studies in appropriate species (Rupniak *et al.*, 1996).

In preclinical studies evidence has been limited by the prevalence of tests utilizing rats and mice, whose NK1 receptor pharmacology differs to that of the human receptor (Beresford *et al.*, 1991; Gitter *et al.*, 1991). The majority of NK1 antagonist compounds display only low affinity for the rat receptor whereas they have high affinity for the human NK1 receptor. For example, L-733,060 in species that possess the human-like NK1 receptor (notably gerbils and guinea pigs), displays sub-nanomolar affinity while it does not possess affinity for the rat NK1 receptor (IC50nM) (Rupniak *et al.*, 1993). A small number of compounds have been described that possess nanomolar affinity for the rat receptor (RP 67580 and SR 140333), but their utility for *in vivo* studies is severely limited by their short half-life and poor brain penetration (Rupniak *et al.*, 1997). Nonetheless, data show an anxiolytic-like profile of the TK receptor antagonist in rodents. MK-869 reduces vocalization in guinea pig pups after maternal separation, suggesting an involvement in the integration of emotional responses to stress by amygdala or related brain areas (Saria, 1999). (±)-CP-96,345 has been reported to increase the time spent in the more aversive light compartment in the mouse light/dark test, albeit at high doses which caused sedation and motor impairment (Zernig, Troger and Saria, 1993). File (1997) also reported the anxiolytic activity of the TK NK1 antagonist, CGP 49823, in the rat social interaction test.

However, the picture is less clear with other selective NK1 receptor antagonists such as FK 888. Although the drug produced anxiolytic-like activity in the mouse elevated plus-maze (De Lima *et al.*, 1995; Teixeira *et al.*, 1996), these effects were not confirmed in a subsequent experiment in rats (De Lima and Ribeiro, 1996). Only at non-consecutive doses and only on one index of anxiety (time on open arms) was there an anxiolytic-like activity found after FK 888 administration in studies. Chronic treatment of NK1 antagonists were also studied in animal models of anxiety (File, 2000). After NKP608 administration, rats were tested in various conditions of the social interaction test. NKP608 administration had significant anxiolytic effects at 0.01, 0.03 and 0.01 mg kg^{-1} (p.o.) in an unfamiliar arena lit by both high and low light test conditions,

but was without effect in the familiar arena under low light illumination. The anxiolytic effect of 0.03 mg kg^{-1} remained after 3 weeks of chronic treatment and there was no anxiogenic effect after 24 h of drug withdrawal. At 6 weeks tolerance had developed, but no anxiogenic withdrawal effect was seen 24 h after the last dose.

Foot taping may be elicited in gerbils (a species whose NK1 receptor resembles that of the human (Beresford *et al.*, 1991; Gitter *et al.*, 1991)), by an aversive stimulus, i.e. electroshock and notably by cues paired with unconditioned stimulus. Recently, data supported the view that shock-induced foot taping in the gerbil is robustly inhibited by two NK1 antagonists. MK-869 (0.3–3 mg kg^{-1}, i.p.) dose-dependently blocked this foot tapping response and CP-99,994 (3 mg kg^{-1}, i.p.) inhibited foot tapping whereas its less active enantiomer (CP-100,263) had no effect (Ballard, Sänger and Higgins, 2001).

In contrast with NK1 studies, which can have contradictory results, NK2 antagonist studies show more reliable results. However, all results were obtained in exploration and social investigation procedures, but not conflict tests. Indeed, NK2 antagonists could possess antianxiety activity. The selective NK2 antagonist, SR 48968, increased the time spent in open arms and also increased the frequency of entries into the open arms in the mouse elevated plus-maze (Teixeira *et al.*, 1996). GR 159897, another non-peptide NK2 receptor antagonist was evaluated in two models of anxiety (the mouse light–dark box and the marmoset human intruder response test) and demonstrated in both rodent and primate species the ability to restore behaviour which had been suppressed by novel aversive environments (Walsh *et al.*, 1995). The effects of GR 159897 (0.0005–50 μg kg^{-1}, s.c.) are similar to SR 48968 (0.0005–50 μg kg^{-1}, s.c.) and diazepam (1–1.75 mg kg^{-1}, s.c.), as the three molecules increased time spent in the light compartment, without affecting locomotor activity. In the marmoset human intruder response, GR 159897 (0.2–50 μg kg^{-1}, s.c.), SR 48968 (10–50 μg kg^{-1}, s.c.) and chlordiazepoxide (0.3–3.0 mg kg^{-1}, s.c.) significantly increased the amount of time marmosets spent at the front of the cage during confrontation with humans. In fact, GR 159897 and SR 48968 produced similar effects to the classical antianxiety agents diazepam or chlordiazepoxide, but these two molecules produced positive effects over a wide dose range, with minimum dose levels in the microgram range. Another compound tested in the light–dark paradigm, GR 100679 (0.02–200 μg kg^{-1}, s.c.) which dose-dependently increased the time mice spent in the light side of the light–dark paradigm, had a similar effect to diazepam (Stratton *et al.*, 1993).

The present review suggests that centrally acting NK antagonists may have clinical utility in a number of psychiatric disorders that are currently treated with benzodiazepines and 5-HT1A agonists. The variable effects produced by NK1 receptor ligands in various anxiety models requires further investigation. The use of NK1 receptor knockout mice could be an alternative for this investigation.

Humans Studies

Investigators in a study undertaken by Merck Research Laboratories, studying 213 patients over 6 weeks, reported antidepressant effects for MK-869, a selective NK-1 antagonist, in the first week. By contrast, the study by California Clinical Trials, which took place also over a similar period, reported that statistically significant antidepressant activity of MK-869 was initially observed at week 4 and was maintained at week 6. The Californian trial formed part of a multicentre study comparing MK-869 to placebo in patients with Major Depressive Disorder (Hung, 2000).

NATRIURETIC PEPTIDES AND ANXIETY

There are at least three peptides constituting the natriuretic peptides (NP) system, namely the atrial natriuretic peptide (ANP), the brain natriuretic peptide (BNP) and the C-type natriuretic peptide (CNP). These peptides are natriuretic factors in the periphery and have a role in regulation of homeostasis of body fluid electrolytic balance and blood pressure in the CNS (Nicholls, 1994). The corresponding natriuretic peptide receptors NPR-A, NPR-B and NPR-C have been identified in the brain (Imura, Nakao and Itoh, 1992). In the CNS of rodents NPR-A is found mainly in the cortex and hippocampus, whereas NPR-B is present in the amygdala and several brainstem regulatory sites. NPR-C is found widely within the CNS, i.e. in the neocortex, limbic cortex, the hippocampal area and the amygdala. These peptides and their receptors represent an important neuromodulatory system within the CNS which is involved not only in the regulation of fluid homeostasis but also directly influences emotional behaviour such as anxiety and arousal.

Animal Models

Strohle *et al.* (1997) studied the effects of the central and peripheral administration of atriopeptin II, an aminoacid residue peptide of atrial natriuretic peptide (Ser103-Arg125), on anxiety-related behaviour and locomotor activity. Its behaviour on the elevated plus-maze after social deficit stress indicated that i.c.v. (2.5 and 5 μg) and i.p. (50 μg) administration of atriopeptin II produced anxiolysis. A low dose of 0.25 μg atriopeptin II administered bilaterally into the central nucleus of amygdala was also found to be anxiolytic.

The anxiolytic effects observed after central and peripheral administration support the hypothesis that atrial natriuretic peptide, which is increased in panic anxiety, may be involved in the tapering of anxiety-related behaviour. In contrast, ANP failed to diminish punished responses in the Geller–Sefter conflict test in rats (Heilig *et al.*, 1992). It was previously found that i.c.v. administration of ANP, PNP and CNP increased the exploratory activity of rats in the elevated plus-maze (Biro, Toth and Telegdy, 1995, 1996; Bhattacharya *et al.*, 1996). ANP-induced anxiolysis was also observed in the open field, social interaction and novelty-induced feeding suppression tests in rats (Bhattacharya *et al.*, 1996).

Isatin is an endogenous indole which has been shown to counteract some of the effects of ANP both *in vitro* and *in vivo*. Given intraperitoneally isatin reduced the effects of both BNP and CNP in a model of passive-avoidance learning in rats (Telegdy, Adamik and Glover, 2000) and the anxiolytic effect of ANP in the elevated plus-maze (Bhattacharya *et al.*, 1996). On the other hand small amounts of isatin are anxiogenic in rodent models. Furthermore, isatin has a wide spectrum of biological properties: a marker of stress and anxiety, an antiseizure agent, an inhibitor of benzodiazepine receptors and ANP binding to its receptors (Hamaue, 2000).

Human Studies

It has been suggested that ANP receptor modulation may have antipanic activity in patients with panic disorder. A double-blind placebo-controlled study was conducted in nine patients presenting with panic disorder and nine healthy control subjects. After pretreatment with an infusion of 150 μg ANP or placebo in random order, each subject received 50 μg CCK-4 (Wiedemann *et al.*, 2001). After pretreatment with ANP, the number of CCK-4-induced panic attacks decreased from eight to six in patients and from five to two in controls. Infusion of ANP significantly curtailed

the CCK-4-induced release of corticotrophin in patients. ANP exerts anxiolytic-like effects on patients with panic disorder. These results are supported by another study (Strohle *et al.*, 2001) using 25 μg CCK-4 in 10 panic disorder patients. These two studies support the antipanic activity of ANP, however it is possible that non-peptidergic ANP receptor ligands may be ultimately used to treat anxiety disorders.

CONCLUSION

The pharmacological, behavioural and molecular investigations of neuropeptides has advanced remarkably the knowledge of the peptidergic transmitter system in recent years. This knowledge has led to the development of new compounds with potential clinical interest and development has reached clinical studies in some examples of anxiety disorders. Although there is little clinical evidence so far of peptidergic involvement in human anxiety, the results from animal studies are encouraging. However, much research is required to address the hypotheses that neuropeptides may represent a new option for pharmacotherapy of stress-related disorders.

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XIX-5

Neuroendocrinology of Anxiety Disorders: Post-traumatic Stress Disorder

W.S. de Loos

INTRODUCTION

History of Neuroendocrinology

The General Adaptation Syndrome

Endocrinology has strongly been linked to stress since Hans Selye formulated his concept of the 'general adaptation syndrome' (GAS) in his initial description of 'A syndrome produced by diverse nocuous agents' in *Nature* in 1936 (Selye, 1936a). He connected the initial response under the name 'alarm reaction' to the stage of resistance during continued exposure to the stressor and the subsequent stage of exhaustion ultimately followed by death. Shortly thereafter, he put the word 'stress' on stage as a concept of 'the nonspecific response of the body to any demand made upon it'. He drew the attention to the hypertrophic response of the adrenal glands, especially their cortical layers, and named the hormonal substances derived from them 'corticoids', such as 'cortisone'. Ever since, the glucocorticoids (cortisol/hydrocortisone and, in rodents, corticosterone) have figured as the stress hormones *in optima forma* and taken a role as the biological parameters or 'proof' of the stress response. Selye did not deny the role of the adrenal medulla and its main product adrenaline/epinephrine (EP) but the scientific community became very much focused on cortisol as the hallmark of the stress response.

Interestingly, Selye's attention was primarily drawn by physical causes of stress such as injuries, cold, restraint, hunger and numerous chemical substances (atropine, morphine, formaldehyde, epinephrine) (Selye, 1936b). Already in this and a further publication, he outlined the role of the pituitary gland in exerting the effect on the adrenal (cortical) hypertrophy marking the road to our present-day interest in the hypothalamo-pituitary-adrenocortical (HPA) axis in psychoneuroendocrinology (Selye and Collip, 1936). He also made the link to the immune system in his observations on thymicolymphatic atrophy laying the base for modern psychoneuroimmunology.

The Defence Reaction

Selye's original focus at physical causes of the GAS is of great value as it draws our attention to the unconditional stimuli that provoke it. Twenty-five years earlier, Walter B. Cannon had already described the physical (= unconditional) and psychological (= conditional) stimuli for adrenomedullary activation (Cannon and de la Paz, 1911; Cannon and Hoskins, 1911). He extended his observations to a concept of the emergency response of the individual by changes that are 'directly serviceable in making the organism more efficient in the struggle which fear or rage or pain may involve' (Cannon, 1914). His description of the 'Bodily Changes in Pain, Hunger, Fear and Rage' (Cannon, 1915) has become classical as the immediate response to the perception of threat mediated by epinephrine which is *sensori strictu* as much a hormone as cortisol. He described these changes here as 'The organic preparation for action ... in fight or conflict — either one requiring perhaps the utmost struggle'. This emergency response has later been named the 'defence reaction' (Hess and Brügger, 1943) and has its organizational centre in the amygdala (LeDoux, 1998).

The defence reaction consists of both behavioural and physiological components. The behavioural components are species specific; the physiological ones are more general, such as: pupillodilation; pilo-erection; increase of muscle tone, ventilation, cardiac output, oxygen consumption and muscle blood flow; decrease of blood flow in the skin, intestine and kidneys; bladder and bowel emptying; and many others like neurohumoral, hormonal, immunological and haemostatic changes. Understanding of the ethology and comparative physiology of the defence reaction permits excellent understanding of the psychophysiology of alarm and anxiety in humans, both under normal and pathological conditions.

The other important survival response is the above-described GAS which is characterized by behavioural inhibition when active ways of surviving a challenge have been blocked off. It can be compared to the psychodynamic concept of unsolvable conflict or to Seligman's behavioural model of learned helplessness (Abramson, Seligman and Teasdale, 1978), while Henry described it from a comparative physiological and ethological point of view as conservation-withdrawal expressing subordinate behaviour (Henry, 1992). The corresponding neuroanatomy has important organizing centres in the septum and hippocampus and has every right to be acronymized as the LHPA axis, for limbic-hypothalamo-pituitary-adrenocortical (Sapolsky, Zola-Morgan and Squire, 1991; López, Akil and Watson, 1999), including important physiological outflow through the vagal motor system. The effect of chronic mild stress on the HPA axis is organized in the hippocampus by modulation of hippocampal inhibition on the paraventricular nucleus (PVN) of the hypothalamus (Bratt *et al.*, 2001).

The emergency, or alarm, or defence reaction and the general adaptation, or conservation-withdrawal, or inhibition syndrome have been renamed 'allostasis' (Charles Kahn, see Sterling and Eyer (1988). Allostatic responses are temporary encroachments on homeostasis, a term we owe to Walter B. Cannon.

Looking back on almost a century of stress hormone research (EP and cortisol), we conclude that EP is a hormone that increases energy expenditure acutely and mobilizes quickly available resources (glucose, free fatty acids). It generates a metabolic response that can only last for a rather short time and should be followed by recuperation under better circumstances. Cortisol,

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however, is a hormone that becomes useful under prolonged circumstances of adversity and generates a slowly catabolic response of long duration which can be used by the organism to consume its own structure as a resource for energy. It causes slow but general breakdown of the entire organism ultimately leading to death.

Syndromal Versus Matrix Diagnosis

Biological Parameters

Although challenging and even inspiring, the parallels between animal models and human disorders are defective. It remains to be seen, however, whether the clinical diagnosis of human psychiatric disorder is so much superior to ethological observation of animal behaviour or the biological responses referred to in the previous paragraph. Certainly, at group level interesting biological properties of psychiatric syndromes have been discovered, one of them the escape from dexamethasone feedback suppression of the HPA axis in major depressive disorder (MDD), a well-known phenomenon nowadays (see Gutman and Nemeroff in this book; Mitchell, 1998). Still, at the individual level this has not given us a tool to 'prove' whether a patient is suffering from MDD or not. We are far from using biological parameters to support psychiatric clinical diagnosis. Often, confusing findings have been reported, even at a group level (*vide infra*), which makes us doubt, at times, the validity of syndromal diagnosis. Biological parameters seem not to parallel the syndromal level and syndromal diagnoses inadequately predict the success of therapeutic strategies, as one of the founders of biological psychiatry, Herman M. van Praag, has pointed out (van Praag *et al.*, 1987). It is even highly questionable what the relevance is of the syndromal diagnostic constructs we use to work with. They seem to be based on a nineteenth century disease model that has been derived mainly from the paradigm of infectious diseases, a one pathogen — one disease model that is not valid any more in a world where this model of syndromal diagnosis is becoming less effective with the growth of our physiological, biochemical and genetic insights. In many cases we should speak, in fact, of common final pathways or end points with heterogeneous aetiologies or initiators. Concerning common final pathways, we know that different mutations within a single gene will often cause the same genetic defect. Different genetic defects may still lead to similar phenotypical disease expressions. Diseases that have been known for ages under a single name, had to be split into sub-groups with significant differences.

Co-morbidity

Another artifact of modern diagnostic classification is that of co-morbidity suggesting that psychiatric diagnoses can simply be added up like we do with somatic pathology, e.g. juvenile diabetes plus hypothyroidism plus Addison's disease, or hypertension plus osteoarthritis plus colorectal cancer. Not without reason van Praag again — 'rebel with a cause' — writes about 'classificatory inflation', a nosological doctrine hanging around psychiatry's neck as an albatross (van Praag, 2000). It seems more fruitful to follow a system of developmental elements as they have emerged during phylogeny and ontogeny, the natural history of biology, to form the basis of nosological diagnosis (de Loos, 2001). A matrix of relevant elements would form a diagnostic matrix that is less sensitive to unvalidated constructs and taxonomic changes.

A Diagnostic Matrix

- Dimension I: the genetic code, the set of unchangeable biological properties is at the basis of this system. It includes not only the structural floorplan and homeostatic regulatory mechanisms but also the inborn psychophysiological responses initially described by Cannon and Selye (*vide supra*). Such responses cannot be learnt or suppressed voluntarily; they can be enhanced by conditioning.
- Dimension II: acquired automated mechanisms (organizing effects, imprinting, long-term potentiation/depression, pruning and dendrite plasticity, association). Imprinting and learning are processes directly based on the genetic make-up of an individual. Imprinting is the creation of read-only-memories (ROMs), a biological parallel of computer 'firmware', and happens at a developmental stage when the nervous system is receptive for information that fulfils certain specifications. It is a process that occurs under circumstances created by the first dimension.
- Dimension III: the declarative faculty (reflexive consciousness, emotional declaration). From a heuristic point of view it seems logical to separate linguistic development from the second dimension considering the phylogeny of homo sapiens. Reflexive consciousness and the development of emotional expression are representations of the declarative faculty including aspects of relational behaviour, literature, etc.
- Dimension IV: complex language-dependent systems (science, logic, mathematics, philosophy and political systems). They can be seen as the present ultimate development of phylogeny and ontogeny. They constitute what we use to call human 'civilization' including structures based on semantically explicit cognition. Although scientific theory development requires economy in calling in assumptions or abstractions, it seems appropriate and efficacious to categorize this level of development in a separate dimension.

Construction of an individual matrix for every patient may seem tedious at first glance but respects individual variability more than our present-day system of syndromes and co-morbidity. Modern DNA technology is unravelling genetic codes of adaptive (healthy) polymorphisms and maladaptive mutations. Although still costly at present, DNA diagnostics are becoming available at the individual patient's level and may fill in Dimension I at a certain time. Dimension II can already be filled in with traits like hypervigilance, increased startle, increased impulsiveness, dissociation, sympathoadrenomedullary activation, (L)HPA activation, intrusive memory, etc. For many of these we have knowledge about neural circuits, transmitters and neuromodulators that are involved and drugs that can or cannot interfere.

Changes or disturbances in the regulation of the HPA axis (Dimension II) do not parallel the classificatory division between mood and anxiety disorders (for an overview see Heim and Nemeroff (1999) Table 1). Within the group of anxiety disorders differences are found between panic disorder (PD) and post-traumatic stress disorder (PTSD) (*vide infra*) while somatoform disorders like chronic fatigue syndrome (Demitrack *et al.*, 1991) and fibromyalgia (Griep, Boersma and De Kloet, 1993) resemble PTSD in the down-regulation of HPA axis activity (Heim, Ehlert and Hellhammer, 2000; Altemus *et al.*, 2001). Chronic fatigue syndrome overlaps with major depressive disorder (MDD) but shows opposite HPA axis regulation (Demitrack, 1997). It would thus be more helpful to assess and value HPA axis regulation as a dimensional feature as it seriously confounds syndromal diagnosis.

Dimension II of the matrix is also the domain of interaction between developmental conditions and the influence of environmental factors on adult outcome. What this implies for rodents, especially the responsiveness of the HPA axis, has been reviewed by numerous authors. The HPA axis of developing rats is, in part, regulated by maternal factors like feeding, licking and grooming the pup after a separation, which all seem to act in concert to limit and prevent the so-called stress hormones and more specifically corticosterone from exceeding some optimal level. The pattern of ACTH releasing factors is affected, not only corticotrophin releasing hormone (CRH) gene (hnRNA and mRNA) and AVP gene

activation, but also immediate early genes (the transcription factor c-*fos* and the growth factor NGF-1a) in a differentiated way demonstrating a significant plasticity of the system (Levine, 2001). Differences between mothers are fairly stable over subsequent litters and are transmitted to the next generation by environmental as well as genetic factors. Environment can thus alter the genetic trajectory demonstrating the adaptive value of plasticity (Francis *et al.*, 1999). Numerous retrospective studies in humans support these animal studies with consistent results suggesting sensitization of the neuroendocrine stress responses after early life stress in humans. Differences found between childhood versus adulthood stress on the results in adulthood underscore the interaction with the developmental stage (Heim and Nemeroff, 2001).

General Principles of Endocrine Regulatory Systems

Classical and Clinical Endocrinology

Base line values of hormonal secretions have limited value in endocrinology as they may be confounded by many factors such as inter-individual variability of normal values while intra-individual variability is much smaller in general. Base line values can be more dependent on unrecognized environmental or intra-individual circumstances than is generally acknowledged. This is known, for instance, for 'normal' morning cortisol values taken from outpatients in hospital surroundings and considerable variation has been recorded in studies with 'base line' 24-hour urine cortisol in patients with PTSD (*vide infra*).

Other problems are met with the difference in analogous (continuous) versus digital (pulse frequency) regulation of end-organ function. Releasing hormones from the hypothalamus and pituitary hormones generally are of the latter type, steroid hormones have properties of both while thyroid hormone may be the most continuous in level. An important role is being played by hormone binding proteins. They strongly dampen the oscillations in total hormone concentrations found but this can be unmasked by measuring free hormone levels in plasma or saliva. Only the free hormone can act biologically.

Understanding of hormone function and assessment of normality of function is often better achieved by stimulation or blocking (feedback sensitivity) tests. The difficulty with the tests generally applied is, however, that at least in clinical endocrinology they are of a pharmacological kind using doses far exceeding physiological levels. This may have value in detecting organ failure in autoimmune or post-operative endocrine disease, or of endocrine tumors but is of very limited value in detecting changes in set point of a regulatory system, or even in so-called subclinical thyroid disease. Modifications have been found by applying 1 in stead of 250 μg of i.v. $ACTH_{1-24}$ for adrenal stimulation and 0.5 or 0.25 instead of 1.0 mg of dexamethasone for the 'overnight' suppression test.

Many problems arise from the existence of pre- and pre-pro-hormones and from split products that may be biologically active or inactive. Examples are pro-opio-melano-corticotropin (POMC), the endorphins and enkephalins and, the endocrinologist's nightmare, parathyroid hormone. Laboratory assays may not find the difference and local transformation may mask the 'visibility' of the substance, e.g. of tri-iodothyronine (T_3) in brain tissue where it is being transformed from its biologically almost inactive precursor L-thyroxine (T_4). Also, the sensitivity of assays may be a serious problem in the range of normal function as it has been with thyroid stimulation hormone or thyrotropin (TSH) until the third generation assay became available. The transformation of pro-hormones may be regulated at multiple levels and differently in different tissues as is the case with thyroid hormone of which only 20% is readily produced in its active form T_3 in the thyroid gland itself. Deiodination from T_4 to T_3 occurs intracellularly in many tissues while especially the liver exports T_3 to the general circulation. Half-life of these substances varies considerably, in the case of T_4 and T_3 from 7 days to a few hours only.

The classical hormones are defined by their signal function transported through the general circulation. The releasing hormones and the intra-cerebral and intra-medullary peptides can hardly be found in the peripheral circulation and even in the cerebrospinal fluid the levels found must be considered as spillover from tissue to the gutter of the brain which helps to clear this organ from products that have not been degraded locally or removed by presynaptic reuptake. Lastly, psychoneuroimmunological research has shown that peptide hormones like CRH and the cytokines are being secreted by lymphocytes at the femtomolar level (10^{-15} mol l^{-1}) where they have a paracrine function and still can influence the brain that way by uptake through the blood–brain barrier at specific sites and connection to specific neuronal circuits (Breder, Dinarello and Saper, 1988).

Neuroendocrinology

Still, when we have taken into account all these technical points, we must realize that by studying a few substances we are only looking to a few links in a long chain of events. In classical ('clinical') endocrinology, hormone systems are described as separate domains. In neuroendocrinology the limitations of this way of thinking become obvious: there are important connections with other neurochemical and neurophysiological systems. The classical hormone concept is also too limited. CRH, for instance, has endocrine, autonomic, behavioural and immune effects. It acts as a classical hormone in the hypothalamo-pituitary portal system, as a neuromodulator in widespread areas of the central nervous system (CNS) confirming David de Wied's neuropeptide concept (de Wied and Jolles, 1982) and paracrine in the immune system.

POST-TRAUMATIC STRESS DISORDER

Neuroendocrinology, Psychophysiology and Post-Traumatic Symptoms

Early Biological Predictors

Peri-traumatic dissociation has been found to be a good predictor of the development of PTSD, better and stronger than depression, anxiety and intrusive symptoms (Shalev *et al.*, 1996). Heart rate in the peri-traumatic phase, i.e. in the emergency room after traffic accidents etc., was also positively correlated to the development of PTSD (Shalev *et al.*, 1998a). In an extension of the study, again peri-traumatic dissociation and heart rate predicted the development of PTSD and were associated with more intrusive symptoms and exaggerated startle (Shalev *et al.*, 1998b). Heart rate is controlled by two extracardial mechanisms. EP increases it and the vagal nerve lowers it. The sympathetic innervation only influences electrical conductance (induction of arrhythmia's), stroke volume and coronary artery blood flow. No acute studies, in the emergency room, have been done on EP production, but assessment after one and six months after an accident has shown elevated EP production rates in men with PTSD symptoms. Catecholamine rates were also associated with intrusive thoughts at one month and avoidance at six months. In this study urinary cortisol production rates were elevated in men at one month and normal at six months. Both in men and women, greater emotional numbing predicted a lower cortisol production rate at six months (Hawk *et al.*, 2000). This is a finding that seems to be confirmed by Mason *et al.* (2001) who linked lower cortisol levels with disengagement defence mechanisms (numbing) in combat veterans with PTSD.

A negative correlation between serum cortisol immediately after traumatization and the development of PTSD was found in two different settings, namely sexual assault and motor vehicle accidents (Resnick *et al.*, 1995; McFarlane, Atchison and Yehuda, 1997). Significantly lowered urinary cortisol over 15 hours at acute assessment in the emergency room predicted PTSD at one month and especially the occurrence of intrusive and avoidant thoughts (Delahanty, Raimonde and Spoonster, 2000). These psychophysiological factors raise the issue of pre-traumatic vulnerability in the development of PTSD (Yehuda *et al.*, 1998).

Acute stress disorder (ASD) is characterized by dissociation and strongly predicts the development of PTSD (Brewin *et al.*, 1999; Koren, Arnon and Klein, 1999). No specific neuroendocrine research focused on ASD *per se* has been done. Studies on initial cortisol responses predicting development of PTSD may be seen as relevant to ASD as such. When combining the elements of dissociation and stress-induced analgesia with the known effects of opioids, it is suggestive to infer a central opioid effect as a core mechanism in the initiation of this disorder (*vide infra*).

Psychophysiological Testing: Heart Rate and Epinephrine

Reference has been made to increased EP production at early stages after traumatic accidents. Analysis of heart rate variability by means of power spectrum analysis in PTSD patients many years after traumatization again showed higher heart rates and lower heart rate variability at rest. This was interpreted as an indication for lower cardiac parasympathetic (vagal) tone and elevated sympathetic activity (Cohen *et al.*, 1997).

In laboratory settings cardiovascular and other psychophysiological responses, mainly galvanic skin response (GSR) and electromyography (EMG), to stimuli of various kind have been studied extensively. Many studies have demonstrated strong specific responses of blood pressure and especially heart rate to startling unspecific noises and to individually significant sensory input in subjects with PTSD, combat veterans from various war theatres (Pallmeyer, Blanchard and Kolb, 1986; Pitman, Orr and Steketee, 1989; Blanchard *et al.*, 1990, 1991a), and in other populations of trauma survivors (Shalev *et al.*, 1993, 1997). Also in Rorschach testing the projection of traumatic content elicited significant increases in skin conductance (sympathetic activation) and heart rate (Goldfinger, Amdur and Liberzon, 1998).

Yohimbine, an α_2-adrenergic receptor antagonist that activates noradrenergic neurons, e.g. in the locus coeruleus, hippocampus and amygdala, increased systolic blood pressure significantly more in PTSD subjects than in healthy controls, especially when they had a flashback and/or a panic reaction after administration of this drug. The same occurred with heart rate which showed no significant response in the controls (Southwick *et al.*, 1993). None of these studies discriminates between sympathetic mediated decrease of vagal tone versus EP mediated cardioacceleration. A role of the latter can only be inferred by the above-mentioned study by Hawk *et al.* (2000). But all these point to a strongly increased sympatho-adrenomedullary activation in PTSD.

Vasopressin and Oxytocin

Vasopressin and oxytocin are two hormones of the central nervous system (neuropeptides) that are of special importance in memory processing. Behavioural and cardiovascular conditioning in animals has shown that vasopressin increases the retention of both appetitive and aversive memory while oxytocin in low doses has the opposite effect (Bohus, Kovacs and De Wied, 1978; Wan *et al.*, 1992). Similar results have been demonstrated in humans with PTSD with respect to psychophysiological parameters in relation to personal traumatic imagery, most specifically exerted by vasopressin on EMG (Pitman, Orr and Lasko, 1993).

Opioids

'Addiction' to the trauma, a strong need to return to memories, memorials and other symbols of the trauma, is a clinical phenomenon in many PTSD patients that was poorly understood until the role of the opioid peptides was discovered. It may also be related to novelty/sensation seeking or risk taking behaviour (Van der Kolk *et al.*, 1985). Pain-induced analgesia was known as an experimental model in pharmacology for a considerable time and has been extended, later, to stress-induced analgesia. In animal experiments it has been connected to learned helplessness (*vide supra*) and shown to be dependent on μ-opioid mechanisms at the time of initial uncontrollable footshock (Hemingway and Reigle, 1987). It can be blocked with the classical morfine antagonist naloxone. These mechanisms have been associated with post-traumatic symptoms, such as numbing (Van der Kolk and Saporta, 1991; Glover, 1992). There are indications that flashbacks and other dissociative phenomena in PTSD patients and also emotional numbing are opioid mediated phenomena that can be blocked by naloxone (Van der Kolk *et al.*, 1989; Pitman *et al.*, 1990). Improvement of many PTSD symptoms has been reported after the administration of nalmefene (Glover, 1993), a relative pure opioid μ-receptor antagonist more potent than naloxone (Reisine and Pasternak, 1996). It is possible although speculative at this moment that clinical phenomena like dissociation, auto-mutilation and conditioned or self-induced analgesia like the fakir syndrome are mental states in which the opioids play an important role. A puzzling finding is that plasma levels of β-endorphin, both in the morning and the evening, were found in one study to be lower than in controls (Hoffman *et al.*, 1989). In this same study morning cortisol levels in PTSD subjects were higher than in controls which is at variance with most later findings (see below). The above reported opioid responses to traumatic flashbacks were not accompanied in that study by detectable changes of opioids in the general circulation (B.A. van der Kolk, personal communication). The effects are confined to the CNS compartment exclusively as was shown in a study in which subarachnoid cerebrospinal fluid (CSF) sampling was compared to peripheral plasma. It was shown that PTSD patients have significantly increased CSF levels of β-endorphin and that higher levels corresponded with less intrusive and avoidance symptoms (Baker *et al.*, 1999). Also, the connection to the hypothalamic-pituitary-adrenal axis is to be considered in the light of its inhibition by opioids at the hypothalamic level (Hockings *et al.*, 1994).

The HPA Axis

Interaction with the Sympatho-Adrenal System

Many studies have addressed the complex interplay between the sympatho-adrenomedullary system and the hypothalamic-pituitary-adrenocortical (HPA) axis. In most studies PTSD is characterized by increased norepinephrine (NE) spillover (Kosten *et al.*, 1987; Blanchard *et al.*, 1991b) on one hand but decreased total daily cortisol production (Mason *et al.*, 1986; Yehuda *et al.*, 1990a, 1995b) and decreased circulating cortisol levels on the other (Yehuda *et al.*, 1996a; Boscarino, 1996; Kanter *et al.*, 2001). Urinary cortisol was also found to be lower in adult offspring from Holocaust survivors with PTSD while offspring with parental PTSD and own lifetime PTSD had the lowest levels (Yehuda *et al.*, 2000).

Daily free cortisol excretion was found to be normal at group level but to correlate inversely with intrusive PTSD symptoms in one study (Baker *et al.*, 1999) while it was increased similarly to patients with major depression and without any correlation to

symptoms in another (Maes *et al.*, 1998). One difference between the last study and the previous ones is that it was done on civilians with a majority of females without control for menstrual cycle phase while most of the previous studies were done on male combat veterans. The study by Hawk *et al.* (2000) has the same limitation mainly due to the set-up, namely emergency room assessment of accident victims. A study that did control for menstrual cycle phase showed no effect of phase on total urinary-free cortisol output, and no effect of PTSD although there was a trend towards an increased cortisol output in PTSD (Rasmusson *et al.*, 2001). It was also argued that single traumatic events might cause an increased HPA axis response while repetitive and prolonged trauma might do the opposite. Some support for this view can be found in other research but this problem has not been solved satisfactorily.

In concordance to the release rates reported above, α_2-adrenergic receptors are down-regulated (Perry, Giller and Southwick, 1987; Yehuda *et al.*, 1990b) and glucocorticoid receptors (GRs) are up-regulated (Yehuda *et al.*, 1991, 1995c). Norepinephrine release and the up-regulation of GRs correlate with the severity of PTSD symptomatology (Kellner, Baker and Yehuda, 1997). A significant negative correlation between 24-hour urinary-free cortisol and PTSD symptoms reported in another study confirmed this (Baker *et al.*, 1999).

Feedback Systems

In PTSD the efficacy of glucocorticoid feedback is increased as demonstrated by a significantly enhanced dexamethasone suppression in comparison to normals (Kudler *et al.*, 1987; Yehuda *et al.*, 1993; Stein *et al.*, 1997b; Heim *et al.*, 1998) and it is opposite to depression which is known for its dexamethasone non-suppression. One would even conclude that PTSD and biological depression as defined in this neuroendocrine way exclude one another. Yet, many studies describe co-morbidity of PTSD and major depressive disorder (MDD) (Shalev *et al.*, 1998b), not merely dysthymia. It should be kept in mind that a biological definition of depression is not by itself concordant with a psychological one. Individuals with PTSD and co-morbid depression are still better than normal suppressors, but less than having PTSD alone (Yehuda *et al.*, 1993). The enhanced negative feedback of dexamethasone is not reflected by lower levels of circulating adrenocorticotropic hormone (ACTH) but the pituitary capacity to release ACTH is markedly enhanced which excludes pituitary insufficiency and confirms the increased feedback sensitivity (Yehuda *et al.*, 1996b).

The high levels of NE in PTSD are interpreted as to reflect high sympathetic activity which corresponds with many findings on cardiovascular stimulation and galvanic skin response (GSR) reactivity. A positive correlation between intrusive PTSD symptoms and urinary excretion of the catecholamines dopamine and EP points in the same direction (Yehuda *et al.*, 1992). Thus, PTSD with or without accompanying symptoms of depression seems to be characterized, on one hand, by sympatho-adrenal arousal which is reflected by increased cardiovascular responsiveness and sweat gland activation as signs of the defence reaction, the paradigm of active survival strategy; on the other hand, it is characterized by a turn-down of the conservation-withdrawal response and its catabolic survival hormone cortisol that induces the organism to consume its intrinsic resources while waiting for a better time.

CRH, the 'Stress Superhormone'

CRH Testing

The response of ACTH to CRH has been found to be blunted in male PTSD patients as in depression and to result in slightly but not significantly lower cortisol responses (Smith *et al.*, 1989) while in panic disorder (PD) enhanced and blunted responses have been reported (Heim and Nemeroff, 1999). In PTSD this cannot be understood as a feedback effect of functional hypercortisolism, as in the case of depression (Gold, Goodwin and Chrousos, 1988). In premenopausal women ACTH response to CRH was increased and slightly prolonged with an increased and prolonged cortisol response. Cortisol response to $ACTH_{1-24}$ was tested in the follicular phase (high progesterone) and also showed an increased response. The cortisol responses correlated to each other and to 24-hour urinary cortisol excretion (Rasmusson *et al.*, 2001). It demonstrates once more the importance of accounting for sex and, as we will see, age.

Children: CRH Testing and Urine Sampling

The neuroendocrine pattern in children has not been investigated as intensively as in adults. In one study CRH testing was performed in children of 7–15 years old who were living in a stable and safe environment but who had been sexually abused 1–12 years earlier. Some of them had concurrent dysthymia and suicidal ideation and had attempted suicide but none of them was reported to have PTSD. They showed smaller than normal ACTH responses but nonetheless normal cortisol responses to this (De Bellis *et al.*, 1994), which resembles the result found in male adults.

A very different finding is the increased ACTH response to CRH in abused children who experienced ongoing chronic adversity and were rated as depressed. They differed from abused depressive children living in a stable environment, depressive non-abused controls and healthy children who all showed the same ACTH response. The increased ACTH response in the first group was not followed by an increased cortisol response which thereby was the same in all four groups (Kaufman *et al.*, 1997).

A group of children of the same age (8–13) with PTSD was compared to normal controls and children with overanxious disorder. Childhood PTSD was associated with greater co-morbid psychopathology including depressive and dissociative symptoms, lower global assessment of functioning and increased suicidal ideation and suicide attempts. The children in this group excreted significantly greater amounts of urinary dopamine and norepinephrine per day than in both comparison groups. Their free cortisol excretion was equal to that of the overanxious group but exceeded the controls. Catecholamine and cortisol excretion was correlated to the duration of traumatization and to PTSD symptoms (De Bellis *et al.*, 1999).

It is unclear what the discrepancies between these studies and the results found in adults imply. One of the possibilities is that the psychobiological development stage is a critical factor. Also in a broader sense, age may be a factor influencing the HPA axis response to challenge (Seeman and Robbins, 1994). Repetition or perseverance of traumatization is likely to influence the neurohumoral response to it as has been observed in rape victims (Resnick *et al.*, 1995). Other possibilities accounting for the discrepancies are that the studies were done on non-patients and patients with different diagnoses (diagnosing PTSD in young children poses its own difficulties), sample sizes, time of the day and baseline values.

Systems Integration

No convincing correlations have been found between HPA axis activity in the morning, when it is as high in PTSD patients as in controls, and circulating catecholamines or psychophysiologic parameters like GSR (which reflects sympathetic activity), heart rate or frontalis EMG (Liberzon *et al.*, 1999). The conclusion was drawn, then, that no integrated, multisystem stress response occurred in PTSD and this conclusion is supported by other

findings when the HPA axis response was studied in connection with CNS noradrenergic activity as represented by 3-methoxy-4-hydroxyphenylglycol (MHPG or MOPEG) spillover (Goenjian *et al.*, 1996; Yehuda *et al.*, 1998) which can be considered a metabolic parameter of central NE turnover reflecting spillover from the CSF compartment into the systemic circulation (Webster, 1989). This may seem, but is not necessarily, at variance with the above-described findings on the HPA axis and catecholamine activity. It means that within an individual these systems are not being coupled per single event. This conclusion is in concordance with the insight that the sympatho-adrenal response system and the HPA axis are not connected to each other through the activation of CRH, as this neurohormone or neuromodulator acts at different locations in the CNS independently, in different circuits and functions (Schulkin, Gold and McEwen, 1998). CRH gene expression in the central nucleus of the amygdala and the bed nucleus of the stria terminalis (BNST) is dissociated from that of the paraventricular nucleus of the hypothalamus which is the classical top of the HPA axis organization. Direct application of CRH by infusion into the third ventricle induces multiple physiological stress responses like increase of plasma epinephrine, norepinephrine, glucose and glucagon, of mean arterial blood pressure and heart rate, and inhibition of gastric acid production, all by autonomic nervous system activation (Lenz *et al.*, 1987). The gastric inhibition could, in part, be inhibited by naloxone or a vasopressin antagonist. This implies involvement of an opioid neuropeptide as a neuromodulator, e.g. a pro-opiomelanocortin (POMC)-derived endorphin (De Wied, 1999). The possibility of a relation with dissociation and flashback-related analgesia is intriguing within this context (Pitman *et al.*, 1990). The role of vasopressin is interesting from the viewpoint of its role in the consolidation of memory (Bohus, Korac and De Wied, 1978; Chepkova *et al.*, 1995), including the psychophysiologic concomitants of emotional memory (Bohus *et al.*, 1983; Pitman, Orr and Lasko, 1993) and its role in the potentiation of CRH-induced ACTH release (Scott, Medbak and Dinan, 1999).

The Role of CRH

The question of the specificity of CRH activity in the CNS is of special importance in the case of PTSD as higher levels of this neurohormone have been found in the cerebrospinal fluid of patients compared to controls, which may seem paradoxical at first sight given the increased feedback sensitivity of the system (Bremner *et al.*, 1997b; Baker *et al.*, 1999). CRH in the CSF is mainly of extrahypothalamic origin, not related to HPA axis activity (Garrick *et al.*, 1987). Interestingly, this was accompanied in patients, but not in controls, by positively correlated CSF levels of somatostatin which often acts as an inhibitory hormone or neuromodulator both in the CNS and peripherally, but its role in these particular circumstances remained unclear. It is also unclear, at this point, what actually causes the increased feedback sensitivity within the HPA axis and whether stimulation of this axis at the level of CRH production by the paraventricular nucleus (PVN) of the hypothalamus is decreased. As mentioned above, the elevated CRH levels in the cerebrospinal fluid are not likely to be generated by the PVN but to be due to spillover from the central amygdala, the bed nucleus of the stria terminalis and the locus coeruleus. The latter three nuclei have important roles in organizing or mediating vigilance, arousal and anxiety reactions and they activate both the central norepinephric system and the sympathetic nervous system (Lenz *et al.*, 1987). Central norepinephric system activation has not systematically been demonstrated (Yehuda *et al.*, 1998). However, frequently repeated activation of the sympathetic nervous system is a general feature of chronic PTSD. Similarly to PTSD symptoms, of panic and flashbacks, yohimbine challenge has indeed produced increases in systolic blood pressure and heart rate, but also MHPG spillover as a putative parameter of central norepinephrine activation (Southwick *et al.*, 1993).

Hippocampal Glucocorticoid Receptors

One of the options for increased HPA feedback sensitivity is increased GR function in the hippocampus which is an important centre for control over the HPA axis function (Meaney *et al.*, 1989). The hippocampus with its dense population of GRs is now broadly recognized as the top of the system by exerting inhibitory control over hypothalamic CRH production (Jacobson and Sapolsky, 1991). GRs may have been up-regulated conforming to a theory derived from the model of neonatal handling in rats in which attenuation of stress responses in adulthood has been observed (Levine, 1957; Denenberg, 1964). This model has been differentiated by more recent studies showing that individual differences in caring behaviour by the mother animal after separation from the litter are responsible for differential effects of such handling. The better the caring attention of the mother after replacement of the pup into the litter, the higher the GR density in the hippocampus and the more efficacious the feedback of circulating glucocorticoid hormone (Liu *et al.*, 1997; Sapolsky, 1997). This process is thought to have a protective effect on the hippocampus against later damage by high glucocorticoid responses under environmental stress or allostasis. The hippocampal atrophy found in PTSD, like in depression and Cushing's disease (Sapolsky, 1996), is not compatible with such protection if, indeed, the damage is due to high glucocorticoid responses under traumatic circumstances.

Hippocampal GRs may respond differently to cortisol than to dexamethasone. This has been shown in an intravenous cortisol inhibition study in PTSD patients in whom adrenal cortisol synthesis was partly blocked with metyrapone. Its precursor, 11-deoxycortisol, can be measured as a parameter of ACTH stimulation of the adrenal cortex. No significant difference in ACTH response to cortisol was found in the PTSD subjects who had lower baseline cortisol and 11-deoxycortisol conforming to the expected HPA axis down-regulation. The PTSD subjects showed no increased feedback sensitivity to cortisol, contrary to expectations from the dexamethasone studies (Kanter *et al.*, 2001). This difference could be based on the lack of effect of dexamethasone on the hippocampus and the PVN as it does not penetrate into the CNS. It implies that the enhanced feedback sensitivity is not present at the level of hippocampal or PVN GR. Other mechanisms must be responsible for the HPA axis down-regulation.

Atrophy of the Hippocampus

A smaller volume of the hippocampus found in several PTSD studies (Bremner *et al.*, 1995, 1997a, 1997b; Stein *et al.*, 1997a), which is enigmatic in the light of the atrophy found in MDD and Cushing's disease with their increased levels of cortisol (Sapolsky, 2000), is the opposite of what is thought to be happening in PTSD. The finding has been confirmed with more sensitive neurochemical methods independent of gross neuroanatomical morphology (Schuff *et al.*, 2001). There is not much doubt about the potential harm of glucocorticoids for the hippocampus, especially the granulosa cells and the dendritic outgrowths and sprouting of the pyramidal cells (McEwen, Gould and Sakai, 1992; Gould and Tanapat, 1999). It has been postulated that the impact of the initial adversive experience may trigger damaging levels of glucocorticoid release thus causing the observed atrophy in PTSD (Bremner, 1999). Other causes for neuronal damage are excitatory amino acid neurotransmitters, especially glutamate, via its N-methyl-D-aspartate (NMDA) receptor and possibly also its kanainate-type feedforward autoreceptor, and serotonin which may also potentiate the NMDA receptor (McEwen and Magariños, 1997; Gould and Tanapat, 1999). Neuroprotection

by GABA-ergic inhibition or by neurotrophins (NT) such as brain-derived neurotrophic factor (BDNF) and NT-3 may decrease under certain stressful circumstances.

A postulated consequence of hippocampal atrophy with respect to the striking down-tuning of the HPA axis is the putative disinhibition of CRH release from the PVN which, then, should result in CRH receptor down-regulation in the pituitary and thereby cause a decrease of ACTH stimulation. From the viewpoint of classical endocrinology, however, it seems improbable that this would result in an absolute decrease of ACTH release from the pituitary, instead of an attenuated increase, and hence produce a decrease of cortisol release from the adrenal and, lastly, an enhanced glucocorticoid feedback effect. Continuous hormonal overstimulation at a pharmacological level produces receptor down-regulation and a sharp and almost complete decline of end-organ activity. This is applied in the treatment of prostatic cancer by the use of a long-acting gonadotropin-releasing hormone agonist that down-regulates testosterone production to almost zero, but in physiological circumstances it is not known to occur and the neuroendocrinology of major depression with its increased activity of the HPA axis does not confirm this either. Moreover, experiments in primates examining the effects of lesions of the hippocampus and other related structures produced chronic glucocorticoid hypersecretion lasting 6 to 15 months (Sapolsky, Zola-Morgan and Squire, 1991).

Vasopressin, Somatostatin and the HPA Axis

Thus, there must be other reasons for the opposite characteristics of PTSD and MDD with respect to the HPA axis. Vasopressin is one candidate for discriminating between PTSD and MDD although this may be part of a very complex pattern of interaction. Vasopressin potentiates the release of ACTH (Antoni, 1993; Aguilera, 1998) and it has been shown to co-occur with CRH in the median eminence in a way modulated by neonatal handling and stress (Bhatnagar and Meaney, 1995). It also has an important role in the consolidation of memory (De Wied, 1999) and could play a role in the conditioned physiologic responses found in PTSD (Pitman, Orr and Lasko, 1993). Arginine vasopressin (AVP) is secreted into the median eminence where it enters the portal blood circulation that brings it to the pituitary. Experiments in rats have shown that this is controlled indepently from CRH by axonal transport through AVP-containing versus AVP-deficient CRH neurons, and that under conditions of chronic or repeated stress plastic changes in hypothalamic CRH neurons evolve resulting in increased AVP stores and co-localization in CRH nerve terminals (De Goeij *et al.*, 1991). Also under conditions of chronic or intermittent stressful stimulation, a shift in hypothalamic signals for ACTH release in favour of AVP may ensue as it has been found in rats (De Goeij, Binnekade and Tilders, 1992). Experimental analysis in rats at the level of CRH and AVP responses in the PVN measured by primary transcript (heteronuclear) RNA and messenger RNA has confirmed that there is a desensitization of CRH, but not AVP transcription responses to repeated restraint stress. It has also been demonstrated that animals that adapted to a chronic homotypic stress show a greater response of CRH and AVP gene transcription in the parvocellular PVN after a novel, heterotypic stress. The hypothalamus clearly has the flexibility to adapt to homotypic stress while at the same time maintaining its ability to respond to novel stressors (Ma, Lightman and Aguilera, 1999). These experiments show that, with regard to the responses of the HPA axis, vasopressin is a mediator for the discrimination between chronic (homotypic) and acute (heterotypic) stressors, which can to some extent be controlled independently from CRH. In human depression not only an increase in CRH expressing neurones in the PVN was found, but also an increased co-expression of AVP and of AVP *per se* (Hoogendijk *et al.*, 2000). If PTSD is indeed the mirror image of depression it seems to be, the feedback of cortisol on the hypothalamus should be enhanced through parallel inhibition by another central mechanism.

CSF levels of the inhibitory neuropeptide somatostatin have been found to be elevated and to be correlated with the elevated CRH levels in PTSD patients but not in controls (Bremner *et al.*, 1997b). Somatostatin is known to inhibit the release of both CRH and ACTH (Richardson and Schonbrunn, 1981; Heisler *et al.*, 1982; Brown, Rivier and Vale, 1984). This points to the possibility that somatostatin is a pivotal link in the down-regulation of the LHPA axis.

The question that remains unanswered is what the advantage for survival of these lowered cortisol responses could be. The answer may be given by the fact that CRH gene expression in the brain is differentially stimulated by glucocorticoids. In the parvocellular region of the PVN, CRH gene expression is inhibited but in the central nucleus of the amygdala and in the lateral bed nucleus of the stria terminalis it is elevated. The elevation of CRH gene expression in these two nuclei may underlie a number of fear/anxiety or pathological states (Schulkin, Gold and McEwen, 1998). This is clearly applicable to PTSD, as we have seen, and it is almost too attractive as an explanation to presume that the organism has found a way to contain this limbic CRH fly-wheel by counter-regulation in order to protect the amygdala from unlimited positive feedback.

Glucocorticoid Receptor Gene Polymorphism

A possibility that has not been considered by researchers in the field of the psychobiology of PTSD until now, is the existence of a receptor polymorphism accounting for lower than expected circulating levels of cortisol and increased dexamethasone feedback sensitivity. In an epidemiological field study of an elderly population a close relationship was found between basal cortisol levels and the feedback sensitivity of the HPA axis to a low dose of dexamethasone, lower cortisol corresponding with higher feedback effect which looks the same, so far, as in PTSD. This suggested a genetic influence on the set point of the HPA axis. Over a two-and-a-half year follow-up period, individual characteristics remained fairly constant denying an effect of ageing on HPA activity or feedback sensitivity (Huizenga *et al.*, 1998a). Among 216 elderly people 13 heterozygotes for the N363S GR gene polymorphism (codon 363) were identified as showing increased cortisol suppression to 0.25 mg dexamethasone but no differences in GR number or ligand binding affinity on peripheral mononuclear leucocytes (Huizenga *et al.*, 1998b). In PTSD patients increased receptor numbers on lymphocytes have been found and a correlation with specific symptomatology which suggests that this is indeed a disease-specific phenomenon (Yehuda *et al.*, 1991). Nevertheless, this finding calls for control of receptor polymorphism in studies on the HPA axis of PTSD patients.

Overall View of HPA Axis Regulatory Abnormalities in PTSD

- The enhanced sensitivity of HPA feedback at the pituitary level and a down-regulation of the set point seem to be consistent.
- The anxiety-related CRH system which is intimately connected to central noradrenergic neurotransmission, is activated and is probably driven by the amygdala.
- The limbic-HPA down-regulation may be mediated by somatostatin and may serve as a counter-regulation to protect the amygdala from too much CRH gene expression by cortisol stimulation.

The Hypothalamo-Pituitary-Thyroid Axis

Much less work has been done on thyroid function in PTSD compared to the HPA axis. The first publication that is relevant

to cite in this work is from John W. Mason's group, which has accumulated the work on this topic so far (Mason *et al.*, 1994, 1995). Mason and his collaborators have substantiated their initial findings in several subsequent studies of which the highlights are that PTSD is accompanied by an increased level of free T_3 which is the biologically active form of thyroid hormone (*vide supra*). It was also correlated with hyperarousal (Wang *et al.*, 1995) and with novelty/sensation seeking and this finding coincided with positive correlations with urinary total NE and NE/cortisol ratio while it correlated negatively with urinary total cortisol (Wang *et al.*, 1997). It was not restricted to the population of American Vietnam veterans but was also found in Israeli veterans (Mason *et al.*, 1996) and older Second World War veterans who had suffered from PTSD over decades (Wang and Mason, 1999). The latter finding was accompanied by slightly, although not statistically significant, higher levels of TSH. The long duration of the psychiatric disorder made it unlikely that it was men with this pattern of thyroid axis hormones that were especially vulnerable to PTSD. Probably these changes are the result of the disorder, or part of the process (Prange, 1999).

In conjunction with the above discussion on CRH and hippocampal function in PTSD it is relevant to note that thyroid hormone causes long-lasting increases in hippocampal GR numbers through intermediate serotonergic projections (Sapolsky, 1997). On the other hand, there is an intricate relationship of thyroid function with noradrenergic neurotransmission which thus seems to support the arousal response and confirms the catecholamine findings by Mason's group. It is suggested that the excess of free T_3 is produced in the thyroid gland itself by direct sympathetic nervous control (Prange, 1999) but an increase of de-iodination by any of the multiple mechanisms that control it, such as transmembrane transport and tissue-specific de-iodinase, cannot be excluded. Free T_3 production has been reported to follow rapidly on the TSH surge after thyrotropin-releasing hormone administration before T_4 responds, which makes it indeed functional as an emergency response hormone.

GENERAL CONCLUSION

PTSD is one of the most extensively studied of the anxiety disorders concerning their psychoneuroendocrine properties. It consists of response patterns that are pathological in the sense that they continue and even may manifest themselves long after the initial stimulus has occurred. They show all features of the emergency response and these are detectable at all levels of neurobehavioural and metabolic regulation. The response patterns have become fixed like fossil imprints in stone although they may not be continuous but many of them phasic in nature. The marker hormones do not always act as we would have expected and this forces us to differentiate our understanding of these substances and their dynamics. The study of PTSD has enriched our knowledge substantially in this respect. It is highly probable that this will also lead to therapeutic innovations of which the CRH antagonists are among the most promising given the central role this neuromodulator plays in the congealed emergency response of post-traumatic stress disorder.

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XIX-6

Neuroimmunology of Anxiety Disorders

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INTRODUCTION

There is unequivocal evidence that stress is both causally related and a concomitant of most psychiatric disorders, including anxiety syndromes. For example, the post-traumatic stress disorder typically arises after unusual stress life experiences. Panic disorder is a syndrome with anxiety as the core of the disease and panic attacks possibly representing repeatedly recurring stress episodes. The obsessive–compulsive disorder involves recurrent thoughts (obsessions), that are experienced as intrusive and senseless, and/or repetitive seemingly purposeful behaviours (compulsions); both obsessions and compulsions cause marked distress. In phobic disorders, the phobic fear is a potent stressor.

There is agreement in the literature that stress profoundly affects immune function. Therefore, since patients with anxiety disorders endure considerable stress, it is plausible to expect alterations of immune competence in these subjects. On the other hand, it has been demonstrated that immune elements, namely some cytokines, are able to induce 'ansiogenic-like' and/or 'depressive-like' effects in the animal (Anisman *et al.*, 1998; Dantzer *et al.*, 1998); hence, it is likely that changes in immune parameters following real-life stress may be involved in the aetiopathology of anxiety syndromes and/or symptoms. Conversely, one would expect that if alterations of immunity occur in anxiety disorder patients, treatments aiming to alleviate anxiety symptoms should restore immune parameters.

The purpose of this review is to first consider the effects of stress on immune function; then, the changes in immune system that are associated with different anxiety syndromes will be discussed; finally, the effects of both pharmacological treatments and psychotherapeutic interventions on immune parameters will be analysed.

STRESS AND IMMUNE FUNCTION

Early evidence for an association between stress and immune function in humans came from the demonstration that human susceptibility to infections was increased by stress events. Kissen (1958) suggested a link between recent loss of a love object and the morbidity and mortality of pulmonary tuberculosis. Other authors suggested that hypnotically induced stress reactivates herpes simplex infection in susceptible individuals (Ullman, 1947), whereas hypnosis itself was found to be able to reverse both immediate-type and delayed-type hypersensitivity to tuberculin (Black, 1963; Black, Humphrey and Niven, 1963).

There is a large body of literature showing that stress has a significant impact on immune function. Initially, human studies examining the effects of experimental (speech task and mental arithmetic task) and real-life stressors (academic examination stress, bereavement, unemployment, divorce, caring for patients with chronic diseases) suggested that stress is associated with reduced immune responsiveness, and therefore it may compromise immune function (Dantzer and Mormede, 1995). However, there is now agreement in the literature that stress may even enhance immune activity.

In bereaved widows and widowers, a suppression of mitogen-induced lymphocyte proliferation was noted (Schleifer *et al.*, 1983); similarly, divorce, separation and unemployment were reported to decrease lymphocyte mitogen reactivity (Arnetz *et al.*, 1987; Kiecolt-Glaser *et al.*, 1987). A blunted unstimulated proliferation of peripheral blood mononuclear cells (PBMC) was observed in Israeli civilians during the period of Scud missile attacks (Weiss *et al.*, 1996), while recently released prisoners of war in Bosnia showed altered immune functions with reduced number of natural killer (NK) cells (Dekaris *et al.*, 1993).

Subacute or chronic real-life stressors were shown to decrease the number or the percentage of T-helper ($CD4^+$) cells, the T-helper/T-suppressor ($CD4^+/CD8^+$) cell ratio, and the number of NK cells in peripheral blood (Glaser *et al.*, 1985; Bachen *et al.*, 1992). To the contrary, acute laboratory and naturalistic stressors were reported to transiently elevate the number of total T and B lymphocytes, $CD8^+$ cells and the NK cells in the peripheral blood of healthy subjects (Fittshen *et al.*, 1990; Brosschot *et al.*, 1992). Moreover, Maes *et al.* (1999a) reported that students who responded to academic examination stress with a strong psychological reaction showed an increased number of activated T cell leukocyte subset profile. Furthermore, in those students, modifications of immune profile were positively and significantly associated with the stress-induced increase in self-rated severity of perceived stress and negative emotions.

Similarly to what occurs in cell-mediated immunity, increases or decreases in humoral immune parameters were reported after stress experiences. Some authors found increased salivary levels of immunoglobulin (Ig) A, while others reported reduced salivary concentrations of these Igs after exposure to acute or subacute stressors (Jemmott and Magloire, 1988; Bosch *et al.*, 1996). Increased concentrations of serum IgA, IgM and IgG were found by Maes *et al.* (1997a) in students with high levels of stress perception following academic examination, but not in those with low stress perception. Moreover, academic examination stress significantly increased serum levels of the C3 complement factor as well as those of the acute phase (AP) protein α_2-macroglobulin. Furthermore, students with high stress perception had a trend toward significant increases in serum levels of the complement C4 factor and AP proteins haptoglobin and α_1-acid glycoprotein, whereas students with low stress perception exhibited a reduction of these immune parameters (Maes *et al.*, 1997a).

Several studies assessed the effects of stress on cytokine production in humans. Early reports showed that psychological stress did not affect plasma interleukin-1 (IL-1) or interleukin-6 (IL-6) concentrations (Dugué *et al.*, 1993) or decreased the production of interferon-γ (IFγ) or increased that of IL-1β (Dobbin *et al.*,

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1991). Maes *et al.* (1998) and Song *et al.* (1999) found that academic examination stress significantly enhanced the production of proinflammatory cytokines, such as IL-6, tumour necrosis factor-α (TNFα) and IFγ, but decreased that of the 16kd Clara cell (CC-16) protein, a natural anti-inflammatory secretory protein. These data support the idea that the immune response to psychological stressors resembles that of organic stressors (injury, infection, necrosis), being characterized by the secretion of proinflammatory cytokines (IL-1, IL-6, TNFα) which, in turn, activate an AP response in order to cope with stress and minimize its impact on the homeostasis of the organism.

In sum, this brief review emphasizes that stress may suppress or enhance immune function depending on a number of variables, including the acute or chronic nature of stressors, the severity of the triggered stress response, the immune variable under consideration, the individual's perception of the stressor, the personality characteristics of the subject, and the nature of the stress-induced affective states.

SUBCLINICAL ANXIETY AND IMMUNE FUNCTION

Studies examining the relationships between subclinical anxiety and immunity are sparse. Linn, Lin and Jensen (1981) reported a negative correlation between anxiety and lymphocyte responses to mitogens in hospitalized patients. Decreased levels of salivary IgA were found in nurses with subclinical anxiety as compared to those without anxiety (Graham *et al.*, 1988); similar results were reported in dental students (Jemmott, Borysenko and Chapman, 1983). Moreover, healthy male students with high levels of subclinical anxiety had a significantly lower lymphocyte proliferative response to concanavalin A (ConA) as well as lower levels of circulating IL-1β (Zorrilla, Redel and DeRubeis, 1994). Among women undergoing adjuvant chemotherapy for breast cancer, those with high trait anxiety evidenced a reduced number of monocytes and $CD4^+/CD8^+$ T cell ratio, and a compromised activity of NK cells (Fredrikson *et al.*, 1993). Finally, a decreased lymphocyte proliferative response to phytohaemoagglutinin (PHA) was observed in healthy students with high anxiety scores independently from a previous history of life events (Gonzales-Quijano *et al.*, 1998).

On the contrary, a few studies suggested that subclinical anxiety may increase immune function. Indeed, a positive correlation was found between NK cell activity, IL-2 production and scores on the SCL-90-R anxiety subscale during an examination period in medical college students (Koh, 1996, 1997).

GENERALIZED ANXIETY DISORDER

La Via, Workman and Lydiard (1992) first reported that subjects with generalized anxiety disorder (GAD) and/or panic disorder (PD) had a significantly higher frequency of upper respiratory infections as compared with normal controls, which suggests a decrease of the immune defences in these individuals. Moreover, the same group of investigators (La Via *et al.*, 1996) showed that a 72-hour stimulation of peripheral lymphocytes with anti-$CD3^+$ induced a significantly lower expression of $CD25^+$ cell subtype in GAD patients with respect to healthy subjects.

Castilla-Cortazar, Castilla and Gurpegui (1998) investigated several immune parameters in 16 patients with GAD and five patients with obsessive–compulsive disorder (OCD). As compared to a control group, anxiety disorder patients showed increased number of monocytes and $CD13^+$ cells, normal counts of T lymphocytes, $CD4^+$ and $CD8^+$ T cell subtypes, and decreased number and activity of $CD16^+$ (NK) cells with a blunted lymphoproliferative response to PHA, and a normal *in vitro* production of IFγ from peripheral blood mononuclear cells (PBMC). Moreover, patients with anxiety displayed an anergy to candidin and tuberculin, alterations in the expression of both monocyte surface HLA-DR molecules (involved in the mechanisms of antigen presentation) and membrane CR1 receptors (important in phagocytosis of opsonized particles and microrganisms) as well as a reduced number of monocytes expressing cytoplasmic vimentin filaments, which are implicated in the cytoskeletal organization. Incubation *in vitro* of these monocytes with naloxone normalized the number of cells expressing vimentin filaments and their ability to ingest *Candida albicans*. These findings suggest that at least some immune alterations detected in patients with GAD may be dependent on an increased opioid tonus. Indeed, plasma levels of β-endorphins were found to be elevated in those patients.

Koh and Lee (1998) investigated cell-mediated immunity in a cohort of 31 patients, 20 with GAD and 11 with PD, and 31 healthy subjects. They found that both GAD patients and subjects with PD had reduced blastogenic responses to PHA and decreased PHA-induced IL-2 production. No significant differences were found in NK cell activity between the two patient groups and healthy subjects.

SIMPLE PHOBIA

It has been recently shown that phobic fear is a potent stressor, which results in increases of autonomic activity, NK cell percentages and cytotoxicity (Gerritsen *et al.*, 1996). These immune effects, however, seem to be influenced by the individual's level of worry. Indeed, people with spider or snake phobia who experienced high levels of trait worry showed a blunted increase in the number of peripheral NK cells after exposure to the phobic stimulus, whereas those with normal levels of worry showed a marked increase in this immune parameter (Segerstrom *et al.*, 1999).

One study reported that patients with agoraphobia had normal counts of peripheral $CD3^+$, $CD4^+$, $CD8^+$ and $CD57^+$ cells, normal value of the $CD4^+/CD8^+$ ratio and an increased frequency of $CD14^+$ cell types (monocytes) (Covelli *et al.*, 1990). Moreover, *in vitro* phagocytosis and killing of *Candida albicans* exerted by polymorphonuclear cells and monocytes were reduced in those patients. After an 8-week treatment with the synthetic thymic hormone, thymopoietin, the phagocytic capacity of monocytes significantly increased, whereas that of polymorphonuclear cells remained unchanged. Moreover, treatment with the thymic hormone led to a significant recovery of polymorphonuclear and monocyte killing capacity, even if values remained below the normal range (Covelli *et al.*, 1990).

SOCIAL PHOBIA

A limited number of studies investigated immune function in people with social phobia.

Rapaport and Stein (1994a) found normal levels of serum IL-2 and soluble IL-2 receptor (sIL-2R) in 15 patients with generalized social phobia, and suggested that these patients do not have evidence of T-lymphocyte activation. This hypothesis was supported by the findings of a subsequent study, in which patients with social phobia exhibited normal values of peripheral $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD25^+$ lymphocyte subtypes and B cells expressing HLA-DR molecules, with only a slight increase in the number of NK ($CD16^+$) cells (Rapaport, 1998).

PANIC DISORDER

The studies of circulating lymphocyte phenotypes in patients with PD have provided conflicting results. In particular, no difference in

total lymphocytes, leukocytes, T cells and B cells or decreased total lymphocytes and increased total T cells have been reported in PD patients (Schleifer *et al.*, 1990; Ramesh *et al.*, 1991; Andreoli *et al.*, 1992; Marazziti *et al.*, 1992). The number of $CD4^+$ and $CD8^+$ cell subtypes and the $CD4^+/CD8^+$ ratio were found to be normal in three studies (Schleifer *et al.*, 1990; Perini *et al.*, 1995; Rapaport, 1998), but decreased in another study (Marazziti *et al.*, 1992). The peripheral NK cell number has been reported to be mostly normal, with only one study reporting an increased value (Schleifer *et al.*, 1990; Marazziti *et al.*, 1992; Perini *et al.*, 1995; Koh and Lee, 1998; Rapaport, 1998). Two groups of investigators found increased values of $CD19^+$ B cells and lymphocytes expressing the HLA-DR antigen (Perini *et al.*, 1995; Rapaport, 1998), whereas Marazziti *et al.* (1992) detected normal values of these cells. Similarly, the $CD57^+$ cell number was reported to be normal in two studies (Marazziti *et al.*, 1992; Rapaport, 1998), but was decreased by another study (Perini *et al.*, 1995). Finally, $CD56^+$ cells and lymphocytes expressing the CD25 molecule, which is the surface receptor for IL-2, were not increased in patients with PD (Perini *et al.*, 1995; Rapaport, 1998).

The *in vitro* mitogen response of lymphocytes from PD patients has been found to vary within a wide range. Two studies reported normal blastogenic responses to PHA (Surman *et al.*, 1986; Brambilla *et al.*, 1992), two others found a reduced proliferative responses to ConA, Pokeweed mitogen (PWM) and PHA (Schleifer *et al.*, 1990; Koh and Lee, 1998), and one found increased blastogenic responses to PHA and ConA (Andreoli *et al.*, 1992).

Serum levels of immunoglobulins were investigated by Ramesh *et al.* (1991) who found increased concentrations of IgA, but normal values of IgM, IgG and IgE in PD patients as compared to normal controls. Brambilla *et al.* (1994) detected increased plasma concentrations of IL-1β in PD patients both before and after successful alprazolam therapy, whereas Rapaport and Stein (1994b) found normal serum levels of IL-1β, IL-1α and sIL-2R, with a slight but not significant increase in serum IL-2 levels. The finding of normal serum sIL-2R is consistent with the normal values of $CD25^+$ lymphocytes reported in these patients (Rapaport, 1998). Moreover, blood levels of TNFα in people with PD were reported to be the same as those from normal controls (Brambilla, Bellodi and Perna, 1999). Finally, *in vitro* experiments have shown a decreased production of PHA-induced IL-2 and a normal unstimulated synthesis of IL-2 and IL-3 from PBMC of panic patients (Koh and Lee, 1998; Weizman *et al.*, 1999).

The patient selection is likely the most relevant factor accounting for this discrepancy. Indeed, studies widely differed in the patient populations since they included subjects with or without agoraphobia, previous histories of major depression, and concomitant depressive disorders.

OBSESSIVE–COMPULSIVE DISORDER

Several lines of evidence suggest that some cases of children-onset obsessive–compulsive disorder (OCD) may have an autoimmune aetiology. Swedo *et al.* (1989, 1998) reported an increased incidence of OCD in paediatric patients with Sydenham's chorea, an autoimmune disease of the basal ganglia. These investigators showed that some children may develop obsessive–compulsive symptoms with motor and/or vocal tics in the aftermath of group A beta-haemolytic streptococcal infection, presumably on the basis of antineuronal antibodies, and introduced the term of paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) to characterize these patients. As in Sydenham's chorea, the pathogenesis of PANDAS is thought to be autoimmune. This idea is supported by the following: (a) autoimmune antibodies reactive with nuclei of the basal ganglia have been detected in some of these patients (Black, Lamke and Walikonis, 1998); (b) a temporal link between symptom exacerbation and streptococcal infection has been described (Allen, Leonard and Swedo, 1995); (c) a progressive clinical improvement has been observed in those patients undergoing antibiotic prophylaxis at dosages effective in preventing recurrent streptococcal infections (Leonard and Swedo, 2001). Furthermore, Murphy *et al.* (1997) found that the B lymphocyte antigen D8/17, a marker of vulnerability to rheumatic fever, was more common in patients with childhood-onset OCD than in comparison subjects, despite the absence of documented Sydenham's chorea or rheumatic fever.

Very recently, a case of a 25-year-old man who developed OCD after a severe antibiotic-responsive pharyngitis and exhibited increased levels of antibodies to group A beta-haemolytic streptococci has been described (Bodner, Morshed and Peterson, 2001). This subject had elevated serum D8/17 lymphocytes, positive antistriatal antibodies and positive anticytoskeletal antibodies. This case report suggests that post-streptococcal OCD may occur also in adult individuals.

Similarly to patients with major depression who produce antibodies against β-endorphin and somatostatin-14 (Roy *et al.*, 1988), patients with OCD have been shown to exhibit high circulating levels of antibodies for somatostatin-14 and prodynorphin 209–240, although the pathophysiological significance of these immune changes has not been clarified (Roy *et al.*, 1994). Finally, Khanna *et al.* (1990, 1997) documented higher levels of IgG in the blood and increased antibodies to herpes virus type-1 in the cerebrospinal fluid of subjects with OCD.

Investigations of cell-mediated and humoral immunity in people with OCD have provided conflicting results. Plasma levels of IL-1β have been reported to be normal by both Maes, Meltzer and Bosmans (1994) and our group (Monteleone *et al.*, 1998), but decreased by Brambilla *et al.* (1997). Serum levels of IL-6, sIL-2R, soluble IL-6 receptor (sIL-6R) and transferrin, an AP protein, have been found to be unchanged (Maes, Meltzer and Bosmans, 1994; Monteleone *et al.*, 1998), whereas plasma concentrations of TNFα have been detected to be reduced (Brambilla *et al.*, 1997; Monteleone *et al.*, 1998). *In vitro* assessment of IL-1β, IL-2 and IL-3 production from unstimulated PBMC of OCD patients did not display any alteration (Weizman *et al.*, 1996). Finally, Barber *et al.* (1996) did not find any change in T-lymphocyte subsets in a small sample of obsessive–compulsive patients, whereas Marazziti *et al.* (1999), in a study involving a larger group of adult patients with OCD, reported a significant increase in $CD8^+$ and a decrease in $CD4^+$ lymphocytes.

POST-TRAUMATIC STRESS DISORDER

Among the anxiety syndromes, post-traumatic stress disorder (PTSD) may be considered the prototype of stress-induced diseases. In fact, by definition, PTSD occurs in response to unusually stressful life events (extreme and catastrophic stresses). Given the above-mentioned relationships between stress events and immune activity, one would expect dramatic changes in immune parameters in people with PTSD.

Watson *et al.* (1993) first reported that Vietnam combat veterans with chronic PTSD, compared with healthy control subjects, had enhanced cell-mediated immunity, as assessed by means of delayed skin responsivity to a panel of antigens (CMI multitest). Ironson *et al.* (1997), instead, found that NK cell cytoxicity, $CD4^+$ and $CD8^+$ cell counts were significantly lower in PTSD patients, while the number of white blood cells was positively and significantly related to the severity of PTSD symptoms. Normal counts of $CD2^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD20^+$ and $CD56^+$ cell subtypes, and increased activity of NK cell activity were detected by Laudenslager *et al.* (1998) in combat veterans with long-term PTSD. Finally, significantly increased counts of total leukocytes, total lymphocytes,

T cells, $CD4^+$ and $CD8^+$ cell subtypes were reported by Boscarino and Chang (1999) in Vietnam veterans with current PTSD; moreover, in this study, patients exhibited a delayed cutaneous hypersensitivity, as assessed by the CMI multitest. This finding confirmed the occurrence of a delayed skin hypersensitivity in PTSD patients, previously reported by Watson *et al.* (1993). Recently, a normal lymphoproliferative response to PHA, but a reduced blastogenic response to tetanus toxoid (a specific recall antigen recognized by memory T cells) has been found in Gulf War veterans with a diagnosis of PTSD (Everson, Kotler and Blackburn, 1999).

With regard to humoral immunity, increased levels of serum IL-1β and normal values of sIL-2R have been detected in Israeli male veterans with PTSD (Spivak *et al.*, 1997). In this study, serum levels of IL-1β were positively correlated with the duration of the illness. Finally, Maes *et al.* (1999b) reported normal plasma levels of soluble IL-1 receptor antagonist, CC-16, glycoprotein 130 (gp130) and soluble CD8 antigen, but increased concentrations of IL-6 and sIL-6R in subjects with PTSD following accidental man-made traumatic events. Since serum sIL-6R mediates IL-6 signals, while serum gp130 has the potential to inhibit IL-6 signalling, the findings of Maes and coworkers suggest an increased IL-6 signal transducing and, consequently, an activation of the inflammatory response system in PTSD. These results, together with the above-reported observation of increased production of IL-1β in war veterans with PTSD (Spivak *et al.*, 1997), support the idea that proinflammatory cytokines may play a role in the development of anxiety subsequent to stressful traumatic events.

ANTIANXIETY TREATMENTS AND IMMUNE FUNCTION

Drug Treatments

Although several pharmacological agents are commonly used in the treatment of anxiety syndromes, we will focus exclusively on the immune effects of benzodiazepines (BDZs) and selective serotonin reuptake inhibitors (SSRIs), that are actually the most widely prescribed drugs for these disorders.

Benzodiazepines

Evidence that BDZs influence immune function emerged from studies showing the presence of BDZ receptors on immune cells. Peripheral-type BDZ receptors have been detected on immune cells of the experimental animal (Zavala, Haumont and Lenfant, 1984) and on human monocytes, polymorphonuclear neutrophils, B cells, NK cells, $CD4^+$ and $CD8^+$ cells (Canat *et al.*, 1992). It has been shown that activation of these receptors stimulates chemotaxis of human monocytes (Ruff *et al.*, 1985), enhances the production of reactive oxygen species (an essential step in oxygen-dependent phagocytic defence against pathogens) (Zavala and Lenfant, 1988), and modulates the production of proinflammatory cytokines such as IL-1β, TNFα, IL-6, IL-8 and granulocyte/macrophage-colony stimulating factor (Taupin *et al.*, 1991; Zavala *et al.*, 1991). Moreover, peripheral-type BDZ receptors have been detected also on microglial cells, which are considered the resident macrophages of the brain (Bender and Hertz, 1985), and their activation has been shown to enhance the production of IL-1, TNFα and IL-6 in the rat cortex after a fluid percussion trauma (Taupin *et al.*, 1993).

A number of studies suggest that anxiolytic BDZs reverse or attenuate the stress-induced immunosuppression. For example, chronic treatment with alprazolam, diazepam or imidazolam protects mice against the stress-induced decreases in thymus cellularity and weight, blastic response to ConA and NK cell activity (Freire-Garabal *et al.*, 1991a, 1991b). Partially consistent with animal data, Lechin *et al.* (1994) recently reported that chronic BDZ users without psychotic or depressive symptoms had lower-than-normal $CD3^+$, $CD4^+$ and $CD16^+ + CD56^+$ (NK) cell counts with a reduced $CD4^+/CD8^+$ ratio, and enhanced NK cell activity and $CD57^+$ cell number. After 15 days of BDZ discontinuation, some of these immune parameters ($CD3^+$, $CD4^+$ cell counts and NK cell activity) returned to normal values, others ($CD16^+$ cell number) persisted significantly lower than controls, while $CD4^+/CD8^+$ ratio and $CD56^+$ cell number exceeded normal values.

Selective Serotonin Reuptake Inhibitors

There are some indications that both acute and chronic administration of SSRIs may compromise cellular immune responses in the animal. For instance, fluoxetine administration has been reported to increase tumour growth and tumour formation following tumour transplantation and exposure to chemical carcinogens in rodents (Brandes *et al.*, 1992). In addition, acute fluoxetine has been shown to decrease immune cell infiltration and inflammation in rats following exposure to irritants (Bianchi *et al.*, 1995). Finally, either fluoxetine or sertraline have been shown to decrease NK cell cytolytic activity and lymphocyte proliferative responses (Pellegrino and Bayer, 1998; Pellegrino and Bayer, 2000).

Other studies, however, point to an immunopotentiating effect of prolonged administration of SSRIs to experimental animals. Indeed, chronic administration of sertraline has been shown to increase the percentage of neutrophils and enhance the proliferative activity of T-lymphocytes (Song and Leonard, 1994), while chronic treatment with fluoxetine has been reported to inhibit the growth of induced and spontaneous neoplasms in rodents (Bendele *et al.*, 1992; Abdul, Logothetis and Hoosein, 1995). It has been suggested that, at least in animals, SSRIs may exhibit both immunoenhancing and immunosuppressive effects depending on the type of drug and the duration of its administration (Kubera *et al.*, 2000).

With regard to human studies, although a direct assessment of the immune effects of SSRIs in people with anxiety disorders is lacking, there are some data *in vitro* and in depressed patients that support an immunosuppressive action of these drugs. For example, *in vitro* studies have shown that SSRIs decreased splenic lymphocyte proliferation (Berkeley *et al.*, 1994), while coincubation of mitogen-stimulated human immunocytes with fluoxetine, sertraline or citalopram significantly reduced the production of proinflammatory cytokines IL-1β, IL-6, IFNγ and TNFα and/or increased the production of IL-10, an anti-inflammatory cytokine, with a consequent decrease of the IFNγ/IL-10 ratio (Xia, DePierre and Nassberger, 1996; Maes *et al.*, 1999c). Moreover, it has been reported that some patients receiving fluoxetine for the treatment of a depressive condition experienced a reactivation of a herpes simplex infection or developed cutaneous pseudolymphoma lesions, that promptly reverted when the drug was discontinued (Reed and Glick, 1991; Crowson and Magro, 1995). In patients chronically treated with SSRIs, a significant reduction in the number of leukocytes and neutrophils, but no significant changes in the absolute number of PBMC and $CD4^+/CD8^+$ cell ratio have been reported (Maes *et al.*, 1997b; Kubera, Van Bockstaele and Maes, 1999). Moreover, subchronic treatment with fluoxetine has been shown to normalize the pathologically elevated blood levels of IL-6, α_1-acid glycoprotein and IL-2R in major depression (Maes *et al.*, 1995; Sluzewska *et al.*, 1995). To the contrary, a 12-week treatment with sertraline has been reported to not affect the abnormally increased plasma concentrations of IL-1β in dysthymic subjects (Anisman *et al.*, 1999), while both fluoxetine and fluvoxamine, administered for 3 months, did not influence plasma levels of IL-1β, IL-6 and TNFα in people with eating disorders (Brambilla *et al.*, 1998).

The exact mechanisms by which SSRIs exert their activity on immune functions are still unknown. It has been suggested that the immune effects of SSRIs are mediated by endogenously released serotonin (5-HT). Indeed, in animals depleted of endogenous 5-HT

stores, the administration of both fluoxetine and sertraline was not able to reduce lymphocyte proliferative responses (Pellegrino and Bayer, 2000). Moreover, it has been shown that T-lymphocytes constitutively express 5-HT receptors as well as the high-affinity 5-HT transporter system (Aune, Golden and McGrath, 1994; Faraj, Olkowski and Jackson, 1994).

Psychotherapeutic Interventions

Further to initial reports of immune effects of hypnosis (Ullman, 1947; Black, 1963; Black, Humphrey and Niven, 1963), the effects of psychotherapeutic interventions on immune function have been assessed in two categories of subjects: (a) patients dealing with different types of cancer; (b) healthy subjects exposed to different kinds of stressors.

The effects of short-term (6 weeks) structured cognitive-behavioural therapy on immune parameters were evaluated by Fawzy *et al.* (1990) in patients with post-surgical malignant melanoma. At the sixth-month evaluation point, along with a reduction in levels of psychological distress and greater use of active coping methods, patients in the intervention group exhibited significant increases in the percent of large granular lymphocytes and NK cells along with an increase in NK cytotoxic activity and a small decrease in the percent of $CD4^+$ cells. In post-surgical early-stage breast cancer patients, a 10-week cognitive-behavioural intervention programme induced a significant increase in the number of circulating lymphocytes (Schedlowski *et al.*, 1994). Finally, in the study of van der Pompe *et al.* (1997), cancer patients who had been treated for early-stage breast cancer and were diagnosed with either positive axillary lymph nodes or distant metastases, were assigned to a 13-week experimental–existential group psychotherapy (EEGP) or to a waiting list control condition (WLC). EEGP patients with a higher percentage of $CD4^+$, $CD8^+$ and $CD16^+/56^+$ T-lymphocytes at baseline had lower post-treatment percentages than their counterparts in the WLC with similar initial levels. Similarly, those EEGP patients with the highest lymphoproliferative response to PWM at baseline had smaller post-treatment responses than patients in the WLC. These authors, on the basis of literature suggestions showing that lower percentages of NK and $CD8^+$ cells were associated with a better prognosis in cancer patients, speculated that EEGP by normalizing increased immune parameters may positively affect the outcome of these patients.

Several groups have studied the effects of psychotherapeutic interventions on stress-induced immune changes. After a 1-month intervention period, geriatric patients who received relaxation training reported a decrease in distress symptoms coupled with increased NK cell cytotoxicity and decreased antibody titres to latent herpes simplex virus (Kiecolt-Glaser *et al.*, 1985). In a study by Peavy, Lawlis and Goven (1985), in subjects with high life stress scores coupled with poor phagocytic capacity, a biofeedback-assisted relaxation training significantly enhanced neutrophil activation capacity, suggesting a qualitative improvement of phagocytic ability. In a subsequent study (Green and Green, 1987) a single 20-minute relaxation session resulted in a significant increase in salivary IgA concentrations. Finally, in males at high risk for HIV-1 infection, significant positive correlations were found between the frequency of relaxation procedures and the number of $CD4^+$, $CD8^+$, NK cells and the $CD4^+/CD8^+$ ratio during the high stress week of serostatus determination (Antoni *et al.*, 1990).

Two studies examined the effects of self-hypnosis/relaxation intervention on immune changes induced by academic examination stress in medical students. The first one (Kiecolt-Glaser *et al.*, 1986) showed that the hypnosis/relaxation programme prevented exam-related increases in distress symptoms, but did not influence immune parameters. Indeed, both the intervention and control groups showed significant decreases in the percentages of $CD4^+$ lymphocytes and NK cell activity at the examination time; however, the frequency of the relaxation procedures was a good predictor of the increases in the $CD4^+/CD8^+$ cell ratio during the exam period. The second study (Whitehouse *et al.*, 1996) demonstrated that significant increases in the number of B lymphocytes, activated T-lymphocytes, PHA-induced and PWM-induced blastogenesis and NK cytotoxicity occurred during the examination period in both students assigned to a self-hypnosis/relaxation intervention and in those assigned to the control group. Nevertheless, within the self-hypnosis group, the quality of relaxation exercises predicted both the number of NK cells and their cytotoxicity.

Finally, it has been very recently reported that, in medical students undergoing academic examination, massage therapy induced a significant increase in the total number of white blood cells with a decrease in the percentage of T cells, no significant changes in the lymphocyte responses to mitogens and a significant increase in the killing activity of NK cells (Zeitlin *et al.*, 2000).

CONCLUSIONS

The existing literature is highly controversial and fraught with several methodological problems. The diagnostic heterogeneity of the subject samples is certainly one of the most relevant factors contributing to the inconsistency of the results. Indeed, in some studies patients were suffering from pure anxiety disorders, in others patients had concomitant major depressive disorder or positive histories of previous depressive episodes. Moreover, most of the studies did not include healthy controls matched to patients on age and gender; as there may be age-dependent alterations (Weksler, 1983) and gender-specific differences (Oyeyinka, 1984) in immune function, these factors must be taken into account in this kind of research. Furthermore, patients differed on the duration and/or severity of the illness, personality characteristics and treatment status. This last variable is particularly relevant in light of the immune effects of both BZD and psychotherapeutic techniques. Differences in both methodology (e.g., evaluation of absolute number of lymphocytes versus lymphocyte percentages, assay techniques) and/or immune parameters investigated may also add to the variability of results. Finally, smoking, excessive drinking, disturbed sleep and poor nutrition are known to affect immune function (Locke and Gorman, 1989), hence variations of these behaviours may further explain controversies among the studies.

All these factors limit the interpretation and generalization of the findings. Notwithstanding, there is unequivocal evidence that immune changes do occur in anxiety disorders, with the majority of studies suggesting an impairment of the immune function in these syndromes. Only PTSD seems to be associated with an immune enhancement rather than immunosuppression. It has been suggested that the increase in immune activity in patients with PTSD may have a pathogenetic role (Spivak *et al.*, 1997; Maes *et al.*, 1999b). Moreover, there is consistent evidence that at least some cases of childhood-onset OCD may have an autoimmune aetiology, possibly linked to the streptococcal infection and rheumatic fever. Finally, it has been almost uniformly reported that psychotherapeutic interventions aiming to alleviate anxiety symptoms potentiate immune functions, whereas antianxiety drugs may have both immunoenhancing and immunosuppressive effects.

In conclusion, whether in anxiety disorders immune changes occur as a consequence of the psychological distress or are primitive and aetiopathogenetically linked to the disorders themselves, it becomes more evident every day that they are part of the biological substrate of these syndromes and, as such, they are worthy of every effort by both clinicians and researchers in order to better

understand their implications in the course and the treatment of anxiety disorders.

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XIX-7

Psychophysiology of Anxiety Disorders

G. Wiedemann and A. Mühlberger

INTRODUCTION

The use of psychophysiological methods in the assessment of a condition and the evaluation of treatment can be separated from research on mechanisms in the aetiology of fear (see also Hugdahl, 1989). In the following section, we will focus on condition assessment and treatment evaluation. For an extended discussion of experimental research on the mechanisms of fear, we refer you to the corresponding section in this book or to the *Handbook of Psychophysiology* (Bradley, 2000; Öhman, Hamm and Hugdahl, 2000).

The investigation of anxiety in psychophysiological research has a long history and is based on the dualistic view of emotions as consisting of both a cognitive and a somatic component (James, 1884). Obvious changes in different physiological systems, such as pounding of the heart, sweaty palms, and trembling hands upon confrontation with fear-eliciting stimuli, indicate the importance of physiological assessments, especially in anxiety disorders. The physiological reactions may tell us not only about the intensity of fear, but also about the quality of fear responses. In current psychophysiology research, a three-system concept of fear and emotion (Lang, 1968; Foa and Kozak, 1993) is widely accepted. The phobic reaction is seen as consisting of three loosely coupled components: psychophysiological responses, cognitive reports, and avoidance behaviour (Lang, 1971). As the three systems do not always react to the same extent or in the same temporal course (e.g. Rachman, 1977), the assessment of fear responses in all three component levels is recommended (e.g. Hugdahl, 1989). Many of the issues arising regarding one anxiety disorder may be equally applicable to other anxiety disorders. Research on panic disorder, for instance, may be difficult because of diagnostic co-morbidity, particularly with depression. On the other hand, the issue of the nature of the stimuli used to elicit physiological responses is illustrated by disorders such as post-traumatic stress disorder.

This paper is subdivided according to the Diagnostic and Statistical Manual (DSM-IV, American Psychiatric Association, 1994) diagnoses of the different anxiety disorders: (1) phobias; (2) panic disorder (PD); (3) generalized anxiety disorder (GAD); (4) post-traumatic stress disorder (PTSD); and (5) obsessive–compulsive disorder (OCD).

PHOBIAS

Phobias are subdivided into three subcategories: specific phobia; social phobia and agoraphobia.

Specific Phobia

No evidence of abnormal reactions of subjects (Ss) with specific phobia was found if no phobic context was present (Klorman, Weissberg and Wiesenfeld, 1977; Lang, Cuthbert and Bradley, 1998b). However, there is clear evidence for differences during exposure to phobic stimuli. The most established paradigm is to compare phobic Ss with control Ss in their reactions to phobic stimuli (e.g. spider pictures) in contrast to neutral, pleasant, or unpleasant stimuli. Early investigations focused on responses of the autonomic nervous system (ANS, e.g. heart rate (HR), skin conductance (SC) and respiration). More recently, focus was drawn to protective reflexes (e.g. startle response (SR), measured by corrugator electromyography (EMG) upon exposure to electric shocks or loud noise) and direct central nervous system (CNS) parameters, e.g. EEG/EP (evoked potentials). Spontaneous EMG measures were most frequently used to measure frontalis or forearm extensor muscle activity. The results were inconsistent (see e.g. review by Sartory and Lader (1981). Hamm *et al.*, (1997) found no significant group-specific differences in corrugator EMG in animal and mutilation phobic Ss during perception of phobic or neutral pictures. This seems to reflect heterogeneity of EMG reactions among subjects. This would be confirmed by subjective evaluations. Phobic Ss quite commonly experience mixed feelings of muscles becoming weak or tense (Hugdahl and Öst, 1985).

Many studies dealing with specific phobias investigated selected fearful, but non-clinical Ss. Since these Ss were comparable to diagnosed phobic Ss in many measures, selected investigations including analogous samples will be included in this section. Fearful samples without a clinical diagnosis are described as fearful Ss whereas diagnosed samples are described as phobic Ss.

According to the DSM IV, phobias can be subdivided in five types: animal type; natural environment type; blood-injection-injury type; situational type and other type. Most research was done on the animal type, the blood/injection/injury type and the situational type (e.g. flight phobia). The following sections will deal with these three types only.

Animal Type Specific Phobia

The autonomic system activity profile is dominated by a strong activation of the sympathetic branch. SC level (SCL) is considered a good candidate for measuring sympathetic activation, because the electrodermal system is thought to be controlled exclusively by the sympathetic part of the autonomic nervous system (Bradley, 2000). Nevertheless, there are some drawbacks. Firstly, sweat gland activity relies on a cholinergic mechanism rather than an adrenergic mechanism like most other sympathetic actions. Secondly, it is assumed that there are different types of sweating (e.g. Dawson, Schell and Filion, 2000). It is possible, for example, that sweating of the palms, which is most commonly measured, is an effect of parasympathetic regulation (Guyton and Hall, 1996). Nevertheless, SC reaction (SCR) is reliably modulated by emotional arousal in perception, anticipation and imagination (Bradley, 2000) and

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many publications used SCL as an index of sympathetic activation (Sartory, 1983; Hugdahl, 1989; Bradley, 2000). In laboratory settings, the SCR upon presentation of emotional (e.g. phobic) pictures is widely investigated. Geer (1966) showed an increased SCL for spider phobic Ss compared to control Ss during the perception of spider pictures. Wilson (1967) was able to show the same effects when pictures were presented tachistoscopically. This suggests that conscious perception is not necessary for this type of reaction.

Special focus was drawn to the underlying mechanism of these autonomic responses. Öhman and Soares (1994) exposed selected snake- and spider-fearful Ss to masked- and non-masked phobic and control pictures in a backward masking paradigm. SCR of phobic Ss was enhanced in the unmasked as well as the masked condition only upon exposure to their phobic stimuli (snakes or spiders). Further, snake phobic Ss rated snakes as more arousing and more negative in valence (with similar results for spider phobic Ss upon exposure to spider stimuli). This suggests that SCRs are individual rather than evolutionally prepared. Guided by these results, Öhman and Soares (1994) proposed a pre-attentive perceptual analysis of physical stimuli characteristics by a supposed '*physical feature detector*'. This feature detector can activate the arousal system. Van den Hout and co-workers (2000) tested the hypothesis of the '*physical feature detector*' (Öhman and Soares, 1994) by exposing spider phobic Ss (DSM-IV) to words (fear-related, general threat and neutral) instead of pictures. Control Ss were not included. As expected, the researchers found the strongest reaction of phobic Ss to phobia-related words, followed by progressively milder reactions to common threat words and neutral words during unmasked presentation. In the masked condition, spider phobic Ss reacted more strongly to general threat words and phobia-related words than to neutral words, but reactions did not differ between spider words and threat words. It was concluded that pictures are processed as words semantically, that the hypothetical significance evaluator (Öhman and Soares, 1994) directly influences the arousal system and that for this process, no conscious perception is required (Van den Hout, De Jong and Kindt, 2000).

Additionally, cardiovascular measures verify the sympathetic dominance in animal phobic Ss during anticipation or perception of phobic pictures. Spider or snake phobic Ss show strong HR acceleration, whereas control Ss show HR deceleration (e.g. Klorman, Weissberg and Wiesenfeld, 1977; Cook *et al.*, 1988). The HR acceleration in phobic subjects is accompanied by an increase in peripheral resistance in skin blood vessels (vasoconstriction measured by a decrease in digit pulse amplitude) and a decrease in peripheral resistance in muscle blood vessels (vasodilatation, measured by an increase of forearm blood volume) (e.g. Kelly, 1980). Furthermore, an increase in average blood pressure was found (Hamm *et al.*, 1997). To summarize, cardiovascular responses and SCR suggest that confrontation with phobic stimuli results in a defensive reaction (DR) (Sokolov, 1963) in phobic Ss, but an orientation reaction (OR) Sokolov, 1963) in non-phobic comparison Ss. For a review of the OR/DR literature see for example Öhman and co-workers (2000). According to the idea that the phobic reaction is a DR and includes the activation of a fight–flight system (Lang, 1968), not only the sympathetic nervous system should be activated, but also behavioural avoidance as well as protective reflexes (e.g. acoustic SR (ASR)) should be facilitated (Lang, Bradley and Cuthbert, 1990). To test this hypothesis, Hamm *et al.* (1997) assessed blood pressure (BP), HR, and eyeblink reflex (ASR, measured by orbicularis occuli EMG reaction to sudden, loud noise) during the perception of spider/snake pictures. In a second part of the experiment, subjects were able to stop the presentation themselves. An increased ASR during the perception of phobic stimuli was found only in animal fearful Ss, but not in control Ss. No differences between groups were found for neutral, pleasant or unpleasant pictures. Additionally, fearful Ss showed a strong avoidance reaction. While control Ss looked at spider/snake pictures and neutral pleasant/unpleasant pictures for the same length of time, fearful Ss stopped examining spider/snake pictures after a few seconds. Globisch *et al.* (1999) studied the temporal course of ASR potentiation. The researchers found a *relative* potentiation of the ASR beginning 300 ms after the onset of phobic pictures in animal fearful Ss. Furthermore, increased SCR, HR and average BP reaction were found. Similar results were observed when pictures were shown only for 150 ms, with the exception of the blood pressure (BP) reaction. Further research is necessary to differentiate whether this fast onset of relative startle potentiation is a result of reduced pre-pulse inhibition due to rapid encoding of the threat cue, or due to the fast onset of fear-potentiated startle response (Globisch *et al.*, 1999).

Central nervous measurements of phobic responses focus on alpha activity (8–13 Hz) and different features of Event-Related Potentials (ERPs). Within the ERPs, the Contingent Negative Variation (CNV), which occurs between signal stimulus, and target stimulus, e.g. in conditioning paradigms, and the P300 after stimulus presentation were investigated. Unfortunately, results are inconsistent. Larger (more negative) CNVs were observed in phobic subjects anticipating phobic stimuli compared to subjects awaiting neutral stimuli (e.g. Dubrovsky, Solyom and Barbas, 1978); however, smaller (less negative) CNVs have also been observed under these conditions (e.g. Lumsden, Howard and Fenton, 1986). To summarize, it seems that smaller CNVs may be associated with phobic fear states (Hugdahl, 1989).

Not only the perception of phobic stimuli results in physiological reactions. Memory retrieval or imagery of phobic stimuli (spiders/snakes) in animal phobic Ss results in sympathetic activation. Imagery is typically realized by presenting a script to the subject with instructions to imagine the presented situation. Scripts can be standardized or created with respect to individual fear stimuli (Cuthbert and Lang, 1989). Different investigations showed that even very short scripts of a few seconds in duration can induce subjective anxiety as well as physiological activation (e.g. Vrana, Cuthbert and Lang, 1986; Bradley, Land and Cuthbert, 1991).

Lang and co-workers (1970) showed accelerated HR in snake phobic Ss during imagery. This acceleration was linearly correlated with subjective fear, and could be enhanced by including more extensive use and description of text portions describing a reaction to stimuli in the imagery script (Lang *et al.*, 1980, 1983). In addition to this HR acceleration, Watson and Marks (1971) and Marks and co-workers (1971) verified an acceleration of spontaneous SC fluctuations (SCF) correlated with the extent of reaction-describing material in imagery scripts. Hamm *et al.* (1992) showed increased HR along with enhanced ASR in spider/snake phobic Ss during presentation of short, 12-second scripts. Compared to control Ss and neutral scripts, this increase was even higher during a period of imagery following the script presentation. This study also involved presentation of different picture categories (e.g. phobic, neutral). No differences between perception and imagination were found.

Another important application of psychophysiology is the evaluation of treatment outcome. As the presentation of real spiders within the laboratory is relatively inexpensive, *in vivo* confrontation as well as Behaviour Avoidance Tests (BAT) have been quite thoroughly investigated. Öst (1996) compared the effects of small and large therapy groups in a one-session exposure treatment of spider phobia. Significant time effects, but no group differences were found in any variable. Self-rating anxiety, assessor's rating of anxiety severity and BAT performance as well as systolic BP (SBP), diastolic BP (DBP) and HR during the BAT were improved after the therapy. The improvements were maintained up to a 6-month follow-up.

Watts, Trezise and Sharrock (1986) compared treated and untreated spider phobic Ss in a modified Stroop Test (Stroop, 1938). In this reaction time paradigm, they presented spider-related and control words in different colours. The Ss' task was to name

the colour while ignoring the semantic word. Untreated Ss were severely retarded in naming the colour only for spider-related words (see also Mathews and MacLeod, 1985), while treated Ss were not. This effect probably is suppressed when measured directly before or during exposure treatment (Armir *et al.*, 1996). This paradigm allows the investigation of attentional aspects through psychophysiological methods. De Jong *et al.* (1992) additionally showed that the amount of covariation bias (Tomarken, Mineka and Cook, 1989), which is found in untreated spider phobic Ss, has decreased in treated Ss. The covariation bias seems to measure cognitive changes and may be used in outcome or relapse prediction (De Jong *et al.*, 1992). Another noteworthy predictor of treatment success is the initial HR response during perception of phobic pictures, as found by Lang (Lang, Bradley and Cuthbert, 1998a).

Blood/Injection/Injury Type Specific Phobia

Symptoms such as weakness, dizziness, and fainting or near-fainting while being confronted with phobic stimuli are more common in blood/injury phobia than in any other type of phobia. This very different type of reaction is also shown in psychophysiological measures. Enhanced sweat gland activity, measured by SC, was not found in blood/injury phobic Ss as compared to control Ss (e.g. Hamm *et al.*, 1997). Furthermore, no HR accelerations were found, as had been observed in other phobia types. However, after a short acceleration phase, strong HR decelerations were found, accompanied by a drop in mean BP (e.g. Hamm *et al.*, 1997). Blood/injury fearful subjects do not respond with a relative enhancement of sympathetic activation when exposed to their fear-relevant pictures, as do other phobic Ss. Interestingly, enhanced ASR was also found in blood/injury phobic Ss (e.g. Hamm *et al.*, 1997). Therefore, Hamm *et al.* (1997) concluded that the startle probe methodology provides important information for the clinical assessment and the evaluation of treatment outcome in phobias.

Situational Type Specific Phobia

We would like to present flight phobia as one prevalent and specific situational phobia. In the investigation of flight phobia, excellent ambulatory psychophysiological studies have been recently conducted by Wilhelm and Roth at Stanford University.

One study investigated the effect of benzodiazepine (alprazolam) on acute subjective and physiological responses during and following real flights in 28 flight phobic women (Wilhelm and Roth, 1997). Interestingly, alprazolam reduced self-reported anxiety and symptoms more than placebo, but induced an increase of HR and respiratory rate. In a second flight two weeks later, none of the Ss received alprazolam. However, subjects who had received alprazolam before the first flight indicated more anxiety, experienced panic attacks more often, and showed further increased HR during the second flight, compared to the placebo group. Wilhelm and Roth concluded that alprazolam increases physiological activation during acute stress situations and hinders therapeutic effects of exposure.

Another goal was to establish valid measures of HR response due to emotional activation. Therefore, the ability to distinguish whether HR increases are due to exercise or due to emotional response is necessary. This is especially important in ambulatory settings, where body movements or speech behaviour cannot be prevented. These sources of potential artefacts (e.g. walking, speaking) are essential to realize a natural setting. An early attempt to discriminate emotionally induced from exercise-induced HR increases involved an estimate of exercise-induced HR increases by measurement of oxygen consumption (Blix, Stromme and Ursin, 1974). By regression analysis, this source of variance was excluded and the resulting *additional* HR was attributed to emotional activation. Wilhelm and Roth (1998b) substituted the estimation of exercise due to oxygen consumption for practical reasons with a measurement of respiratory minute ventilation. Twenty-eight flight phobics and 15 control Ss were examined in different situations before and during a real flight. Previously, Ss completed different exercise tasks in the laboratory to obtain a function for the estimation of exercise-induced HR increases. The computed additional HR could be used to distinguish the phobic group from the control group during the walk to the airplane, which was not possible by examining HR or HR difference. During the flight, there were no differences, because exercise variation was negligible. One shortcoming of this technique is that anxiety-induced hyperventilation may distort the results. In another article, Wilhelm and Roth (1998a) described different physiological measures that were recorded in flight phobics and control Ss before, during and after a real flight. They were able to distinguish flight phobics from control Ss by measuring increased additional HR, more SC fluctuations, and less respiratory sinus arrhythmia during flight. By using the additional HR measure, they were able to classify 90% of flight phobics correctly. No differences between groups were found when respiratory rate or minute ventilation was recorded. For comparison, Sartory, Roth and Kopell (1992) also found encouraging results while investigating ambulatory assessment of driving phobia. They compared driving phobic Ss (e.g. avoidance of freeway drives for more than one year) with control Ss while performing different driving-related tasks within a BAT. Increased HR and respiratory minute volume were observed, but no differences in T-wave amplitude, respiratory sinus arrhythmia or respiratory rate were observed.

A new tool for the investigation and treatment of phobias is virtual reality. Virtual reality makes it possible to investigate states of phobic anxiety within a naturalistic, but highly controllable and repeatable setting. Mühlberger *et al.* (2001) compared the effect of repeated exposure to flights in virtual reality (VR) on flight phobics with effects resulting from relaxation training (RT). To test the effects, fear reports and physiological response were assessed during additional short virtual flights before and after the treatment. A decrease in subjective fear, SCL and HR reaction was found in both groups, but SCL and fear reports in the VR group were reduced compared to the RT group. Furthermore, a stronger initial HR response correlated highly with post-treatment fear of flying reduction at least up until a 3-month follow-up. Substantial correlations were only found in the VR group, and not in the RT group (Mühlberger, 1997). This may be in part due to the smaller changes within this group. However, it may also be the case that the predictive value of initial HR response is especially high in exposure-based treatments.

Social Phobia

An overview of earlier research on psychophysiology in social phobia is given by Öst (1989). Typically, giving a speech (e.g. in the paradigm of public speaking) or having a conversation with an unknown person (especially of opposite sex) is used as an anxiety-provoking stimulus in laboratory settings. Turner, Beidel and Larkin (1986) compared 26 socially anxious Ss and 26 non-socially anxious Ss with a clinical sample of 17 social phobics. During an impromptu speech, both the anxious and phobic group had higher SBP than the non-socially anxious Ss. DBP and HR did not differ between groups. In selected socially anxious and non-socially anxious students, Panayiotou and Vrana (1998) found no HR differences due to self-focus instructions while performing a digit rehearsal task, and an increased ASR only in socially anxious Ss. Lang (1993) compared speech fearful Ss with spider-fearful Ss during an impromptu speech *in vivo*

as well as *in sensu*. Increased HR was found in speech fearful Ss and also in spider-fearful Ss, with no difference between the two groups. McNeil *et al.* (1993) were able to verify these results in a clinical sample. They found an increase in HR during the imagination of a scene most fearful for the individual being tested, but observed no difference in HR increase compared to dental phobic Ss. In a second analysis, Ss were divided based on questionnaires into two subgroups: a fear group and an avoidance group. The fear group (14% assigned) showed a strong increase in HR correlating with their subjective fear, whereas the avoidance group (71% assigned) showed only a small increase. However, both groups subjectively stated experiencing strong anxiety and vivid imaginations. Turner and Beidel (1985) also found a high correlation between strong physiological reactions (BP) and subjective fear as major symptom after splitting their sample into high and low physiological responders. Similar results were described by Heimberg *et al.* (1990). They found stronger HR accelerations but less subjective fear in speech fearful Ss giving a talk or interacting socially than in Ss with a diagnosis of social phobia of the *generalized type* (Specifier of DSM-IV diagnosis). Levin *et al.* (1993) replicated these results by comparing specific social phobics and generalized social phobics. Hofmann *et al.* (1995) compared social phobics with and without avoidance personality structure and control Ss. Phobics without personality disorder reacted with a stronger HR increase than the other two groups, whereas phobics with additional personality disorder reacted with more subjective anxiety than the other two groups while presenting a public speech.

In treatment outcome studies, usually a decrease in physiological response, but no differences in treatment comparison tests were found. Jerremalm, Jansson and Öst (1986) divided 38 patients into physiological or cognitive reactors and assigned them either to applied relaxation (AR), self-instructional training (SIT), or waiting list (WL) group. They found a reduced HR reaction during a conversation test after AR and SIT compared to the WL group in physiological reactors, but no treatment group differences.

One major challenge in research involving social phobia or agoraphobia is the confusion of cognitive demand or motor exercise with emotional activation. One strategy for dealing with this difficulty is to disentangle these two sources of variance by manipulating the anxiety (e.g. Ss are observed by authorities or not) or the demand parameters (e.g. known or unknown speech topic (e.g. Erdmann and Baumann, 1996). Another strategy is to simply use the anticipatory anxiety, measuring the physiological states during expectation of giving an impromptu talk (e.g. Davidson *et al.*, 2000). Recently, Davidson *et al.* (2000) published an interesting article comparing the ANS responses and spontaneous EEG alpha power values of 18 social phobics with those of 10 control Ss. HR was elevated among phobics in most conditions. Furthermore, a marked increase in right-side activation of the anterior temporal and lateral prefrontal brain regions was found in social phobics. These EEG and HR measures explained more than 48% of the variance of the negative affect during speech anticipation. Like the results of Wilhelm and Roth, this indicates that when using physiological parameters within the correct paradigm, subjective anxiety can be predicted very well.

The results indicate that based on the phobic reaction, social phobia can be divided into two subgroups, simple phobia-like and generalized or agoraphobia-like. The two subgroups seem to reflect the different diagnoses in social phobia. One subgroup may reflect the simple diagnosis social phobia, the other one the diagnosis social phobia with specifier *generalized* (or even the additional diagnosis of a personality disorder). These promising results in distinguishing different types of social phobia might prompt a broader use of psychophysiology in further research of social phobia.

Agoraphobia

An overview of the earlier research on the psychophysiology in agoraphobia is given by Öst (1990). In some laboratory assessments, a general increased ANS activation without anxiety-eliciting stimulation could be found (Öst, 1990). This increase is accompanied by reduced habituation. Interestingly, the reactions to phobic stimuli or imagery tend to be less intensive than in specific phobia. Cook *et al.* (1988) compared the reaction of specific phobic, social phobic and agoraphobic (with PD) patients during imagery of phobic scripts describing their most feared scene. It was found that all three groups of patients rated their subjective fear-related cues as very arousing and highly unpleasant. However, when ANS responses were examined, simple phobics showed the largest and agoraphobics the smallest overall reactivity (HR and SCL changes). Specific phobics also showed a strong correlation between subjective anxiety and physiological reactions, whereas agoraphobic Ss did not. Cuthbert *et al.* (1994) replicated these results and found in addition a general increased level of ASR reactions during phobic and neutral imagery as well as between imageries, but the modulation of the ASR by emotional content as in social or specific phobics was not found (see Figure XIX-7.1, adapted by permission of the author). The presentation of external stimuli led to the same results as *in vivo* exposure (e.g. Fisher and Wilson, 1985). Unfortunately, like in social phobia, *in vivo* exposure or ambulatory assessments are often confounded by motor exercise. For instance, having a walk is a common paradigm in the assessment of agoraphobia. As discussed above, it is difficult to disentangle the different sources of physiological changes. It is not possible to state whether the HR acceleration during *in vivo* exposition (e.g. having a walk) commonly found not only in agoraphobics but also in control Ss is due to artefacts or the lack of differences between these groups. Therefore, the use of different physiological measurements (e.g. including respiratory) and methodological improvements in ambulatory measurements (see e.g. Wilhelm and Roth, 1998b) is strongly recommended in the assessment of agoraphobia.

In treatment studies, an overall HR reduction during different tasks was found (see Öst, 1990). Öst, Jerremalm and Jansson (1984) found a reduction of HR reactivity through both exposure and relaxation training. Bonn, Readhead and Timmons (1984) found (upon follow-up measurement) *in vivo* exposition combined with breathing retraining to be superior to *in vivo* exposition alone. Michelson *et al.* (1990) compared paradoxical intervention (PI), graduated exposure (GE) and relaxation training (RT). Only RT and

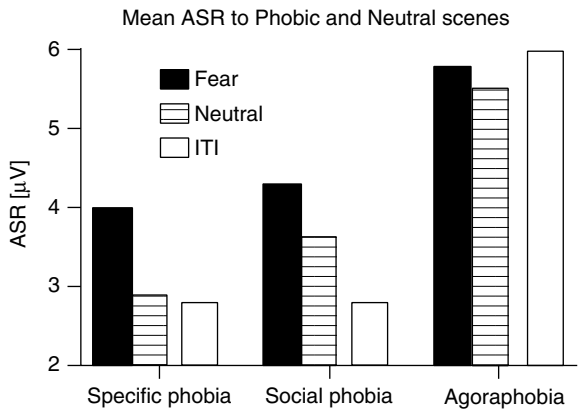

Figure XIX-7.1 Average acoustic startle responses (ASR) during the imagery of phobic or neutral scenes and during the Inter-Stimulus-Interval (ITI) for patients of these phobias. Adapted by permission from Cuthbert *et al.*, © 1994 by B. Cuthbert

GE led to substantial reductions in HR reactivity. No correlation between pre-treatment physiological reactivity and therapy outcome was observed. Additionally, the authors categorized persons with improvements across all three systems of phobic reactions (physiology, behaviour, self-report; see e.g. Lang, 1968) as synchronizers. Ss that exhibited discrepant patterns of improvement and worsening were classified as desynchronizers. Treatment outcome of synchronizers (e.g. Michelson *et al.*, 1990) was found to be superior to that of desynchronizers (e.g. Michelson *et al.*, 1990) in all but the physiological (HR) measures. In a comparison of behavioural and pharmacological (imipramine) treatment, Michelson, Mavissakalian and Marchione (1985) observed substantial decreases in HR through the behavioural treatment. Conversely, HR increases in imipramine-treated Ss were found.

Interestingly, in some investigations a substantial correlation of pre-treatment HR and post-treatment as well as follow-up treatment response was found (e.g. Craske, Sanderson and Barlow, 1987; Roth *et al.*, 1988), but also contrary results were reported (see Michelson *et al.*, 1990).

To summarize, strong evidence has been found that psychophysiological measures in agoraphobia are important. Nevertheless, there is still a dearth of investigations using psychophysiological measures in assessment or outcome prediction.

Direct Comparison of Phobias

Some studies were able to show in direct comparisons that different phobias are related to different physiological reactivity (Cook *et al.*, 1988; Cuthbert *et al.* 1994; Lang, Bradley and Cuthbert, 1998a). *Specific phobia* is generally characterized by a marked sympathetic activation and potentiated protective reflexes (e.g. ASR) during presentation or imagination of phobic stimuli. This was attributed to the activation of a DR (Lang, Bradley and Cuthbert, 1997), which protects against potentially dangerous stimuli. The phobic reactions were very reliable and accompanied by strong avoidance tendencies. However, no abnormal signs have been found in baseline reactions. Also, in *social phobia*, no abnormal ANS or ASR baseline reactivity was found (Lang, Bradley and Cuthbert, 1998a). Reactions upon phobic cues were somehow less salient than in simple phobia. In *agoraphobia*, more baseline ANS activation and enhanced ASR accompanied by smaller reactions during the presentation or the imagery of phobic stimuli was discovered (see Figure XIX-7.1, adapted by permission of the author) (Lang, Bradley and Cuthbert, 1998a).

Recent findings in animal research led to the conclusion that different neurophysiological pathways are involved in fear behaviour under different paradigms. It was assumed that fear can be divided into cue-explicit fear and more generalized context-related fear (Lang, Bradley and Cuthbert, 1998a). This implies that object-related phobias (e.g. snakes, spiders phobia) differ fundamentally from more general phobias (e.g. agoraphobia). They differ not only in a quantitative matter, i.e. in amount of subjective, physiological and behavioural reactions, but also in a qualitative matter, in the underlying mechanisms of fear processing. This would imply that very different therapy strategies may be effective for different types of anxiety disorders.

PANIC DISORDER WITH OR WITHOUT AGORAPHOBIA

Discrepant Findings in Resting Heart Rate as an Example for Different Explanations

Many studies of panic disorder (PD) have found higher resting heart rates (HR) among patients in comparison to healthy control participants (Freedman *et al.*, 1984; Liebowitz *et al.*, 1985; Ehlers *et al.*, 1986; Castellani *et al.*, 1988; Aronson *et al.*, 1989; Nutt, 1989; Uhde *et al.*, 1989; Yeragani *et al.*, 1990; Hoehn-Saric, McLeod and Zimmerli, 1991; Stein, Tancer and Uhde, 1992). However, several other authors did not find such differences (Charney, Heninger and Breier, 1984; Cowley *et al.*, 1987; Villacres *et al.*, 1987; Gaffney *et al.*, 1988; Stein and Uhde, 1991; Stein, Tancer and Uhde, 1992; Gurguis, Vitton and Uhde, 1997). Some authors claim underlying physiological differences are responsible (Stein and Uhde, 1998; Ballenger, 1990), others suggest that these discrepant findings may result from anticipatory anxiety in response to the experimental environment (Margraf, Ehlers and Roth, 1986; McNally, 1994). Ambulatory monitoring studies have found that the resting HR of patients with PD did not differ from those of healthy control individuals (Freedman *et al.*, 1985; Cameron *et al.*, 1987; Taylor *et al.*, 1987; Pauli *et al.*, 1991). Therefore, Larson *et al.* (1998) compared the resting HR of patients with PD, social phobia, and healthy control participants during two consecutive laboratory sessions. The authors found no significant differences in resting HR between groups on either day. Patients with PD had significantly higher mean resting HR during their first laboratory session, which may be due to elevated anticipatory anxiety in these patients. This study highlights a potential confounding factor in many studies. It suggests that anticipatory anxiety and its effect on baseline physiological reactivity in patients with PD may be reduced by pre-exposing them to the experimental environment.

Theoretical Models for the Development of Panic Disorder and Psychophysiological Response Patterns

A uniform theoretical model for the development of panic disorder and/or phobia has not yet been established, but according to research thus far, two important theories have emerged: a learning-theory based conditioning model and a cognitive model. Classic conditioning processes and dysfunctional cognitions are thought to be vulnerability factors for the development of panic disorders with or without agoraphobia (Beck, Emery and Greenberg, 1985; Ehlers *et al.*, 1986; Clark, 1988; Carr, Lehrer and Hochron, 1992).

Mowrer (1939) developed a two-factor theory. Firstly, common and naturally triggering factors for an anxiety response become linked to neutral stimuli by spatial and temporal association. Thus, these neutral situations are able to set into action an anxiety reaction without necessitating the presence of a 'natural' trigger (classical conditioning). Secondly, avoidance behaviour is developed, which prevents the learned anxiety reaction from becoming habituated. According to operant conditioning models, this avoidance behaviour acts as negative reinforcement and thus maintains the pathological anxiety response. In fact, retrospective assessments of agoraphobia cases point to conditioning experiences as triggering factors (Öst and Hugdahl, 1983).

Ehlers *et al.* (1986) were able to show that patients with a panic disorder exhibit altered perception of their normal interoceptive stimuli, such as heart beat. This selective focusing on bodily reactions did not correspond to a real improvement in their ability to appraise such changes (Rapee *et al.*, 1992), but this readiness to appraise physical experiences as anxiety-provoking events leads to fears which themselves provoke psychophysiological changes, such as even further accelerated heart beat or hyperventilation. These observations again confirm the original fear and act as reinforcement. Thus, a vicious circle has been established, which ultimately leads to a panic attack. These theories are based on studies examining psychophysiological response patterns in patients with panic disorders compared to healthy, depressive, or somatically ill control participants (e.g. Hoehn-Saric, McLeod and Zimmerli, 1991; Yeragani *et al.*, 1993; Klein *et al.*, 1995).

Respiratory Psychophysiology and its Explanatory Capacity in Panic Disorder

In the context of respiratory psychophysiology, Ley outlined a dyspnoea/suffocation-fear theory of panic which later led to a tripartite categorization of panic attacks (Hibbert and Pilsbury, 1988; Ley, 1989; Sanderson, Rapee and Barlow, 1989; Craske and Barlow, 1990; Ley, 1994a, 1994b). In contrast, Klein's false suffocation alarm theory hypothesizes a deranged suffocation monitor, which is assumed to trigger a hypothetical suffocation alarm (Klein, 1993; Taylor and Rachman, 1994; McNally, Hornig and Donnell, 1995; Stein *et al.*, 1995). This hypothesis relies on the physiological mechanism whereby potential suffocation is detected by increasing P_{CO_2} and brain lactate.

In his critique of Klein's theory, Ley emphasizes the distinction between dyspnoea, the sensation of difficulties in breathing, and suffocation, the condition that may give rise to dyspnoea (Ley, 1998).

Asmundson and Stein (1994) found an association between a measure of pulmonary function, the forced expiratory flow rate, and the severity of panic symptoms in patients with PD. Thus, they provided relatively strong support for the dyspnoea/suffocation-fear theory, stating that uncontrollable dyspnoea is the source of panic fear. On the contrary, Spinhoven, Onstein and Sterk (1995) did not succeed in replicating these findings; however, this study was criticized by Ley as containing flawed methodology and statistical anomalies. Although the significant findings of Asmundson and coworkers might be subject to type I error, the study of Spinhoven and co-workers cannot be seen as proof for this.

Papp *et al.* (1997) challenged patients with PD and healthy individuals with 5% and 7% CO_2 inhalation and hyperventilation of room air. Patients were more sensitive to the anxiogenic effects of CO_2 compared to healthy participants. Furthermore, CO_2 was a more potent panic stimulus than hyperventilation. The authors identified several respiratory abnormalities, and concluded that patients with PD are behaviourally and physiologically more sensitive to CO_2 inhalation. Panic attacks are explained by inefficient compensatory mechanisms of respiratory rate.

In summation, many studies have shown that patients with PD have a greater propensity to experience increased anxiety and more panic attacks during inhalation of CO_2 compared to healthy participants (Griez *et al.*, 1987; Sanderson and Wetzler, 1990; Perna *et al.*, 1994; Gorman *et al.*, 1994, 1997). In patients with PD, rates of panic during CO_2 inhalation decrease after successful treatment of the disorder (Sanderson, Wetzler and Asnis, 1994; Perna *et al.*, 1997; Bocola *et al.*, 1998). Some authors even claim that CO_2-induced panic is specific to patients with PD (Griez *et al.*, 1990; Perna *et al.*, 1995a, 1995b), but others could show that other patients are also susceptible, e.g. in social phobia (Caldirola *et al.*, 1997) or premenstrual dysphoric disorder (Harrison *et al.*, 1989).

CO_2 sensitivity is increased even in clinically asymptomatic, first-degree relatives of patients with PD (Perna *et al.*, 1995c; Coryell and Arndt, 1999). This could represent a specific abnormality in the afferent neural pathways that respond to increased levels of CO_2 (Klein, 1993). Others argue that the non-specific somatic distress produced by CO_2 inhalation triggers the panic attack (Roth *et al.*, 1992), but studies that addressed this controversy led to conflicting results (Gorman *et al.*, 1988; Pain, Biddle and Tilter, 1988; Papp *et al.*, 1995, 1997). As a result, the mechanism of action is still a matter of intense debate.

Therefore, Gorman *et al.* (2001) investigated differences in respiratory response to CO_2 breathing by comparing patients with PD, healthy control participants, patients with major depression (MD), and patients with premenstrual dysphoric disorder (PMDD). Both latter groups had no co-morbidity with panic disorder. Patients with MD generally do not respond to panic-producing agents such as CO_2, whereas patients with PMDD do.

In sum, patients with PD exhibit higher rates of panic attacks during CO_2 inhalation than other groups, but have no fundamental abnormality in their ventilatory physiology. The exaggerated respiratory responses are more a function if a panic attack occurs than the diagnostic group. The panic response to CO_2 involves a generalized fear response that is not specific to PD. These results are compatible with preclinical fear conditioning models.

These CO_2 studies have pointed the way for neuroimaging studies of cortical and subcortical sites.

Is Regional Brain Asymmetry Associated with (Different) Anxiety Disorders?

Evidence has accumulated from non-psychiatric and psychiatric populations, that individual differences in electroencephalogram (EEG) resting frontal brain asymmetry are associated with differences in basic dimensions of emotion, i.e. in dispositional mood and temperament (see Davidson, 1992; for review, Schaffer, Davidson and Saron, 1983; Henriques and Davidson, 1990; Tomarken *et al.*, 1990; Henriques and Davidson, 1991; Wheeler, Davidson and Tomarken, 1993; Heller, Etienne and Miller, 1995). In particular, increased relative activation of right hemisphere anterior regions is linked to negative affects, such as depressive or sad emotions. In contrast, relatively higher activation of the left frontal area is associated with positive affects, i.e. cheerful emotions. Davidson (1992) has conceptualized this frontal brain asymmetry as reflecting specialized systems in the right hemisphere for withdrawal behaviour coupled with negative emotions and in the left frontal region for approach behaviour associated with positive affects. Much of the data supporting this relationship comes from studies measuring EEG alpha activity during different emotional states (Davidson *et al.*, 1990; Tomarken *et al.*, 1992).

Hemisphere Asymmetry and General Negative Emotional Dispositions

Davidson found that sub-clinically as well as clinically depressed individuals have significantly higher right frontal hemispheric activation than non-depressed subjects (Schaffer, Davidson and Saron, 1983; Henriques and Davidson, 1991, 1992). This difference did not depend on the acute phase of the disorder. Also, remitted depressives that were upon examination normothymic showed increased right frontal activation (Henriques and Davidson, 1990). Thus, this seems to be a trait marker of individuals reacting with negative affect.

Hemisphere Asymmetry and Negative Emotional States

However, individuals differ dramatically in their affective responses to emotional provocations, as they do in their propensity to approach or withdraw from unfamiliar situations. These differences in action have been linked with corresponding differences in emotional reactivity. Therefore, in response to elicitors of dimensions of positive emotion, there should be more intense positive affect and less intense negative affect in subjects with strong left-sided frontal activation. The opposite pattern of reactivity should occur in individuals with stronger right frontal activation. So, increased right frontal hemisphere activation is not only a trait marker for depressives, but also a state marker in conditions predominated by negative affect.

Laterality Findings in Anxiety Disorders

Tucker and co-workers found that anxiety-producing conditions in healthy volunteers were linked to significantly more left than right lateral eye movements (Tucker *et al.*, 1977) and resulted specifically

in decreased performance in the right visual field (Tucker *et al.*, 1978). Tucker *et al.* (1978) also showed that high trait-anxious subjects reported tones at the right ear as significantly louder compared to low trait-anxious people with the same tone in quantity and quality (Tucker *et al.*, 1978). Moreover, high trait-anxious individuals showed reduced left lateral eye movements. So, Tucker and co-workers argue that anxiety is associated with greater left-hemisphere activation. Therefore, left-hemisphere tasks are more poorly performed, as there is a concomitant processing overload. In contrast, further studies with imaging techniques during anxious states revealed more right than left activation in parietotemporal regions (Buchsbaum and Wu, 1987; Naveteur *et al.*, 1992), while others found no asymmetry (Fredrikson *et al.*, 1993). One EEG study even showed increased left brain activation (Carter, Johnson and Borkovec, 1986).

These patterns of hemisphere activation therefore also vary as a function of anxious mood. The findings, however, have been inconsistent (Heller, Etienne and Miller, 1995), and this variability has made generalizations difficult. According to reviews by Heller (1990, 1993), this could be due to the possibility that anxiety and depression may be linked to opposite patterns of activation in the cerebral hemispheres. Depressive mood might be associated with right-hemisphere activation and anxiety with left-hemisphere activation. In populations with much co-morbidity of anxiety and depression, these patterns might suppress each other. Such opposing tendencies could account for the variable patterns in different studies, depending on the ratio of these two emotional states in the particular sample. Because studies of anxiety-depressive conditions and depression studies do not account for anxiety, this might have led to confounded effects. More so, because the co-morbidity of depression and anxiety is high: 40% to 80% of patients with panic disorder have had an episode of major depression (Katon and Roy-Byrne, 1991). However, the overlap between symptoms of anxiety and depression decreases, as the severity of symptoms increases (Hiller, Zaudig and von Bose, 1989).

Heller and co-workers (1995) classified more than 1000 subjects as either high- or low-depressive and high- or low-anxious, and gave a face-processing task that elicits left hemispatial bias (chimeric faces task, CFT). High-depressed probands showed smaller left hemispatial biases than low-depressed ones (corresponding to lower right hemisphere activation), whereas high-anxious subjects had larger left hemispatial biases than low-anxious individuals (corresponding to higher right hemisphere activation). As there was no interaction effect, the number of depressive symptoms cannot be made responsible for the right or left hemispheric asymmetry of anxiety.

Consequently, Heller and co-workers (1995) hypothesize that different kinds of anxiety might be associated with different patterns of hemispheric asymmetry. Left-hemisphere functions may be linked to experiences of worry involving verbal rumination, i.e. cognitive and anticipatory anxiety (Öhman, 1993), whereas right hemisphere activation may be associated with fear or panic responses, i.e. more autonomic and physiological changes (Spence, Shapiro and Zaidel, 1996). These subtypings have been based primarily on behavioural data from lateralized tasks and on lateralized EEG findings. Beyond Heller and colleagues, this distinction has not yet been systematically assessed.

Right frontal brain activation seems to represent an activation of an avoidance–withdrawal system and appears to be associated with negative emotions. Since panic patients are characterized by negative emotions and by avoidance behaviour, this hemispheric asymmetry might be expected. Therefore, Wiedemann *et al.* (1999) compared the spontaneous EEG of 23 panic patients and 25 healthy control subjects during resting phases and while being confronted with neutral, panic-relevant, anxiety-relevant but panic-irrelevant, or anxiety-irrelevant but emotionally relevant stimuli, or when performing a motor task. This study showed that in

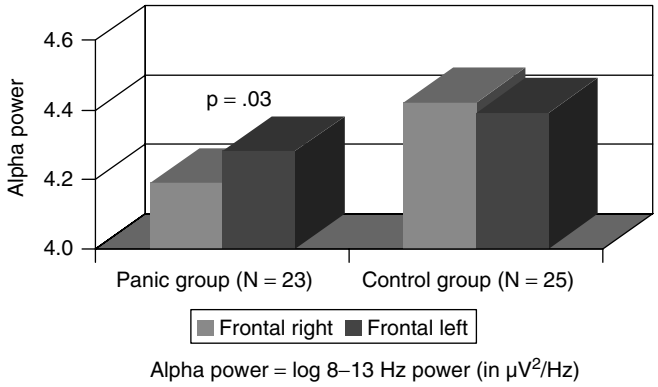

Figure XIX-7.2 Frontal alpha power during resting phases in patients with panic disorder. Adapted by permission

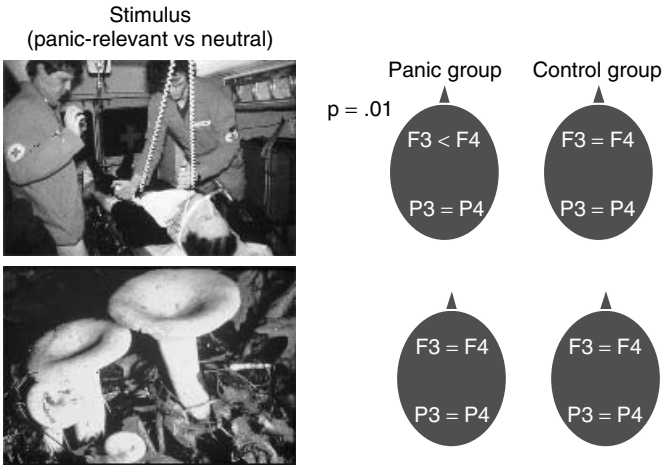

Figure XIX-7.3 Frontal and parietal hemispheric activation in patients with panic disorder when being confronted with panic-relevant and neutral stimuli. Adapted by permission

the spontaneous electroencephalogram of panic patients there are asymmetries in frontal hemispheric activation during resting phases (see Figure XIX-7.2, adapted by permission of the author) and when being confronted with anxiety-relevant stimuli (see Figure XIX-7.3, adapted by permission of the author).

The right frontal alpha power was significantly decreased in these patients compared to that of the left side, while control subjects did not show a frontal asymmetry during these phases.

There was no frontal asymmetry when patients observed an emotionally neutral picture or while performing a motor task. Thus, panic patients were characterized by greater activation of a right frontal avoidance–withdrawal system in negatively valenced situations.

Studies that investigate both central and autonomic measures are warranted to further develop the theory of distinct, psychophysiologically defined subtypes of anxiety disorders.

GENERALIZED ANXIETY DISORDER

In generalized anxiety disorder (GAD), persistent, uncontrollable worry represents the central defining feature. This state of apprehension is maintained by defensive attentional mechanisms such

as pre-attentive bias for threat information and poor habituation to novel stimuli (Mathews, 1990). The persistent cognitive anxiety of GAD may be based on an adaptive coping strategy gone awry. Thayer *et al.* (2000) tried to bridge the attentional and physiological underpinnings of GAD by examining phasic heart period responses to cued threat and non-threat word stimuli in an S1–S2 procedure. The GAD group showed HR acceleration in response to threat stimuli, and a conditioned anticipatory HR deceleration to threat stimuli over repeated trials, in comparison to healthy control participants. Additionally, the GAD group showed smaller and impaired habituation of cardiac orienting responses to neutral stimuli. These findings are interpreted as a diminished global adaptive variability in GAD patients.

Although it is reasonable to assume that patients with GAD exhibit physiological hyperarousal at rest and heightened responses to stressors, this is not the case. Patients with GAD show increased muscle tension (Hoehn-Saric, McLeod and Zimmerli, 1989; Hazlett, McLeod and Hoehn, 1994), but not autonomic hyperarousal. Interestingly, GAD patients under stress show less efficient and more rigid but not higher autonomic responses compared to healthy individuals (Hoehn-Saric, 1998). This physiological rigidity may result from a physiological adaptation to chronic arousal, or represent a constitutional predisposition. On the other hand, a patient with GAD may not be aware of stressful situations that are not incorporated into his pathological preoccupations. Furthermore, the relationship between physiological changes and their perception is weak. This inconsistency may be due to alterations of body sensations by psychological factors. Excessive attention to and expectation of body states may exaggerate body sensations on the one hand, but may on a long-term basis (in the long run) lead to a perceptual distortion and to a disregard of the body states.

POST-TRAUMATIC STRESS DISORDER (PTSD)

According to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) symptoms of PTSD include irritability, hypervigilance, exaggerated startle response, and intense physiological reactivity upon re-exposure to events that symbolize or resemble an aspect of the traumatic event (American Psychiatric Association, 1994). In a series of studies, heart rate (HR), blood pressure (BP), skin conductance (SC), and electromyogram (EMG) have been used as outcome measures. The paradigms differed in the nature of the stimulus used to elicit physiological responses: external stimuli reminiscent of the trauma, internal (mental) imagery of the trauma, and intense but neutral stimulations, e.g. auditory startle stimulus (Shalev, Orr and Pitman, 1993a). Following this subdivision, the corresponding studies will be described.

Psychophysiological Responses to External Stimuli

World War II veterans with ($n = 8$) and without ($n = 13$) decompensated combat neurosis and student control individuals ($n = 10$) were exposed to a tape of artillery barrage, small arms fire, and aerial bombardment sounds (Dobbs and Wilson, 1960). While only one of the student control subjects showed a moderate or marked increase in pulse rate, eight of the healthy veterans did so, and five of the decompensated veterans even asked that the tape be turned off. Vietnam veterans with PTSD and non-veteran control individuals underwent mental arithmetic and combat sounds from an audiotape (Blanchard *et al.*, 1982). The mental arithmetic aroused both groups (heart rate, electromyogram, and blood pressure responses), whereas the combat sounds did so only in the PTSD group. However, this study did not differentiate the effect of combat from that of PTSD, as there was no control group with combat exposure.

Therefore, Malloy, Fairbank and Keane (1983) compared the physiological responses of Vietnam veterans with PTSD, psychiatric inpatients, and veterans without PTSD. The audio-visual presentation of combat sounds resulted in significantly greater heart rate and skin conductance responses to combat scenes in the group with PTSD only.

The methodology was further refined by comparing Vietnam veterans with PTSD, veterans with other psychiatric disorders, veterans with combat experience, Vietnam-era veterans, and non-veterans patients with anxiety disorders (Pallmeyer, Blanchard and Kolb, 1986). Physiological responses (HR, BP, SC) were compared during rest, mental arithmetic, and combat sounds. Larger HR responses to combat sounds were found in the PTSD group compared to all other groups. McFall *et al.* (1990) used additional outcome measures by comparing the group's (Vietnam veterans with and without PTSD) HR, BP, subjective responses, and plasma epinephrine upon viewing of combat and non-combat stress films. Greater increases in all outcome measures were found in the PTSD group during the combat films.

Blanchard *et al.* (1991) tested the reliability of the psychophysiological measures. They compared HR and BP responses to combat sounds in two large samples ($n = 104$ and 96, respectively) of Vietnam veterans with and without PTSD. The cumulative difference in HR and BP response to combat sounds correctly classified 80% in the first and 83% in the second sample of testees as individuals with or without PTSD.

These studies resulted in a neuropsychological hypothesis explaining PTSD (Kolb, 1987). The disorder was defined as a conditioned emotional response to overwhelming stressful exposure. Excessive stimulation was thought to result in permanent neuronal changes. These might lead to impairment of learning, habituation, and stimulus discrimination. Thus, the disorder results from strong associative learning. Originally, individuals react to a traumatic event (unconditioned stimulus, UCS) with arousal and fear (unconditioned response, UCR). Associative learning implies that finally, individuals show the same response to external cues associated with the stressful experience. By then, these cues have become conditioned stimuli (CS). The physiological response generated by these cues represents the equivalent of a conditioned response (CR).

However, combat is only one condition capable of provoking PTSD. Rape, motor vehicle accidents, torture, natural disasters etc., are other conditions considered to be potential stimuli.

Psychophysiological Responses to Mental Imagery

Brende (1982) found that hypnotically induced imagery of past traumatic events in PTSD patients was associated with increased electrodermal response of the left side of the body. According to Brende, this lateralization might be explained by the preferential processing of images by the right brain hemisphere.

Pitman hypothesized that in PTSD, there is a pathological emotional network, which can be provoked by mental imagery, resulting in physiological and other responses (Pitman, 1988). Lang and co-workers had shown that emotionally loaded imagery correlates with HR, SC, and EMG responses in general (Lang *et al.*, 1983). According to their theory, emotional memories are stored as an associative network. These networks consist of memory traces of the sensory input, the meaning derived from this input, and the physiological response.

Following this theoretical approach, Pitman and co-workers conducted a series of studies. Vietnam veterans with and without PTSD listened to recorded scripts describing traumatic events. Some of these were standardized, others were based on the

individual's own experience. Imagery of the personal events resulted in strong HR, SC, and EMG responses in PTSD patients only (Pitman *et al.*, 1987). In a further study, Pitman and co-workers compared Vietnam veterans with PTSD and Vietnam veterans with other anxiety disorders (Pitman *et al.*, 1990). The response to combat imagery was specific to PTSD. Shalev and co-workers assessed the psychophysiological response to traumatic imagery in Israeli trauma survivors. These subjects were of both sexes with PTSD of different origin, i.e. rape, road accidents, terrorist acts (Shalev and Rogel-Fuchs, 1993b). In subjects whose disorder was the result of non-military events, increases in HR and EMG in response to traumatic imagery were observed compared with the response of survivors without PTSD, the same trend seen in the studies of Pitman *et al.*

Response to mental imagery was also used as a measure of treatment outcome. Keane and Kaloupek (1982) treated a Vietnam veteran suffering from PTSD with imaginal flooding. The heart rate response to imaginal scenes of combat memories was reduced after therapy. Similarly, Fairbank and Keane (1982) showed that imaginal flooding reduced heart rate and skin conductance response to combat-related imaginal scenes in PTSD patients. Boudewyns and Heyr (1990) compared direct therapeutic exposure and counselling in Vietnam veterans with PTSD. Regardless of treatment protocol, clinical improvement as an outcome measure is correlated with decreases in physiological responses to combat imagery. However, Shalev *et al.* (1992) also showed that such reductions might not generalize to recollections of other traumatic events, although their treatment of desensitization of the specific trauma resulted in a decrease of HR, SC, and EMG responses to that trauma.

In a further study, Shalev (1997) explored the physiological responses of PTSD patients to reminders of a significant stressor that had preceded the onset of the disorder and was not related to its cause. They compared outpatients with PTSD, survivors of traumatic events who had not developed PTSD, panic disorder patients, and mentally healthy individuals with no history of trauma. PTSD individuals showed higher SC and EMG responses than the other groups. The authors concluded that PTSD patients may acquire and maintain prolonged conditioned responses to various stressors during their lifetime or become sensitized to reminders of past traumata following the onset of their illness. Heightened susceptibility to acquire conditioned responses or an inability to extinguish such responses may be expressed before the trauma in individuals who are liable to develop PTSD.

In a recent study, Orr *et al.* (2000) found evidence that individuals with PTSD show greater conditionability than those without. This stronger conditionability could reflect pre-trauma vulnerability to developing PTSD.

Keane *et al.* (1998) were able to show that more severe PTSD symptomatology is associated with greater physiological responsivity to trauma-related cues.

Blanchard *et al.* (1996) found that higher physiological responsivity to imagery one-to-four months after a motor vehicle accident predicted the persistence of chronic PTSD after one year.

In a recent study, Orr *et al.* (1998) showed that PTSD and non-PTSD groups do not differ in the magnitude of their physiological responsivity to standard stressors (mental arithmetic, postural challenge).

The consistency of the results across various studies suggests that the method of using responses to mental imagery represents a good measurement of the traumatic experiences. Mental representations of the trauma may constitute a mental network of recollections, verbal representations and images. These may be able to reinforce learning and conditioning even in the absence of external reinforcement. Thus, cogitations and nightmares of recollections may result in endless sequences of self-induced reinforcement of the conditioned emotional response.

Psychophysiological Responses to Intense but Neutral Stimulation (Auditory Startle)

The acoustic startle response (ASR) consists of a characteristic sequence of muscular and autonomic responses to sudden, intense stimuli. It is measured as the magnitude of the eyeblink reflexes.

The ASR is modified by non-startling effects that precede the startling stimulus. Such warning stimuli may facilitate or inhibit the magnitude of the ASR. If such a stimulus had previously been paired with a shock, the presence of such a cue augmented the amplitude of the ASR. Conditioning of this type is called fear-potentiated startle effect.

Affective valence and arousal can be induced by background information. Lang and co-workers (1990) studied the extent to which this modulates the acoustic startle response. They found that the ASR is associated with affective valence, whereas the skin conductance response is reflective of arousal.

Increased ASR is one of the cardinal features of PTSD.

Ornitz and Guthrie (1989) found impaired pre-pulse modulation, i.e. a significant loss of normal inhibitory modulation of the ASR by warning stimuli, in children with PTSD compared to age-matched, healthy control children.

In a group of Vietnam veterans with combat-related PTSD, Butler *et al.* (1990) found a higher eyeblink EMG response to an acoustic stimulus of intermediate intensity compared to a group of Vietnam veterans without PTSD. Paige *et al.* (1990) found larger HR responses to ASR in Vietnam veteran PTSD patients. Ross *et al.* (1989), however, found no difference in habituation of the ASR to a series of 100 dB tones between PTSD patients and healthy volunteers.

A study by Morgan *et al.* (1995) showed that infusion of yohimbine, which stimulates noradrenaline transmission in the brain, facilitated the acoustic startle response in combat veterans with PTSD. Another study by Grillon *et al.* (1997) found that darkness facilitated the acoustic startle reflex in combat veterans both with and without PTSD in comparison to non-combat control individuals. There seem to be important influences of contextual fear on the startle response.

Shalev *et al.* (1992) compared autonomic (ANS)- and central nervous system (CNS)-mediated responses to ASR in Israeli PTSD patients, patients with other anxiety disorders, healthy individuals with past traumatic experiences, and healthy individuals having experienced no traumatic event. They found a larger HR and SC response, and an impaired habituation of the SC response to the consecutive presentation of auditory stimuli in the PTSD patients compared to all other groups.

In a recent study, Shalev *et al.* (2000) showed that patients showed differential effects after a psychologically traumatic event. Patients who developed PTSD showed increased HR responses and slower habituation of SC responses to startling stimuli one and four months after the trauma. However, these responses were not seen one week after the trauma. These results suggest a progressive sensitization of the autonomic response to startling stimuli. These studies of ASR in PTSD have some characteristics which differentiate them from the previously mentioned studies. Firstly, the startle reflex represents an elementary stimulus, which is not associated with the traumatic experience and for which previous conditioning is unlikely. Secondly, ASR studies do not require deliberate mental activity by the individual. Thus, confounding by (un)conscious manipulations is less likely. Thirdly, it reflects impairments in structures such as the amygdala and other mesocortical areas, which are responsible for evaluation of the stimulus, memory, and arousal. Therefore, these studies suggest that in PTSD, there is an alteration of the responsiveness of the central nervous system to elementary stimuli. In addition, the capacity to effectively appraise intensive but redundant stimuli and to regulate the arousal response seems to be impaired, as habituation of the SC response is slow.

Summary

The studies using external stimuli support the associative learning paradigm, studies using the imagery technique extend the conditioning paradigm to mental representations of traumatic experiences, and studies of intense but neutral stimulations (ASR) show the abnormal responsiveness to elementary stimuli in PTSD patients.

Event-Related Electroencephalographic Potentials (ERPs)

The latency and amplitude of ERP components index specific aspects of stimulus and information processing. In contrast to responses by the autonomic nervous system, which occur within seconds, those of the central nervous system occur within milliseconds. The studies of Paige *et al.* (1990) and Lewine *et al.* (1997) showed an augmentation–reduction of P2 responses. Increases in acoustic stimulus intensity are normally directly correlated to increasing P2 amplitude responses. In contrast to such an augmenting response pattern, PTSD combat veterans showed a decreasing response pattern, i.e. decreasing P2 amplitudes. Such a pattern reflects a protectively tuned sensory system that dampens increased stimulation. Similar to findings in other diseases (schizophrenia, cocaine abusers), combat veterans with PTSD exhibited less habituation of the P50 potential to the second click in comparison to control individuals (Gillette *et al.*, 1997). This auditory P50 habituation was inversely correlated with re-experiencing symptoms within the PTSD group.

In the auditory oddball paradigm, PTSD patients were exposed to a series of high-frequency non-target tones, low-frequency distractor tones, and low-frequency target tones. PTSD patients showed longer reaction times, delayed N2, and attenuated P3 amplitudes to target and distractor stimuli (McFarlane *et al.*, 1993; Charles *et al.*, 1995; Metzger *et al.*, 1997a, 1997b). It is unclear whether this response abnormality is secondary to a motivational deficit or reflects a higher order cognitive impairment.

In summation, ERP findings provide evidence for increased cortical inhibition in response to high-intensity stimuli, auditory gating deficits, impairments in memory and concentration, and increased selective attention to trauma-related stimuli.

This psychophysiological research into PTSD supports the validity of the diagnostic entity, but more work needs to be done to test the robustness of the various results. Some of the stimulus presentation techniques are now being incorporated into neuroimaging symptom provocation studies. These are described elsewhere in this book.

OBSESSIVE–COMPULSIVE DISORDER (OCD)

Two loosely related goals have been pursued in the research of OCD. Firstly, the search for the biological basis of OCD led to an intense investigation of the EEG properties of OCD sufferers. Secondly, ANS measures were used in the assessment of OCD and in the treatment evaluation. An overview of the earlier research on psychophysiology in OCD is given by Sartory (1989).

EEG Studies

In OCD patients, some abnormalities in sleep EEG have been found. OCD patients have longer Stage 1 sleep and reduced Stage 3 and 4 sleep, which is indicated by the amount of delta activity (review by Sartory, 1989). However, these findings are not disorder-specific and have been found in depression and schizophrenia as well.

Furthermore, awake resting EEG measurements are variable and conflicting (Sartory, 1989; Towey *et al.*, 1994; Simpson *et al.*, 2000). One possible reason is that different subgroups of OCD patients exist. All show the symptoms of OCD, but perhaps differ in psychophysiological measures. Prichep *et al.* (1993) assumed two distinct groups of OCD patients. One group of patients is thought to be characterized by an excess in relative theta power and a second group by increased relative alpha power. By dividing 27 patients into two groups according to this classification, the response to treatment with serotonin reuptake inhibitors (fluvoxamine, fluoxetine, or clomipramine) could be predicted. Eighty percent of patients characterized by increased theta power were non-responders, whereas 82% of patients characterized by increased alpha power were responders. Interestingly, this result is consistent with the finding that serotonin reuptake inhibitors decrease alpha activity, but increase slow and fast activity (Prichep *et al.*, 1993). It might be helpful to follow-up on such a differentiation, in order to ensure more validity in further research results. Once established, such psychophysiological measures may be for use in clinical treatment assignment and the prediction of treatment response.

Symptom provocation studies constitute a further area of research. Possibly the first symptom provocation study including EEG measures was done by Simpson *et al.* (2000). Significant changes in alpha activity during live exposure were found. Unfortunately, only six patients and no control group were investigated so, as yet, no concrete evidence for specific EEG changes has been given. Future research is necessary to identify potential differences in power bands and to specify activated brain regions during sensitive stimulation.

In the investigation of event-related potentials (ERPs), consistent results were found for some components. Different studies found reduced latencies of the N200 and the P300 components (see Sartory, 1989; Towey *et al.*, 1990, 1994). The increase in N200 and P300 latency as a function of task difficulty, which is found in control Ss, was not found in OCD patients (Sartory, 1989; Towey *et al.*, 1990). These results are interesting, since other psychiatric disorders (depression, schizophrenia) are not accompanied by reduced, but rather by increased, ERP latencies. The reduced ERP latencies are interpreted as indicators of cortical hyperarousal. Towey *et al.* (1994) also found that attention-related processing negativity (PN) began earlier, lasted longer and was increased in OCD patients compared to control Ss. This may be interpreted as overfocused attention in OCD patients (Towey *et al.*, 1994). Another well-established finding is the enhanced contingent negative variation (CNV) during preparation of a (motor) response. This enhanced CNV also seems to be specific for OCD patients (Sartory, 1989). Sartory and Master (1984) found that with an interval of 5 s between warning and imperative stimulus, only the late, but not the early component of the CNV was affected. They proposed that this is due to the uncertainty of the situation and that this reflects the doubts of OCD patients regarding the requirements made of them.

ANS Activity

Emotional arousal or anxiety is a common observation in OCD during exposure to obsession-related stimuli, prevention of ritual behaviour, or while experiencing obsessive thoughts. The most effective therapy for OCD is exposure with response prevention, as is effective in severe phobias (Foa and Kozak, 1985). Therefore, the relevance for investigation of ANS responses is obvious.

One hypothesis was that OCD patients are in a state of abnormal arousal which, in the end, results in a failure to adapt to unwanted thoughts (Beech, Ciesielski and Gordon, 1983). This should lead to elevated baseline ANS activation, or at least to an elevated responsiveness to stressful stimuli. Although earlier studies found a generally increased ANS activation (e.g. Kelly, 1980; Insel, Zahn and Murphy, 1985), more recent studies were not able to confirm the hypothesis of a general hyperactivation (Berg *et al.*, 1986; Hoehn-Saric *et al.*, 1995; Zahn *et al.*, 1996). The hypothesis of a general

hyper-responsiveness of OCD patients to stress was investigated during stressful mental activity. Despite some positive results (e.g. Insel, Zahn and Murphy, 1985), this hypothesis remains empirically unconfirmed (Kelly, 1980; Zahn *et al.*, 1996). On the contrary, patients with OCD react with less physiological flexibility to general stressful tasks (Hoehn-Saric, McLeod and Hipsley, 1995), but this is not specific for OCD patients, having also been found in other anxiety disorders (Hoehn-Saric, McLeod and Hipsley, 1995). Nevertheless, a significant positive correlation between symptom severity and general ANS reactivity was found in children and adolescents with OCD (Zahn *et al.*, 1996).

However, disorder-specific stimuli as well as obsessive thoughts increase ANS activity (SCF, SCL, HR) in OCD patients (Rabavilas and Boulougouris, 1974; Boulougouris, Rabavilas and Stefanis, 1977; Haines *et al.*, 1998). Patients with obsessional ruminations react with HR accelerations during the presentation of sensitive words in the same way as phobic Ss do (Eves and Tata, 1989). Words that are used to neutralize the obsessions evoked no HR reactions, whereas neutral words resulted in an HR deceleration (OR) (Eves and Tata, 1989).

A few studies investigated the effect of psychological treatment on ANS responses. A substantially greater reduction of HR and SCF was found for long-duration *in vivo* exposition compared to short-duration *in vivo* exposition (Boulougouris, Rabavilas and Stefanis, 1977). A study by Grayson, Foa and Steketee, (1982) focused on the investigation of the treatment process. A group of patients with washing compulsions performed *in vivo* exposure with focused attention, while a second group was distracted during exposure by playing a video game. Interestingly, HR reactions were attenuated during exposure in both conditions. The improvement was maintained in the focusing condition, but not in the distraction condition between the sessions (Grayson, Foa and Steketee, 1982).

Recent studies focused on the changes occurring during a pharmacological treatment. One major challenge in these studies was to disentangle disorder-independent drug effects from clinical changes, as psychoactive substances are known to induce changes in the ANS. In one study, clomipramine (a tricyclic antidepressant and serotonin reuptake inhibitor) effectively decreased symptoms in patients with OCD (Hoehn-Saric *et al.*, 1993). But tricyclic antidepressants are also known to generally induce HR accelerations (Sartory 1989). Zahn, Insel and Murphy, (1984) were able to demonstrate more clinical benefits and a marked reduction of SCL in clomipramine compared to placebo or clogyline (a monoamine oxidase (MAO) inhibitor) treatment time periods (crossover design). The number of non-specific SCR was reduced after clomipramine and clogyline compared to placebo periods. This was found during rest and during a threshold discrimination task. Additionally, after clomipramine periods, the OR (measured in amplitude of SCR) to startle-like loud noise was reduced and habituation was hastened. In contrast to the reduction in SC activity, a substantial increase of HR was found after treatment with clomipramine (12 beats per minute) and clogyline (six beats per minute), compared to placebo periods. In another study, Hoehn-Saric *et al.* (1993) found no effects of clomipramine during rest in a broad range of ANS measures. The only exception was the HR, which increased during clomipramine treatment and decreased during placebo treatment. However, the ANS reactivity to non-specific and to pathology-specific stressful stimuli compared to placebo was reduced. The conclusion was that the ANS effect of clomipramine is not disorder-specific. The effect might reflect a generally heightened indifference to psychological stressors.

It would be interesting to observe whether ANS reactivity differences can be found in subgroups of OCD patients (with groups divided, e.g. according to EEG power).

In summary, the hypotheses of a general hyperactivity or a general hyper-reactivity in OCD patients were not confirmed. Future research may focus on symptom provocation studies, to determine differences in OCD patient reactions to obsessive cues compared to control Ss and Ss suffering from other anxiety disorders. Especially interesting will be the investigation of EEG differences within symptom provocation studies. ANS measures may be valuable for complete assessment of OCD and evaluation of treatment outcome.

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The Neuropsychology of Anxiety Disorders: Affect, Cognition, and Neural Circuitry

Jack B. Nitschke and Wendy Heller

INTRODUCTION

In attempting to construct a neuropsychology of anxiety, findings can be drawn from several related, yet often perceived as separate, domains of research, including cognitive science and neuroscience. The relatively new fields of cognitive neuroscience and affective neuroscience are concerned with very similar questions regarding brain–behaviour relationships as were fundamental to the older field of neuropsychology, and the neuroimaging tools central to those disciplines are no less pertinent to neuropsychology than are traditional neuropsychological test batteries or cognitive/behavioural paradigms. Thus, this review of the neuropsychological findings in anxiety disorders covers a wide array of methods that together inform knowledge of the brain mechanisms involved in the circuitry governing pathological forms of anxiety.

Although often overlooked by neuroscientists studying brain function in anxiety, cognitive research over the past two decades has contributed substantially to knowledge about brain function in anxiety. A large body of work demonstrates that anxiety disorders are characterized by cognitive biases, indicating a heightened response to the possibility of threat (for review, see McNally, 1998). Attentional biases have been elicited very reliably across a variety of paradigms in which potentially threatening information is associated with greater attentional capture in individuals with anxiety disorders than in controls. The interference of this attentional capture with other cognitive processing serves as the operationalization of this bias in research studies. Furthermore, attentional biases have been found to disappear upon remission (for review, see McNally, 1998), suggesting that such biases are state-dependent. Cognitive biases have also been observed in the form of interpretation and memory biases. Across a number of different paradigms involving ambiguous stimuli that can be interpreted as threatening or neutral, anxious people choose the threatening meaning. Accruing evidence suggests that anxiety disorders are also accompanied by enhanced memory for negative or threatening information under certain conditions. These cognitive data suggest dysfunctional activation of a right hemisphere system involved in threat perception (for review, see Nitschke, Heller and Miller, 2000; see also Compton *et al.*, 2000, 2002).

In addition to these cognitive biases, cognitive deficits have been documented in anxiety disorders. One is a tendency to do poorly on tasks that require selective attention and concentration. This deficit has been suggested to reflect a general problem of preoccupation and distraction due to worry or rumination that interferes with other mental processes (for review, see Nitschke, Heller and Miller, 2000). Compromised visual–spatial functioning has also been reported. In addition, individuals with posttraumatic stress disorder often exhibit deficits in explicit memory. Taken together, these cognitive deficits suggest aberrant frontal, anterior cingulate, right parietal, and hippocampal functioning. Building on this cognitive research as well as on behavioural and electroencephalographic (EEG) findings (for review, see Nitschke, Heller and Miller, 2000) and an extensive literature in non-human animals examining fear and anxiety (for reviews, see LeDoux, 1996; Davis and Lee, 1998), haemodynamic neuroimaging research has implicated a number of the suggested regions.

Although emotional, cognitive, and neural commonalities are apparent, the diversity of findings also warrants the importance of respecting unique patterns and heterogeneity both among and within the various anxiety disorders. An observation that has become increasingly salient in the burgeoning neuropsychological literature on anxiety and its disorders is the lack of clarity and specificity about what anxiety is. Views of anxiety range from its usage in contemporary clinical research as a rubric term that encompasses fear, panic, worry, and all the anxiety disorders listed in the DSM-IV to its very specific operationalization referring to context conditioning and long-term sensitization (e.g., Davis and Lee, 1998) to a more generic personality dimension closely linked to neuroticism (e.g., Gray, 1982). Further, the heterogeneity within each of the different anxiety disorders has become increasingly apparent and represents a major problem for investigators attempting to uncover the neurobiological correlates of individual anxiety disorders. Inconsistencies across studies may be explained by the fact that anxiety is not a unitary phenomenon and that different types and symptoms of anxiety are associated with particular cognitive patterns (Heller and Nitschke, 1998; Nitschke, Heller and Miller, 2000). An important mission of neuroscience research in this area is to help unravel the inchoate notions of anxiety that currently exist. Thus, although it is important to look for generalizations regarding the neural mechanisms of anxiety, it is also necessary to consider the possibility of heterogeneity by being as specific as possible regarding the disorder or type of anxiety under investigation.

The aim of this chapter is to assess what is known about the neuropsychology and neural circuitry of anxiety disorders by examining the relevant cognitive research. Structural and functional neuroimaging data will also be reviewed, including morphometric magnetic resonance imaging (MRI), functional MRI (fMRI), positron emission tomography (PET) using various radiotracers such as [^{18}F]fluorodeoxyglucose (FDG) for glucose metabolism and ^{15}O-labelled water for blood flow, single-photon emission computed tomography (SPECT) with ^{133}Xenon or ^{99m}Tc-HMPAO, and scalp-recorded EEG. This review of the cognitive and neuroscience literatures reveals that the anxiety disorders engage brain regions involved in threat perception (e.g., right hemisphere

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regions; Compton *et al.*, 2000, 2002; Nitschke, Heller and Miller, 2000), anxious arousal (right posterior regions; Nitschke, Heller and Miller, 2000), fear (e.g., amygdala; LeDoux, 1996), vigilance for motivationally salient events (e.g., amygdala; Whalen *et al.*, 1998; Davis and Whalen, 2001), decoding of motivationally relevant emotional information such as the reward and punishment value of a stimulus [e.g., orbital frontal cortex (OFC); Rolls, 1999], worry (e.g., left-hemisphere regions; Nitschke, Heller and Miller, 2000), response conflict [e.g., anterior cingulate cortex (ACC); Carter *et al.*, 1999, 2000; Davidson *et al.*, 2002], and memory (e.g., hippocampus; Squire, 1992). The aforementioned heterogeneity should also lead to some diverse findings for the different anxiety disorders. The focus here is on the anxiety disorders as defined by the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) although consistent patterns have emerged in studies using nonclinical and brain-lesioned human populations (for review, see Nitschke, Heller and Miller, 2000).

OBSESSIVE–COMPULSIVE DISORDER

The most widely investigated anxiety disorder from a neuropsychological perspective has been obsessive–compulsive disorder (OCD). The emphasis on obsessions and compulsions in connection with the experienced anxiety and distress reported by individuals suffering from OCD is unique among the anxiety disorders and can be linked to a number of neuropsychological abnormalities.

Cognitive Studies

An extensive cognitive literature on OCD points most strongly to non-verbal memory and other visual–spatial deficits (e.g., Boone *et al.*, 1991; Zielinski, Taylor and Juzwin, 1991; Christensen *et al.*, 1992; Cohen *et al.*, 1996; Purcell *et al.*, 1998; Savage *et al.*, 1996, 1999; see also McNally and Kohlbeck, 1993; Constans *et al.*, 1995). No evidence of a verbal memory deficit has been found (Foa *et al.*, 1997). There is also ample documentation of impaired executive functions (e.g., Head, Bolton and Hymas, 1989; Veale, Owen and Marks, 1996; Abbruzzese, Ferri and Scarone, 1997; Purcell *et al.*, 1998), with trends reported by Cohen *et al.* (1996) for several neuropsychological tests. It is possible that problems in executive function could account for at least some of the visual–spatial deficits found. For example, Savage *et al.* (1999) found that poor organizational strategies for copying a figure mediated the non-verbal memory deficit for reproducing a figure among OCD patients. Morphometric data for OCD subjects by the same group suggests that larger right prefrontal volumes are associated with worse non-verbal memory using the same task (Grachev *et al.*, 1998). Should this finding be replicated, one possible explanation is that the heightened threat perception and negative affect accompanying OCD occupy the resources in the right prefrontal cortex (PFC) that are normally dedicated to non-verbal memory and also lead to structural changes.

Of additional relevance to cognitive functioning in OCD, Foa *et al.* (1993) documented that the attentional bias toward threat-related material seen across all the anxiety disorders for the emotional Stroop paradigm also emerges in OCD. In this paradigm, subjects are asked to name the colour of words varying in emotional content while ignoring their meanings. Foa *et al.* (1993) found that OCD patients with washing rituals took longer to name the colour for contamination words than for neutral words, suggesting that the threatening nature of the contamination words interfered with the task of naming the colour. They also had longer response latencies to contamination words than did OCD non-washers or non-psychiatric controls. On the other hand, OCD non-washers had longer latencies to negative than neutral words, whereas the opposite pattern was seen in controls. In a similar study in which the contamination words did not reflect the primary concerns of the OCD patients, no interference effects were observed (McNally *et al.*, 1990). These attentional findings implicate the involvement of right hemisphere regions important for threat perception (Compton *et al.*, 2000, 2002; Nitschke, Heller and Miller, 2000).

With regard to memory biases, Foa *et al.* (1997) found no bias for contamination sentences for either explicit or implicit memory. However, they did replicate the finding that OCD patients are less confident than non-psychiatric controls about memory-related judgements (McNally and Kohlbeck, 1993; Constans *et al.*, 1995). Thus, the cognitive literature is fairly conclusive in demonstrating that the memory concerns frequently voiced by OCD patients (e.g., 'Did I lock the door?') are not the result of a memory deficit or a memory bias but rather a lack of confidence in their memory. This lack of confidence is likely to be related to the characteristic fear of forgetting some activity that has become a target for compulsive behaviour, and thus is a reflection of the underlying anxiety in OCD. As such, the degree to which confidence is lacking might correlate with activity in neural structures associated with fear and other anxiety-related features.

Neuroimaging Studies

The most common finding to emerge in morphometric MRI studies to date is a reduction in caudate volume (Robinson *et al.*, 1995; Rosenberg *et al.*, 1997), with a trend also reported by Jenike *et al.* (1996). However, Aylward *et al.* (1996) found no caudate differences, and Scarone *et al.* (1992) reported an increase in right caudate volume (see Table XIX-9.3A in Martis *et al.*, Chapter XIX-9). Similar inconsistencies for the caudate have emerged in functional imaging studies examining resting states using PET and SPECT to measure glucose metabolism and blood flow. Increases were reported in three samples (Baxter *et al.*, 1987, 1988; Rubin *et al.*, 1992), with Perani *et al.* (1995) reporting a trend in the same direction. However, Lucey *et al.* (1997a, 1997b) found a reduction, and others observed no differences from non-psychiatric controls (e.g., Swedo *et al.*, 1989). In contrast, symptom provocation paradigms employing PET (McGuire *et al.*, 1994; Rauch, Jenike and Alpert, 1994) and fMRI (Breiter *et al.*, 1996) have consistently shown caudate activation.

The cortico-striatal model of OCD proposed by Rauch *et al.* (1998) posits that pathology within the caudate results in OFC and ACC hyperactivity via inefficient thalamic gating. An OFC-caudate loop may comprise much of the neural circuitry associated with the repetitive and perseverative nature of obsessions and compulsions (see also Alexander, Crutcher and DeLong, 1991). Further pursuing the evidence of caudate abnormalities, Rauch and colleagues employed PET and fMRI while OCD patients performed an implicit learning task shown to be dependent on striatal function in non-psychiatric volunteers (Rauch *et al.*, 1995b, 1997c). As noted by Martis *et al.* (Chapter XIX-9), the striatum was not activated in OCD subjects (Rauch *et al.*, 1997a), suggesting that OCD symptoms pertinent to perseveration occupy the resources normally allocated to implicit learning. The caudate activation observed in the symptom provocation studies suggests that inconsistencies in other reported findings may be due to heterogeneity in the degree of symptom severity among OCD patient samples. Taken together, these data suggest that augmented caudate activation is associated with the perseverative nature of obsessions and compulsions, which also may serve to enlarge that structure.

Haemodynamic studies of OCD have implicated a number of other regions, most consistently OFC and ventral ACC, areas of the brain frequently found to be involved in aspects of emotion and

attention. PET and SPECT studies using protocols not involving a task have revealed that patients with OCD have more blood flow or glucose metabolism than non-psychiatric controls in OFC (Baxter *et al.*, 1987, 1988; Swedo *et al.*, 1989; Rubin *et al.*, 1992; but see Machlin *et al.*, 1991; Busatto *et al.*, 2001) and ventral ACC (Machlin *et al.*, 1991; Perani *et al.*, 1995; but see Busatto *et al.*, 2001; see Table XIX-9.3B in Martis *et al.*, Chapter XIX-9).

Similar findings for the OFC were also observed during an auditory continuous performance task in a PET study measuring glucose metabolism (Nordahl *et al.*, 1989). OFC and ventral ACC activations have also been reported in fMRI (Breiter *et al.*, 1996; Adler *et al.*, 2000) and PET (Rauch, Jenike and Alpert, 1994) studies employing symptom provocation paradigms with actual obsessional stimuli. In another study employing symptom provocation via presentation of individually specified contaminants in OCD patients, McGuire *et al.* (1994) found symptom intensity to be correlated with right inferior frontal/OFC but not ACC activation. Busatto *et al.* (2001) also found that obsessive–compulsive symptoms correlated positively with left OFC blood flow. A less potent experimental elicitation of symptoms via auditory presentation of obsessional material did not induce blood flow changes in these areas using PET (Cottraux *et al.*, 1996).

With the amygdala often highlighted in models of the neural circuitry of fear, anxiety and emotion (e.g., LeDoux, 1996; Charney, Grillon and Bremner, 1998), it is worth noting that amygdala activation has been documented in only one study examining OCD, that conducted by Breiter *et al.* (1996), who exposed 10 OCD subjects to stimuli highly relevant to their obsessions. One of the subjects studied by McGuire *et al.* (1994) also showed amygdala activation, as did two of the seven OCD patients examined by Adler *et al.* (2000). Further evidence of frontal and ACC dysfunction in OCD can be inferred from two EEG studies examining event-related potentials (ERPs) in a Go–NoGo task (Malloy *et al.*, 1989) and a selective attention task (Towey *et al.*, 1994).

Treatment studies further inform the neural circuitry characterizing OCD (see also Martis *et al.*, Chapter XIX-9). Both cognitive-behavioural and pharmacological therapies have been associated with normalized (i.e., decreased) glucose metabolism in the caudate nucleus (Benkelfat *et al.*, 1990; Baxter *et al.*, 1992; Schwartz *et al.*, 1996; Saxena *et al.*, 1999; but see Baxter *et al.*, 1987; Swedo *et al.*, 1992), OFC (Benkelfat *et al.*, 1990; Swedo *et al.*, 1992; Saxena *et al.*, 1999; but see Baxter *et al.*, 1987, 1992; Schwartz *et al.*, 1996), and ventral ACC (Perani *et al.*, 1995; marginally significant in Baxter *et al.*, 1992, and Swedo *et al.*, 1992). Similar findings have emerged for blood flow measured by SPECT in the OFC (Rubin *et al.*, 1995) and ventral ACC (Hoehn-Saric *et al.*, 1991). Baxter and colleagues have reported that pre-treatment correlations between caudate and orbital regions ranging from 0.44 to 0.74 decreased significantly after effective treatment (Baxter *et al.*, 1992; Schwartz *et al.*, 1996). In addition, lower pre-treatment OFC glucose metabolism may be associated with better response to medications, whereas the converse may be true for psychotherapy (Swedo *et al.*, 1989; Brody *et al.*, 1998; Saxena *et al.*, 1999). Response to pharmacotherapy has also been predicted by glucose metabolic reductions in the ACC (Swedo *et al.*, 1989) and left caudate (Benkelfat *et al.*, 1990); however, Brody *et al.* (1998) did not replicate those findings Saxena *et al.*, 1999, only reported conducting tests for the OFC). Overall, treatment studies further implicate the caudate, OFC, and ACC in OCD. They suggest that the hyperactivity of these structures in OCD is state-dependent and that pre-treatment levels of activity may have prognostic value. The inconsistencies in findings remain to be addressed in further research.

The cognitive data implicating right hemisphere regions suggest the importance of threat perception and evaluation in OCD. The functional significance of the caudate, OFC, and ACC hyperactivity often reported prior to treatment are consistent with their roles in the perseverative nature of obsessions and compulsions, in decoding reward and punishment values of perceived and real events (c.f. Rauch, in press), and in response conflict about whether to perform some mental activity or compulsive behaviour. As noted above, the cognitive data suggest the engagement of right hemisphere regions involved in threat perception. The absence of more right-sided effects in the imaging data should be interpreted with caution, as it may be due to the difficulty of conducting adequate tests of asymmetry (Davidson and Irwin, 1999).

A final important consideration is the high level of comorbid depression in people with OCD. Visual–spatial (including non-verbal memory) and executive deficits in depression are well established and are congruent with the reduced activity in right parietal and bilateral frontal regions often reported for depression (Heller and Nitschke, 1997, 1998). The extent to which the non-verbal memory and executive deficits in OCD can be attributed to depression, anxiety, obsessions, or compulsions has not been determined, in part because the co-occurrence of these various symptoms makes disentangling their effects exceedingly difficult. Furthermore, the pronounced brain abnormalities accompanying depression (for reviews, see Davidson *et al.*, 2002; Mohanty and Heller, 2002, Chapter XVIII-7) certainly have consequences for the neuropsychology of OCD. For example, Martinot *et al.* (1990) reported a bilateral diminution of PFC glucose metabolism in 16 OCD patients as compared to eight non-psychiatric controls and no effects for OFC; however, despite not meeting criteria for DSM-III current major depressive episode, these patients were characterized by significantly higher levels of depression than the controls.

POSTTRAUMATIC STRESS DISORDER

The past decade has witnessed an explosion of research examining the neurobiological mechanisms and neuropsychological, behavioural, and cognitive concomitants of posttraumatic stress disorder (PTSD). The diagnostic requirement of exposure to a traumatic event makes this disorder an ideal candidate for testing aetiological hypotheses based on the rich conditioning literature, including classical cue conditioning, operant conditioning, and context conditioning. However, the array of re-experiencing, avoidance, and arousal symptoms and the common comorbidity with depression (and substance abuse in war veterans) add layers of complexity that make unraveling the neural circuitry of PTSD seem an intractable enterprise. Moreover, classification of PTSD remains a highly controversial topic, not only with regard to prototypic symptoms and subtyping but also with regard to whether it should be considered an anxiety disorder at all. Despite these obstacles, the emerging body of research is contributing to understanding this elusive condition.

Cognitive Studies

As with OCD, a commonly reported cognitive abnormality in PTSD is an attentional bias towards threat-related stimuli on tasks such as the emotional Stroop test. This effect has been reported for rape victims (e.g., Foa *et al.*, 1991), combat veterans (e.g., McNally *et al.*, 1990, 1993, 1996; Kips *et al.*, 1995; Vrana, Roodman and Beckham, 1995), motor vehicle accident victims (Bryant and Harvey, 1995), and people involved in a ferry disaster (Thrasher, Dalgleish and Yule, 1994). Recovery from PTSD has been shown to eliminate the attentional bias (Foa *et al.*, 1991), whereas PTSD patients who have not recovered continue to show the bias toward threat cues when retested (McNally and Kohlbeck, 1993). A memory bias toward trauma-relevant material has also been found in PTSD patients for explicit memory (Vrana, Roodman and Beckham, 1995) and conceptual implicit memory (Amir, McNally and Wiegartz, 1996c), suggesting a more pervasive proclivity towards

threat-related material that is not confined to the frequently reported attentional effect. No bias was found on an implicit-memory task that depended more on physical, perceptual features of the words than on their meaning (McNally and Amir, 1996). Consistent with these cognitive data, a recent ERP study using threat words as the low-probability stimulus type in an oddball paradigm reported that PTSD patients had larger P3 amplitudes than non-psychiatric controls for trauma-relevant but not trauma-irrelevant threat words (Stanford *et al.*, 2001). The oddball paradigm is comprised of frequent presentations of one stimulus type and infrequent presentations of a second stimulus type, which typically elicits an enlarged ERP component known as P3 or P300. Taken together, these data are suggestive of right hemisphere abnormalities pertinent to threat perception.

The other salient cognitive finding in PTSD is an explicit memory deficit. Compromised memory performance has been observed in combat veterans (e.g., Bremner *et al.*, 1993; Uddo *et al.*, 1993; McNally *et al.*, 1994, 1995; Yehuda *et al.*, 1995), rape victims (Jenkins *et al.*, 1998), and adult survivors of childhood abuse (e.g., Bremner *et al.*, 1995b; but see Stein *et al.*, 1997). These data corroborate the reports of reduced hippocampal volume in PTSD to be reviewed next.

Neuroimaging Studies

As covered by Martis *et al.* (Chapter XIX-9), the handful of studies examining structural abnormalities in PTSD consistently implicate the hippocampi, with reduced volume ranging from 8% to 30% (Bremner *et al.*, 1995a, 1997; Gurvits *et al.*, 1996). A 5% reduction in the left hippocampus was observed by Stein *et al.* (1997) in 21 adult survivors of childhood abuse, 15 of whom met DSM-IV criteria for PTSD. Schuff *et al.* (1997) also reported a trend for a 6% right hippocampal reduction in combat veterans. It is not known whether this smaller hippocampal size is due to cell loss, cell atrophy, or to some other cause (Rajkowska, 2000; Sapolsky, 2000; Sheline, 2000). Controversy persists with regard to the role of cortisol as a causative factor in the hippocampal reductions observed in PTSD (Yehuda, 1997). Regardless of this, these hippocampal data are clearly linked to the aforementioned explicit memory deficit in PTSD. Indeed, Bremner *et al.* (1995a) reported a strong correlation ($r = 0.64$) between verbal memory and right hippocampal volume in combat veterans with PTSD.

In contrast to the above morphometric data, functional neuroimaging studies examining PTSD have implicated a host of structures (see Table XIX-9.1B in Martis *et al.*, Chapter XIX-9). Two recent symptom provocation studies used script-driven imagery in conjunction with PET in adult female victims of childhood sexual abuse with and without PTSD (Bremner *et al.*, 1999a; Shin *et al.*, 1999). Bremner *et al.* (1999a) found that personalized traumatic scripts were associated with less blood flow in the right hippocampus and more blood flow in ventral ACC, PFC, insula, posterior cingulate, and motor cortex for women with PTSD than those without. Shin *et al.* (1999) reported more blood flow in the ventral ACC, OFC, and insula for childhood abuse victims with PTSD than those without. Two studies reported activation of the ventral ACC in combat veterans with PTSD as well as in combat controls without PTSD (Bremner *et al.*, 1999b; Liberzon *et al.*, 1999). Using SPECT, Liberzon and coworkers observed activation of the ventral ACC/medial PFC in non-psychiatric controls as well. Another report from this group indicated that only PTSD subjects showed more blood flow in the medial PFC, whereas both PTSD subjects and non-psychiatric controls showed a trend for increased blood flow in the ventral ACC (Zubieta *et al.*, 1999). Using PET, Bremner *et al.* (1999b) also found PTSD to be associated with increased blood flow in parietal, posterior cingulate, and motor areas. It remains to be seen whether activation in some of these regions (e.g., ventral ACC) is specific to PTSD, or has more to do with task demands or other phenomena (e.g., mood, comorbid depression, the presence of other types of anxiety).

Several symptom provocation studies of PTSD have found amygdala activation (Rauch *et al.*, 1996; Shin *et al.*, 1997; Liberzon *et al.*, 1999). Other areas implicated by Rauch *et al.* (1996) in a PET study using script-driven imagery were the ventral ACC and right OFC, insula, and temporal cortex. The same group also found increased blood flow in the ventral ACC in another sample of combat veterans for a paradigm involving combat, negative, and neutral pictures (Shin *et al.*, 1997a, 1997b). Both those studies also reported a blood flow decrease in Broca's area (see also Fischer, Wik and Fredrikson, 1996), perhaps indicative of downregulation of this verbal generation region in the service of more effective recruitment of phylogenetically older structures more appropriate for the extreme fear and horrific traumas experienced by people who go on to develop PTSD.

The importance of the amygdala and OFC for the circuitry implicated in PTSD is further underscored by research not targeting symptom-related stimuli. Using fMRI and a backward masking paradigm previously shown to activate the amygdala in non-psychiatric volunteers (Whalen *et al.*, 1998), Rauch *et al.* (2000) found that combat veterans with PTSD had larger right amygdala responses to fearful faces masked by neutral faces than did combat controls without PTSD. These responses to fear expressions are consistent with cognitive biases toward threat discussed above for PTSD patients. An older study conducted by Semple *et al.* (1993) reported more OFC blood flow as measured by PET during an auditory continuous performance task and a word generation task in combat veterans with PTSD and substance abuse than non-psychiatric controls. Less parietal blood flow during the continuous performance task was also observed (Semple *et al.*, 1996). A newer study from that group found that a similar sample of PTSD patients had more right amygdalar and left parahippocampal blood flow during the same continuous performance task than non-psychiatric controls (Semple *et al.*, 2000), adding further support to the symptom provocation findings above.

In sum, both cognitive and neuroimaging findings suggest the engagement of several right hemisphere regions, consistent with evidence that these areas are differentially involved in responding to threat. In addition, the neuroimaging data highlight a distributed array of structures not clearly lateralized, including the OFC, ACC, amygdala, and hippocampus, regions associated with decoding motivationally salient material, response conflict, fear and vigilance for motivationally salient events, and memory. As with OCD, the OFC and ventral ACC appear to be involved in the brain circuitry associated with the pathogenesis and expression of PTSD. Important points of divergence between the two disorders emerge in the subcortex, with the caudate specific to OCD and the amygdala and hippocampus implicated in numerous studies examining PTSD. It is unclear whether the decrease in Broca's area is unique to PTSD, in part because deactivations often are not reported. As with OCD, the rates of depressive disorders in PTSD populations is extremely high, which again warrants attention to the known cognitive and neurobiological correlates of depression in any discussion of the brain circuitry central to PTSD.

PANIC DISORDER

Characterized by recurrent unexpected panic attacks that share many features with basic fear responses, panic disorder has been viewed as the pre-eminent candidate condition for postulating dysfunction of the fear circuitry identified in research with non-human animals. However, the literature has shown this to be

a disappointing enterprise, and the neural machinery involved remains largely a mystery. It is important to note that even in the majority of individuals experiencing frequent panic attacks (once or more per day), more time is spent worrying about having future attacks or about the implications of those attacks than having actual attacks. For obvious reasons, animal models are not particularly conducive to tracking the circuitry associated with worry, although research on context conditioning, long-term sensitization, and anticipatory anxiety is certainly relevant (e.g., Davis and Lee, 1998; Nitschke *et al.*, 2001). The various neuropsychological research tools now available with humans may hold the most promise for identifying the circuitry affected in panic disorder.

Cognitive Studies

Cognitive reports in the literature on panic disorder have been more sparse than for OCD or for PTSD. The most common finding is a bias for panic-relevant words on implicit and explicit memory tasks (e.g., McNally, Foa and Donnell, 1989; Cloitre and Liebowitz, 1991; Cloitre *et al.*, 1994; Amir *et al.*, 1996b; Becker *et al.*, 1994, 1999), although negative findings have been reported (Otto *et al.*, 1994; Rapee, *et al.*, 1994). Perceptual asymmetry on a dichotic listening task suggestive of more left than right hemisphere activity was associated with better memory for threat words in panic disorder patients but not in non-psychiatric controls (Otto *et al.*, 1994). These results suggest a pattern of brain activity akin to that found for generalized anxiety disorder (see below), anxious apprehension, and worry (for review, see Nitschke, Heller and Miller, 2000). There is also evidence of a bias towards threatening words in a priming task involving lexical and non-lexical word pairs, one presented above the other (McNally *et al.*, 1997). Panic patients showed faster reaction times in naming the threat targets following the threat prime but only when the target was in the bottom position. Emotional Stroop interference has also been observed in panic disorder patients (Ehlers *et al.*, 1988; McNally *et al.*, 1990, 1994). These cognitive biases again point to the involvement of right hemisphere systems corresponding to threat, with dichotic listening data suggesting left-hemisphere engagement, perhaps reflecting anxious apprehension.

Neuroimaging Studies

The one known quantitative morphometric study found that panic disorder patients had smaller temporal lobes than non-psychiatric controls but no hippocampal differences (Vythilingam *et al.*, 2000). Evidence for temporal lobe aberrations has also been documented using qualitative grading methods (Fontaine *et al.*, 1990). Eleven patients exhibited abnormal signal activity in the temporal lobes, which was most prominent at the interface of the right medial temporal lobe and parahippocampal cortex (see Table XIX-9.2A in Martis *et al.*, Chapter XIX-9).

Consistent with these data, haemodynamic imaging studies have repeatedly implicated abnormalities in hippocampal and parahippocampal regions. The first report was a PET study finding more right than left parahippocampal blood flow in panic disorder patients who responded to lactate infusion (Reiman *et al.*, 1984). This finding held for the full sample, with right-sided parahippocampal asymmetries also observed for blood volume and oxygen metabolism (Reiman *et al.*, 1986). Differential hippocampal asymmetries in the same direction were found for glucose metabolism in panic disorder patients while engaged in an auditory continuous performance task (Nordahl *et al.*, 1990, 1998; see Table XIX-9.2B in Martis *et al.*, Chapter XIX-9). In the first study, patients also exhibited more right frontal and occipital metabolism and less left parietal metabolism than non-psychiatric controls. An inferior frontal asymmetry with more right than left metabolism was observed in both patient samples. Similar group differences in inferior PFC asymmetry (right > left), right frontal (marginally significant), and occipital cortex were reported in a SPECT study conducted by De Cristofaro *et al.* (1993). There were no differences in hippocampal asymmetry, but rather patients showed bilateral decreases.

Consistent with the reports of hippocampal and parahippocampal asymmetries, Bisaga *et al.* (1998) found that panic disorder patients exhibited more glucose metabolism in the left hippocampus and parahippocampal area than non-psychiatric controls. Those patients also had less metabolism in right inferior parietal and right superior temporal regions, which could be due to comorbid depression (Heller and Nitschke, 1998). In light of hippocampal involvement in explicit memory, these findings suggest that hippocampal and parahippocampal asymmetries may play a role in the explicit memory bias toward threat emerging in the cognitive literature.

The first quantitative EEG study on panic disorder documented abnormal patterns of asymmetry in both frontal and parietal regions, with patients exhibiting relatively more right-sided activity than non-psychiatric controls (Wiedemann *et al.*, 1999). More right than left frontal activity was documented for the patients but not the controls, whereas the patients did not exhibit the parietal left > right asymmetry observed in controls. Furthermore, the same frontal asymmetry was also present while the patients viewed a spider, an erotic, and an emergency picture, but not a mushroom.

Symptom provocation studies of panic disorder employing haemodynamic methods have assumed the form of pharmacological challenges. Using SPECT during sodium lactate infusion that induced global blood flow increases, Stewart *et al.* (1988) found that patients who panicked following infusion exhibited larger occipital increases, especially on the right, than non-panicking subjects, whereas the non-panicking subjects showed larger global increases, especially over the left hemisphere. In a PET study, Reiman *et al.* (1989) found no blood flow increases following sodium lactate infusion among non-panicking subjects, whereas the panic disorder patients who had panic attacks exhibited increased blood flow in anterior temporal, insula/claustrum/putamen, superior colliculus/periacquenductal grey, and cerebellar vermis regions (see also Table XIX-9.2C in Martis *et al.*, Chapter XIX-9). Of note, the anterior temporal findings may be an artifact of muscular contraction of the jaw (Drevets, Videen and MacLeod, 1992; Benkelfat *et al.*, 1995), such that recent imaging studies on anxiety often employ teeth-clenching control conditions (e.g., Rauch *et al.*, 1996; Reiman, 1997; Javanmard *et al.*, 1999).

The parallel between the most frequently observed cognitive and neuroimaging findings is noteworthy. As the only anxiety disorder with a memory bias toward threat just as reliable as an attentional bias, if not more so, panic disorder also is unique with regard to the consistent hippocampal findings across several functional imaging studies. With the hippocampus known to be the critical structure for explicit memory function, these findings suggest that the commitment of certain right hemisphere regions to threat may extend to the hippocampus. Consistent with the argument forwarded for OCD and PTSD, the involvement of broader right hemisphere systems encompassing various territories governing threat perception corresponds to findings of memory and attentional biases. The PFC asymmetry observed in three studies using different technologies is in concordance with that position. The OFC, ACC, and caudate regions highlighted in the above sections for OCD and PTSD have not emerged with any consistency in research on patients with panic disorder.

Again, the issue of comorbidity with depression deserves mention, because the explicit memory bias and the PFC asymmetry are commonly seen in depression. However, evidence reviewed above suggests that increases in right PFC activity are driving this asymmetry in panic disorder, whereas the preponderance of literature on

major depressive disorder indicates that decreased left PFC activity likely contributes to that asymmetry in depression.

SPECIFIC PHOBIA (SIMPLE PHOBIA)

Characterized by a persistent, excessive, and unreasonable fear of a specific object or situation, this disorder is very amenable to research investigation both with regard to experimental designs (e.g., presenting subjects with phobic stimuli) and subject sampling due to the prevalence of specific phobias and the relatively low rates of comorbidity with other mental disorders. However, studies with phobics are few, perhaps due to minimal public health interest in specific phobias because they generally do not compromise the occupational or social functioning of affected individuals to the same extent as other anxiety disorders. The preponderance of physiological research to date has focused on peripheral psychophysiological measures such as skin conductance, cardiovascular, and neuroendocrine activity (for review, see Fyer, 1998). No structural imaging data are available for specific phobias, and other neuropsychological research has been quite limited.

Cognitive Studies

The handful of studies investigating cognitive function in phobic individuals has documented the presence of an attentional bias but no memory bias. In women with spider phobia, Van den Hout *et al.* (1997) documented interference for both masked and unmasked words associated with spiders on a modified Stroop task similar to those employed in the OCD, PTSD, and panic disorder studies above. Using Stroop tests involving spider, general negative, and neutral words, Watts *et al.* (1986) and Lavy, Van den Hout and Arntz (1993) found larger interference for the spider words in spider phobics than matched non-anxious controls. No Stroop interference effects were observed in driving phobics for motor vehicle accident words; however, the words did not reflect their primary concerns but rather were designed for accident victims who developed PTSD (Bryant and Harvey, 1995). Evidence of a memory bias in spider phobia has not been reported (Watts and Coyle, 1993).

Neuroimaging Studies

Consistent with the conclusion drawn by Martis *et al.* (Chapter XIX-9), functional neuroimaging data have been inconsistent across studies. When small animal phobics were exposed to containers housing the feared animal, Rauch *et al.* (1995a) found blood flow increases using PET in a number of regions implicated in the above studies for OCD and PTSD (see Rauch *et al.*, 1997b), including the right ACC, left insular cortex, and left OFC. Conversely, two earlier PET studies by Fredrikson and colleagues using film clips of the feared stimuli with snake and spider phobics did not find blood flow increases in any region except the secondary visual cortex (Fredrikson *et al.*, 1993, 1995; Wik *et al.*, 1993). The only other PET study conducted with specific phobics found that confronting animal phobics with their feared animal did not elicit blood flow changes in any region of the brain although significant cardiovascular and self-reported anxiety changes were observed (Mountz *et al.*, 1989). They also reported no resting baseline differences between the phobics and non-psychiatric controls. In a SPECT study of women with spider phobia, those reporting panic while watching a video of spiders exhibited less frontal blood flow, especially on the right side, than during a neutral film (Johanson *et al.*, 1998). The remaining phobic women who reported anxiety but did not panic showed more right frontal blood flow to the spider film (although significance level was not reported). The sole published EEG study of specific phobia found more right than left parietal activity to be associated with higher pre-treatment spider phobia scores, whereas frontal activity was not related to pre-treatment or post-treatment clinical measures (Merckelbach *et al.*, 1998). There have been no published findings of amygdala activation in specific phobia despite the clear relevance of that structure for the fear response evoked by confronting phobic stimuli.

Due to the dearth of cognitive and neuroimaging research investigating specific phobias, little is known about the neuropsychology accompanying such intense, long-standing fear of an object that is often harmless. The attentional bias suggests the involvement of right hemisphere regions oriented towards threat; however, the imaging data are inconsistent. Although the structures implicated in the study by Rauch *et al.* (1995a, 1997b) suggest some commonality with other anxiety disorders, those findings have not been supported by the other studies examining specific phobia. It may be that the circuitry implicated is much less pronounced or complex than appears to be the case for the other anxiety disorders, just as the impact on everyday functioning is on average far less than for the others.

SOCIAL PHOBIA (SOCIAL ANXIETY DISORDER)

Now often referred to as social anxiety disorder, social phobia can be viewed as a variant of specific phobia that pertains to social or performance situations. Individuals suffering from social phobia fear that they will act in a humiliating or embarrassing way when in the presence of other people. Recent epidemiological studies have identified it as the third largest psychological disorder in the United States, after depression and alcoholism. Accordingly, the past five years has witnessed an explosion of research interest in the disorder, with efforts to identify the affected neural circuitry very much in their infancy.

Cognitive Studies

Cognitive research has implicated a number of abnormalities in social phobia, the majority of which are consistent with the information processing biases described in other anxiety disorders. The numerous studies examining attention, interpretation, and memory biases in social phobia have recently been reviewed (Heinrichs and Hofmann, 2001). This literature abounds in evidence of attention and interpretation biases towards social threat across multiple different paradigms.

One relevant study not reviewed by Heinrichs and Hofmann (2001) examined attention bias for facial expressions in generalized social phobia (Gilboa-Schechtman, Foa and Amir, 1999). Consistent with the dot probe and Stroop studies reviewed, phobic subjects showed an attentional bias toward angry faces as measured via several metrics using the face-in-the-crowd paradigm, whereas non-anxious controls did not. Moreover, successful treatment has resulted in the attenuation of attention (e.g., Mattia, Heimberg and Hope, 1993) and interpretation (e.g., Foa *et al.*, 1996) biases.

In other findings, Amir *et al.* (1996a) found suppression of Stroop interference to social-threat words in social phobics but not non-psychiatric controls prior to giving a speech (see Mathews and Sebastian, 1993, for comparable findings in snake-fearful subjects when in the presence of a snake they are told they will have to approach upon completion of the Stroop task). The authors suggested that subjects might increase their efforts when anxious, thereby compensating for the interference. Another possibility is that the right hemisphere resources devoted to threat might all be allocated to the situation surrounding the impending social performance, such that the threat words no longer are perceived as threatening (relative to the impending speech) to the same degree as they are under non-anxious experimental conditions.

Research eliciting anticipatory anxiety in interpretation/judgement bias paradigms is needed to determine if this phenomenon extends to other domains of information processing.

Until very recently, it was widely accepted that social phobia was not accompanied by a memory bias (e.g., Rapee *et al.*, 1994). However, recent evidence suggests otherwise (Amir *et al.*, 2000, 2001). Two reports using face stimuli provide further evidence for an explicit memory bias in social phobia. Lündh and Öst (1996) first documented the effect in a paradigm where social phobics and non-psychiatric controls were asked to judge faces as either critical or accepting. Unlike the controls, the phobics showed a memory bias for faces they had previously judged as critical. In two elegant experiments following up the seminal report by Lündh and Öst (1996), Foa *et al.* (2000) found that social phobics recognized more angry and disgust faces than happy or neutral ones, whereas no differences were observed for non-anxious controls. The same pattern was seen for reaction time data, with social phobics showing longer latencies in making a decision about the negative than the non-negative facial expressions. Furthermore, phobic subjects had longer latencies for angry than disgust faces, whereas controls did not. Similar specificity was observed in the attentional paradigm employing faces mentioned above, with social phobics detecting anger faces faster than disgust ones, whereas controls showed no difference (Gilboa-Schechtman, Foa and Amir, 1999). Taken together, data from these face paradigms suggest a general negativity bias (e.g., all negative emotion expressions) that is amplified by faces connoting threat (e.g., anger expressions), again implying that the right hemisphere regions involved in threat perception should be involved.

Consistent with cognitive findings for OCD, visual–spatial impairment including non-verbal memory deficits has been documented in social phobia (Cohen *et al.*, 1996; Hollander *et al.*, 1996), as has executive dysfunction (Cohen *et al.*, 1996). Along with other findings of left-sided neurological soft signs (Hollander *et al.*, 1996), these visual–spatial deficits are consistent with right hemisphere dysfunction in social phobia. Possibly, these deficits are produced by the augmented engagement of the right hemisphere in threat perception with a consequent lack of resources for other processes lateralized to the right hemisphere such as visual–spatial functions.

Neuroimaging Studies

Structural abnormalities of the brain have not been observed in social phobics (Potts *et al.*, 1994); however, a set of recent functional neuroimaging studies point to several critical regions. Surveying EEG at the scalp, Davidson *et al.* (2000) found that social phobics exhibited a larger anterior temporal right > left asymmetry (marginally significant for lateral frontal and parietal sites) during anticipation of making a public speech than non-psychiatric controls. Using PET to measure blood flow in social phobics, Reiman (1997) reported that singing in front of observers activated a number of cortical and subcortical regions, including lateral PFC, anterior temporal, and posterior cingulate regions, with trends noted in the ACC, medial PFC, amygdala, and hippocampus. In another PET study, Tillfors *et al.* (2001) found that social phobics exhibited larger blood flow increases than non-psychiatric controls in the right amygdaloid complex (extending into the hippocampus) while speaking in front of an audience. On the other hand, controls had larger increases in right parietal, restrosplenial, and right secondary visual cortices than social phobics did.

An fMRI study found that social phobics showed greater amygdala activation bilaterally to neutral faces than did non-psychiatric controls despite no differences in subjective ratings of the faces, whereas both groups showed the expected activation of the amygdala to aversive odors (Birbaumer *et al.*, 1998). However, it appears that this effect for the amygdala did not maintain for the full sample (Schneider *et al.*, 1999), with social phobics only exhibiting greater amygdalar and hippocampal activation than controls when the neutral faces were paired with the aversive odors (see Table XIX-9.4A in Martis *et al.*, Chapter XIX-9). Three additional neuroimaging reports presented pilot or preliminary data with mixed results for the structures implicated in the above studies on social phobia (Stein and Leslie, 1996; Van Ameringen *et al.*, 1998; Van der Linden *et al.*, 2000).

Overall, these cognitive and neuroimaging data point most strongly to the right cortical regions and the amygdala, especially in paradigms involving methods that are ecologically relevant to social phobia such as face stimuli and social performance. The concordance of the cognitive findings with the right-sided brain activation reported by Davidson *et al.* (2000) suggests that the circuitry of social phobia includes right hemisphere regions involved in threat perception. The involvement of the amygdala in fear and in vigilance for motivationally salient events is certainly applicable for the paradigms involving anticipatory anxiety and social performance.

GENERALIZED ANXIETY DISORDER

The salience of worry and verbal rumination in generalized anxiety disorder (GAD) suggests the involvement of left-hemisphere structures dedicated to language. In contrast to the other anxiety disorders which may involve varying degrees of worry about disorder-specific content, worry is the hallmark of GAD. Although worry about everyday problems is not pathological in itself, the person with GAD worries excessively, has difficulty controlling the worry, and experiences significant distress and impaired social and occupational functioning as a result. The exceedingly high rates of comorbidity with depression have made it very difficult to isolate brain abnormalities in GAD. Both cognitive and neuroimaging studies have therefore often been quite compromised in terms of diagnostic specificity.

Cognitive Studies

As with the other anxiety disorders covered above, GAD is characterized by an attentional bias towards threat in Stroop (Mathews and MacLeod, 1985; Mogg *et al.*, 1987, 1993; Martin, Williams and Clark, 1991; Bradley *et al.*, 1995; Mathews *et al.*, 1995), dot probe (MacLeod, Mathews and Lata, 1986), distractor (Mathews *et al.*, 1990, 1995), and dichotic listening (Mathews and MacLeod, 1986) paradigms. Consistent findings have emerged in two newer paradigms using emotional faces. Using a variant of the dot probe task, Bradley *et al.* (1999) reported that GAD patients had slower reaction times for threatening than neutral faces, compared to controls. Using a similar probe detection task, Mogg, Miller and Bradley (2000) measured eye movements and found that GAD subjects showed a bias toward threat faces for the two eye-movement metrics employed, but they did not replicate the reaction time differences documented by Bradley *et al.* (1999). Several of these studies reported the absence of an attentional bias in comparison groups with clinical depression (Mogg *et al.*, 1993, 2000) or with comorbid GAD and depression (Bradley *et al.*, 1995). Evidence for general rather than threat-specific distractibility has also been found (Bradley *et al.*, 1999; see also Mathews *et al.*, 1990, 1995), although even these studies found results for threat conditions to be more robust than for non-threat conditions. Despite earlier evidence to the contrary (Mathews *et al.*, 1990), recovery from GAD does not appear to be accompanied by a residual attentional bias (Mathews *et al.*, 1995; see also Mogg, Mathews

and Eysenck, 1992), consistent with findings reviewed above for other anxiety disorders.

Findings of a memory bias in GAD have been mixed. A bias towards threat has generally not been observed for explicit memory tasks (Mogg, Mathews and Weinman, 1987; Mathews *et al.*, 1989a; Otto *et al.*, 1994; MacLeod and McLaughlin, 1995; Becker *et al.*, 1999). However, Friedman, Thayer and Borkovec (2000) found an explicit memory bias in two separate GAD samples with extremely low rates of comorbid depression (although comorbidity with social phobia was 60%). Several important methodological differences from earlier studies (e.g., incidental learning task, no imagery instructions, longer stimulus exposure) suggest the presence of an explicit memory bias in GAD under conditions optimal for detecting memory biases in clinical anxiety (see Becker *et al.*, 1999). In addition, Otto *et al.* (1994) documented the same relationship between auditory perceptual asymmetry and memory bias toward threat discussed above for panic disorder in a sample of GAD patients. The inferred pattern of more left than right hemisphere activity was associated with better memory for threat words, consistent with left-sided neuroimaging findings for GAD reviewed below.

Implicit-memory bias has emerged for GAD under some conditions (MacLeod and McLaughlin, 1995; Mathews *et al.*, 1989a) but not others (Mathews *et al.*, 1995), a discrepancy that cannot be explained by the type of implicit-memory tested (see above discussion contrasting conceptual and perceptual implicit-memory in PTSD). There is also evidence that GAD patients have a bias to interpret ambiguous stimuli as threatening (Mathews, Richards and Eysenck, 1989b; Eysenck *et al.*, 1991). Recovered patients do not show implicit memory or interpretive biases (Mathews, Richards and Eysenck, 1989b; Eysenck *et al.*, 1991). Again, the cognitive bias literature suggests state-dependent recruitment of right hemisphere regions involved in threat perception, perhaps superimposed upon the left-sided perceptual asymmetry and neuroimaging findings also observed in GAD patients.

There is some indication of mild cognitive deficits in GAD that are consistent with the notion that worry occupies cognitive resources that otherwise might be deployed for various experimental tasks and everyday functions. Wolski and Maj (1998) documented performance deficits on a modified Sternberg memory task in a group of 87 anxiety patients, 77 of whom had GAD. The general distractibility effects reviewed above (e.g., Bradley *et al.*, 1999) provide further support for this position. However, overall performance deficits are generally not seen on attention and memory tasks (e.g., Mathews *et al.*, 1990; Otto *et al.*, 1994).

Neuroimaging Studies

The one published morphometric MRI study on GAD was conducted with children and adolescents (De Bellis *et al.*, 2000). The right amygdala was larger in patients than in matched non-psychiatric controls. No differences were found in the temporal lobe, hippocampi, corpus callosum, or basal ganglia or for total intracranial or total cerebral volumes.

In contrast to the other anxiety disorders covered here, functional neuroimaging studies are older, with no work published in the past decade. Wu *et al.* (1991) found that patients had less glucose metabolism in the basal ganglia (comprised of caudate, putamen, and globus pallidus) and more in left inferior frontal, left inferior occipital, right posterior temporal, and right precentral regions than non-psychiatric controls during a passive viewing task. The left inferior frontal finding and concomitant greater left than right frontal metabolism are in line with the hypothesis that language centres involved in worry (e.g., Broca's area) are activated. During a visual continuous performance task using degraded stimuli performed only by the patients, basal ganglia and right parietal metabolism increased, whereas decreases were seen in right temporal and occipital lobes. Consistent with their earlier report (Buchsbaum *et al.*, 1987), which they claimed was on the same GAD sample (the gender breakdown was slightly different), benzodiazepine therapy resulted in decreased occipital, basal ganglia, and limbic system (comprised of the amygdala, hippocampus, and cingulate) metabolism. In a SPECT study, GAD patients showed increased left orbital frontal blood flow when asked to freely associate about threatening pictures presented prior to rCBF measurement (Johanson *et al.*, 1992). The specificity of the effects to GAD in the latter two studies is not clear because neither one included a control group.

Involvement of different brain areas in GAD can also be gleaned from several EEG studies. EEG topography from 32 sites revealed no baseline differences between GAD patients and non-psychiatric controls (Grillon and Buchsbaum, 1987). When presented with neutral lights in a basic orienting response paradigm, patients showed less alpha suppression (presumably reflecting decreased mental activity) than controls, especially over the occipital lobe, perhaps reflecting a diminution of attention to external stimulation because of competing processes devoted to worry. An earlier EEG study by the same group examined benzodiazepine treatment effects in patients with random assignment to placebo or drug group and in non-psychiatric controls (Buchsbaum *et al.*, 1985). Using 16 midline and left-hemisphere sites, they found that patients had less delta and alpha (more activity) than controls, especially over left posterior temporal cortex. Drug effects were seen in different bands across several regions of the brain but were of limited utility in isolating patterns of brain activity critical for GAD because only four of the nine patients administered benzodiazepines showed clinical improvement as measured by the Hamilton Anxiety Scale (none of the 11 patients taking placebo improved). However, correlational analyses revealed that increased left frontal alpha (decreased activity) was associated with clinical improvement for patients in the drug group, consistent with above findings of left frontal involvement in GAD and worry.

Of relevance to imaging research despite only recording from three midline electrodes, a recent treatment study of GAD explored frontal midline theta activity, which is thought to reflect reduction of anxiety during task performance (Suetsugi *et al.*, 2000). Criteria for frontal midline theta at the midfrontal site were not met for any of the 28 patients at the initial visit. The 26 patients for whom frontal midline theta appeared following psychotherapy or pharmacotherapy showed dramatic clinical improvement, whereas the remaining two individuals continued to exhibit high levels of anxiety. Although these data are certainly preliminary, they again implicate the frontal cortex and suggest that worry interferes with the production of frontal midline theta.

The dearth of recent neuroimaging data for GAD — also noted by Martis *et al.* (Chapter XIX-9) — is striking when compared to the proliferation of such research conducted with the other five anxiety disorders covered in this review. The few studies conducted, along with the more extensive cognitive science literature examining GAD, point to several brain regions deserving further investigation. Based on the cognitive deficit and left-sided neuroimaging findings, the circuitry involved in worry and the structures overlapping with attention and working memory (e.g., PFC, parietal regions, particularly left hemisphere) are conspicuous candidates for uncovering brain aberrations in GAD. In addition, the right hemisphere territories implicated by the cognitive biases accompanying GAD are also likely constituents of the brain circuitry involved in the pathophysiology of GAD.

DISCUSSION

Across the many cognitive and neuroimaging studies reviewed here, cognitive biases toward threat is the one attribute common to all six

anxiety disorders covered. Attentional biases have been observed in all disorders, whereas data for explicit and implicit-memory biases have been mixed. Findings of a memory bias have been replicated most consistently for panic disorder, with substantial evidence also reported for PTSD, social phobia, and GAD. On the other hand, no studies have found a memory bias in OCD or specific phobia. Interpretation (i.e., judgement) biases have not been extensively examined among clinical populations, although there is ample evidence of such a bias in OCD, social phobia, and GAD. This orientation towards threat in anxiety disorder populations suggests the involvement of particular anterior and posterior right hemisphere regions (for reviews, see Compton *et al.*, 2000, 2001; Nitschke, Heller and Miller, 2000). As described by Nitschke and coworkers (2000), these biases may be related to an emotion surveillance system of the right hemisphere designed to evaluate the presence of a threat in the external environment. This right hemisphere system may correspond to the cortical processes that McNally (1998) postulated to accompany a subcortical circuit involved in attentional biases toward threat. The hyperactivation of this right hemisphere system may interfere with visual–spatial functions for which right posterior regions are specialized, as seen in OCD and social phobia. The right-sided increases in activation reported in many of the neuroimaging studies examining anxiety disorders — with the notable exception of GAD, which likely invokes left-hemisphere regions devoted to verbal processes needed for worry — may be a manifestation of the heightened reliance on this emotional surveillance system governing threat perception and evaluation.

The anxiety disorders covered here are further characterized by a number of divergent neuropsychological patterns. In contrast to the morphometric and functional studies on OCD, the caudate nucleus is not implicated in any other anxiety disorders. PTSD is the only disorder to be accompanied by memory deficits and by reduced hippocampal volume. Findings of hippocampal asymmetries have been reported exclusively for panic disorder. Unlike the other disorders, the preponderance of imaging findings for GAD implicates left-hemisphere regions. Amygdala activation has not been observed with any inconsistency, except in PTSD and social phobia. OFC and ventral ACC activations have been reliably found only in OCD and PTSD. Finally, visual–spatial deficits have been observed for OCD and social phobia but not the others. This summary of the findings points to the substantial heterogeneity among the anxiety disorders.

Although anxiety is often referred to as a homogenous construct, neuropsychological data clearly indicate the importance of noting distinctions and variable symptom expression both across and within diagnoses. Several useful neurobiological models have been proposed, including one proposed by Rauch *et al.* (1998) on OCD and another proposed by Charney, Grillon and Bremner (1998) concentrating primarily on PTSD. We have proposed a neuropsychological framework positing a distinction between two types of anxiety (e.g., Nitschke *et al.*, 2000). Anxious apprehension is characterized primarily by worry and relies on left-hemisphere processes, whereas anxious arousal is characterized by immediate fear and panic symptoms and closely aligned with the emotion surveillance system of the right hemisphere. In general, GAD is characterized more strongly by anxious apprehension than are the other disorders, whereas panic disorder is likely accompanied by the highest levels of anxious arousal. However, it is important to note that these two forms of anxiety are not mutually exclusive and likely exist in all individuals with anxiety disorders to varying degrees. Pronounced individual differences within a disorder in the expression of both forms of anxiety are also likely, as are intra-individual differences across time. Although several models explain some of the variability in the neuropsychological findings, no current formulation can account for all the heterogeneity.

Attending to psychological and biological mechanisms should inform this heterogeneity that impedes attempts to unravel the neuropsychology and neural circuitry of clinical anxiety. One means of accomplishing this is research with clinical populations that rigorously examines the brain correlates of specific anxiety symptoms, such as worry, contamination obsessions, and avoidance of feared objects or situations. Another approach is to appeal to knowledge about which brain regions govern specific functions relevant to anxiety pathology (see Davidson *et al.*, 2002). Basic research with humans and non-human animals has uncovered some of the circuitry involved in those psychological phenomena central to anxiety disorders and showcased in this review (e.g., threat evaluation, fear, response conflict). This emphasis on mechanisms is also promising for research examining the interface with other neurobiological systems shown to be critical for the expression of fear and to manifest irregularities in anxiety disorders, such as cortisol, corticotropin-releasing factor (CRF), cholecystokinin (CCK), tachykinins, neuropeptide-Y, serotonin, norepinephrine, gamma-aminobutyric acid (GABA), and *N*-methyl-D-aspartate (NMDA). These are some of the areas that await synthesis with the neuropsychological concomitants of anxiety that have been identified in the large corpus of cognitive and neuroimaging research examining anxiety disorders.

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XIX-9

Functional Neuroanatomy of Anxiety Disorders

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INTRODUCTION

In this chapter, current hypotheses regarding the functional neuroanatomy of anxiety disorders are examined. Initially, the concepts of anxiety and stress as well as anxiety disorders are discussed. Next, relevant research on animal fear conditioning as well as human anxiety studies are briefly reviewed. This provides the necessary foundation for the ensuing coverage of human data on anxiety disorders and related contemporary neurobiological models.

Anxiety and Fear

In humans, anxiety is a normal unpleasant affective state with experiential, cognitive, autonomic, neuroendocrine and behavioural components. Fear behaviour is the response to specific environmental stimuli that are perceived as potentially dangerous. Though details are still debated, fear behaviour refers to a stimulus-bound response while anxiety is understood as a state of anticipatory apprehension and dread in humans (Davis, 1998; Nitschke, Heller and Miller, 2000). Fear processes are innate and mediated by rapid-response neurobiological systems in animals and humans. It is appealing to consider that these adaptive mechanisms have evolved to enable harm avoidance and enhance chances of survival. Animal research findings suggest that the fear system is a dynamic, integrated network of neural circuits, comprised of processing nodes (specific brain areas), that detects and responds to danger (LeDoux, 2000a). Given the wealth of behavioural and neurobiological similarities between fear responses in animals and humans, animal research in this area plays an important role in guiding anxiety research in humans. However, the conscious and cognitive aspects of human anxiety are undeniably complex and unique, thereby warranting caution when extrapolating from fear research in animals to the neurobiology of anxiety in humans. Similarly, there are substantial inferential leaps to be acknowledged when moving from theories regarding normal human anxiety to the anxiety disorders, which are complex, multifactorially determined syndromes.

Stress

Stress is generally defined as a circumstance that disturbs the normal physiological or psychological functioning of an individual, as well as the disturbed state that results (derived from The Oxford English Dictionary, Second Edition, 1989). Physiological stress responses are coordinated, multisystem (e.g. autonomic, neuroendocrine and immune) adaptive changes in organisms. Stress inducing stimuli or 'stressors' can be positive or adverse. Stress research in humans is particularly challenging due to the convergent and less well described effects of psychological, social and cultural stressors on biological systems. Excessive stress in humans contributes to increased morbidity and mortality and is a significant predictor of adverse outcomes in many medical and psychiatric disorders, e.g. ischaemic heart disease and major depression (Chrousos and Gold, 1998; O'Connor, Gurbel and Serebrauny, 2000; Monroe *et al.*, 2001). Stress responses are closely related to anxiety disorders; this being inferred mainly from overt exposure to stressful life events or trauma. The effects of chronic low level stress are less well established in relation to psychiatric disorders. In the current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; APA, 1994), two disorders, Acute Stress Disorder and Post-Traumatic Stress Disorder, specifically require exposure to overt traumatic stress as a diagnostic criterion.

Anxiety Disorders

Anxiety disorders, (DSM-IV, (APA, 1994); ICD 10, under 'neurotic, stress-related and somatoform disorders', Chapter V (F), WHO, 1992), are characterized by exaggerated anxiety and fear responses occurring either spontaneously or to relatively innocuous stimuli. Additional hallmarks of these disorders include hypervigilance, hyperarousal, ritualized avoidance and subjective distress. When severe, these manifestations can be disabling. Sufferers tend to display attention and interpretation biases resulting in hypervigilance and overestimation of risk (Nitschke, Heller and Miller, 2000). Psychiatric comorbidity is common among individuals who suffer from an anxiety disorder; the most prevalent comorbid conditions include another anxiety disorder, depression, and substance use disorders. People with anxiety disorders are also at increased risk of suicide and exhibit increased medical service utilization (Regier *et al.*, 1998; Dunner, 2001). Discrete anxiety disorder diagnoses in the DSM-IV include: panic disorder (with and without agoraphobia), acute stress and post traumatic stress disorder, specific and social phobias (social anxiety disorder), generalized anxiety disorder and obsessive compulsive disorder (APA, 1994). Emerging neurobiological evidence provides an opportunity to critically review and refine these nosological constructs, though making sense of inconsistent evidence from studies that involve different modalities and paradigms in heterogeneous populations is a challenge.

FUNCTIONAL NEUROANATOMY OF FEAR AND ANXIETY

Fear Conditioning: Animal Research

The fear-conditioning behavioural paradigm has been a centrepiece for advancing science in this domain via hypothesis-driven research in both animals and humans. Fear conditioning involves the temporal pairing of a neutral stimulus (known as a conditioned stimulus (CS); e.g. a tone), with an aversive stimulus (the unconditioned stimulus (US); e.g. a shock) that can innately elicit fear responses.

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After one or several paired presentations, the CS alone can trigger the fear responses. This process can be shown to habituate (i.e. repeated presentations cause decremental intensity in the response). Moreover, if the CS is subsequently repeatedly presented without the paired US, the fear responses decline and cease, a process referred to as 'extinction'. These phenomena and pathways subserving them have been reliably demonstrated in multiple animal species as well as in humans (LeDoux, 2000a).

Evidence accumulated from neuroanatomical tract tracing, ablation, and unit-recording studies of animal fear conditioning implicates the amygdala as central in the 'fear network'. The critical role of the amygdala is further supported by evidence of its abundant reciprocal connections with other brain areas (e.g. sensory thalamus, primary somatosensory cortex, prefrontal cortex, striatum, and brain stem) that comprise the fear network, subserving the various elements of the characteristic fear response (see below).

The amygdala also houses an internal circuitry, involving its various nuclei. Based on animal findings (best mapped out in the context of auditory CS in rats), sensory information from the sensory thalamus is rapidly conveyed to the lateral nucleus of the amygdala (LA). Further transmission occurs from the LA to the central nucleus of the amygdala (CeA), both directly and via the accessory basal (abA) and basal (bA) amygdalar nuclei. The CeA has projections to multiple output areas which subserve the characteristic components of the conditioned fear response such as autonomic activity (subserved by the brainstem autonomic centres including the locus caeruleus), the stress neuroendocrine response (lateral hypothalamic nuclei), attentional processes and response choice (the anterior cingulate cortex) as well as templates of action tendency and overt response behaviour (periaqueductal gray, the striatum) (Kapp *et al.*, 1992; Maren and Fanselow, 1996; LeDoux, 2000b; Davis, 2000).

Animals thus conditioned also express similar responses to the context in which the conditioning occurred. This phenomenon, called 'contextual fear conditioning' is evidenced when the same characteristic fear responses can be elicited following fear conditioning, simply by exposing the subject to the situation where conditioning occurred, even in the absence of re-exposure to a discrete CS. Lesion studies have demonstrated the crucial role of the hippocampal projections to the amygdala in contextual fear conditioning (Maren and Fanselow, 1995).

The neurobiology of fear conditioning has been further elucidated by studies that assess changes occurring at the cellular and molecular levels within the amygdala, hippocampus and other relevant structures. Plasticity has been studied via cell recordings in various amygdalar nuclei as well as at other nodes within the fear-conditioning network. Long-term potentiation (LTP) has been demonstrated in the amygdalo-hippocampal pathways in fear conditioning though these results are mixed and less well characterized. Specifically, the NMDA receptor activation is implicated in the mediation of LTP in some subnuclei of the amygdala (Chapman and Chattarji, 2000). The amygdala is considered to be essential in fear-related learning and thus may have an important role in decision making (by emotional weighting) and in the early stages of memory formation for emotionally salient events. However, much remains to be understood about the role of the amygdala and relevant neural pathways in fear learning and memory in humans.

The orbitofrontal cortex (OFC) is implicated in the extinction of conditioned fear response. There is evidence for a dynamic interaction between the OFC and the amygdala with regard to processing of fear-conditioning responses. When the potentially threatening nature of a particular stimulus diminishes, the OFC is purported to attenuate responses to that stimulus via projections to the amygdala. Thus prefrontal connections of the amygdala are believed to modulate threat-related information (Wilson and Rolls, 1990; Morgan, Romanski and LeDoux, 1993; Morgan and LeDoux, 1995), conferring flexibility and adaptability needed to successfully navigate a rapidly changing environment.

Primate Studies

Extending fear-conditioning work in rats to primates could be viewed as an essential intermediate step in the understanding of human anxiety and fear. The seminal work of Kluver and Bucy (1939) in describing the behavioural effects of bilateral anterior temporal lobectomy in monkeys set the stage for more detailed investigations into amygdala involvement in fear behaviour. It is important to note that subsequent studies, involving more selective lesions of the amygdala, have shown the ensuing deficits to share some but not all features of the Kluver–Bucy syndrome, confirming that some of the features were due to lesions in the adjacent brain regions. Contemporary data from primates has, however, strengthened the argument that the amygdala is critically involved in stimulus–affect association (i.e. in the elicitation of learned emotional responses; see Baxter and Murray (2000) for a detailed review of these newer studies).

Much of the work on fear conditioning in rodents with specific reference to the role of the amygdala in stimulus-reinforcer associations has been extended in primates. The amygdala neurons have been shown to respond to primary reinforcers (e.g. taste), visual stimuli previously paired with rewarding primary reinforcer, novel stimuli and faces (Rolls, 1999). The interactions between the OFC and amygdala have been demonstrated to similarly contribute to acquisition and extinction of fear conditioning. However, more recent studies highlight the anticipated difficulty in making inferential leaps across different species towards human brain functioning. For example, Kalin *et al.* (2001) reported that rhesus monkeys with previously characterized 'anxious temperament' endophenotype, demonstrated blunted acute unconditioned fear responses in the face of >70% bilateral amygdalar destruction, but were unchanged on unconditioned trait-like anxiety-fear responses in the face of >95% bilateral amygdala destruction. Thus, caution must be exercised while using findings from animal research to hypothesize about human anxiety and its disorders.

Studies of Humans with Brain Lesions

Brain lesions that arise in humans are rarely neuroanatomically configured to involve only the entirety of a brain region of interest. Furthermore, the capacity of spared regions to compensate for lost structures complicates meaningful interpretation of regional function. Nevertheless, the study of humans with discrete brain lesions offers valuable clues to functional neuroanatomy.

Adolphs *et al.* (1994, 1995) reported studies with a single subject (SM), a 32-year-old lady with a rare heritable disorder of primarily epithelial tissue, the Urbach–Wiethe syndrome, characterized by avascular/atrophic mineralizations of medial temporal tissue including the amygdala. SM was reported to have selective, complete and stable bilateral amygdalar damage (as well as minor damage to anterior entorhinal cortices) by magnetic resonance imaging (MRI). Extensive testing on SM revealed a persistent deficit in recognition of fear expression despite the cognitive ability to describe fear and what it entailed. This has been replicated in subsequent studies on humans with bilateral amygdala damage (Calder *et al.*, 1996; Broks *et al.*, 1998; Adolphs *et al.*, 1999b). In humans with discrete lesions of the amygdala or more extensive temporal lobe lesions that include the amygdala, deficits have also been demonstrated involving fear conditioning (Bechara *et al.*, 1995; LaBar *et al.*, 1998). The few studies in subjects with varying amounts of amygdala damage using a task of emotional prosody recognition have yielded mixed results: impairment (Scott *et al.*, 1997) vs no deficits (Anderson and Phelps, 1998; Adolphs *et al.*, 1999a). Thus, taken together, human

lesion studies have extended the research in animals, by demonstrating amygdala involvement in the recognition of facial expressions of negative emotions including but not limited to, fear.

Based on these findings it has been hypothesized that the amygdala is an important component in a specialized human neural system that detects and responds to danger-related stimuli (with or without a social context). The role of the amygdala in this system is to detect the emotional relevance of stimuli and help trigger rapid behavioural responses and conscious awareness of aversive stimuli through its subcortical and cortical connections (Adolphs and Tranel, 2000).

Human brain lesion studies involving other regions germane to the purported fear network, provide further information to guide contemporary circuitry models of anxiety disorders. For instance, OFC lesions are associated with disruption in the rapid flexible response to changes in rule set. This may parallel the established role of the OFC in dynamic response attenuation when stimulus salience has changed (i.e. extinction). This is consistent with a broad role for the OFC in top-down control over subcortical structures in fear processing. Thus, OFC dysfunction in top-down control may lead to an inflexible persistence of fear responses, manifesting as pervasive cognitive misinterpretation of danger even in the face of relatively innocuous stimuli (or conversely a lack of recognition of danger).

Neuroimaging Studies of Anxiety in Healthy Subjects

It is important to understand the neural processing of normal anxiety to gain insight into anxiety disorders where this mechanism is presumably dysfunctional. Recent positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have attempted to map the mediating neuroanatomy associated with perception of threat-related or emotionally valenced stimuli (mainly faces) as well as behaviourally or pharmacologically induced anxiety (see Rauch and Shin, 2002 for review). Such studies of anxiety in healthy volunteers have demonstrated relatively consistent patterns of brain activation in the anterior paralimbic cortical structures (i.e. posterior medial orbitofrontal, anterior temporal, anterior cingulate, and insular cortex) and the amygdala.

Much of the work in this area has involved mapping brain activity in healthy volunteers when presented with emotionally expressive faces (fear, happy, angry and disgust). Perception of fearful faces has been reported to be associated with amygdala activation both when the stimulus was overtly (Breiter *et al.*, 1996a; Morris *et al.*, 1996, 1998a; Phillips *et al.*, 1997) or covertly presented with a backward masking technique (Whalen *et al.*, 1998). Extending this work, Morris, Öhman and Dolan (1998b, 1999) demonstrated significant correlation of right amygdala neural activity (to masked fear-conditioned faces) with activity in visual structures implicated in non conscious processing (pulvinar nucleus of thalamus and superior colliculus). Based on this the authors suggest that processing of behaviourally relevant unseen visual stimuli may be subserved by a right-sided subcortical pathway in parallel to a cortical route necessary for conscious identification.

Habituation in the brain represents an adaptive mechanism by which resources can be preferentially allocated to survival-relevant (e.g. threat-related) stimuli by rapid attenuation of response to stimuli that are no longer salient. Lack of habituation may help to explain some symptoms of anxiety such as persisting anxiety beyond the point of stimulus relevance. Investigators have attempted to demonstrate habituation in fMRI studies of healthy subjects by showing response decrements in various brain areas activated by emotionally relevant stimuli (e.g. faces bearing emotional expressions) (Breiter *et al.*, 1996a; Whalen *et al.*, 1998; Fischer *et al.*, 2000a, 2000b). Although preliminary there seem to be lateralized differences in habituation of amygdala responses (significant signal decrement over time in right but not left amygdala) and differential habituation to emotional valence in the left prefrontal cortex (greater signal decrement to positive vs negatively valenced stimuli) (Wright *et al.*, 2000, 2001).

The phenomena of fear conditioning and extinction are relevant to models of anxiety disorders. Innovative functional neuroimaging paradigms have been employed to study the neural substrates of fear conditioning in humans. Morris *et al.* (1997, 1998a, 1998b) reported association of the fear-conditioned state with increased activity in bilateral amygdala, right thalamus and OFC, further demonstrating lateralized amygdala activation based on subjects' awareness of the fear-related stimuli (right-sided with covert stimuli and left-sided with overt stimuli). Other groups using different conditioning paradigms, have demonstrated anterior paralimbic activation and habituation of conditioning-related signals in the amygdala and hippocampal region (Büchel *et al.*, 1998, 1999; LaBar *et al.*, 1998).

ANXIETY DISORDERS

Overview

The anxiety disorders share many common core features (hypervigilance, excessive anxiety, and avoidance); yet, they are also distinguishable from one another clinically (e.g. spontaneous attacks of terrifying anxiety in panic disorders versus a pervasive state of anxiety in GAD). It is appealing to consider that common neurobiological substrates may underlie the spectrum of anxiety disorders, while unique pathophysiological mechanisms exist that distinguish among them. Investigators have developed tentative models regarding the neurobiology of anxiety disorders drawing from animal research and human imaging data as outlined above.

Currently, neurobiological and neuroanatomical hypotheses for some of these disorders (e.g. PTSD, OCD and PD) are more coherent than for others (e.g. GAD, phobias). A logical starting point is to identify core characteristics of specific anxiety disorders and based on animal and human research findings, test hypotheses of regional dysfunction in specific disorders. Advances in neuroimaging have accelerated this effort. Structural as well as neutral state and symptom provocation functional neuroimaging studies are giving way to more sophisticated cognitive, pharmacological and multimodal paradigms.

In the following section, research findings and current hypotheses pertaining to the functional neuroanatomy of specific anxiety disorders will be discussed. Note that a comprehensive model requires an integration between neurocircuitry and relevant neurochemistry/neuropharmacology of the implicated brain pathways. For clarity of organization, these complementary topics have been addressed in a separate chapter in this volume (Malizia *et al.*, Chapter XIX-10).

POST-TRAUMATIC STRESS DISORDER (PTSD)

PTSD is characterized by a triad of symptom clusters that occur in the aftermath of a precipitating traumatic event: (1) recurrent intrusive 're-experiencing' of the trauma (2) hypervigilance/hyperarousal and (3) emotional numbing/avoidance (DSM-IV; APA, 1994). Neurobiological research in PTSD has mostly involved patients with combat or childhood abuse related PTSD. In fact, there are a wide variety of types of trauma that can cause PTSD, and it is not clear whether different characteristics of the trauma history are reflected in different neurobiological subtypes of the disorder. For instance, the trauma can be a single event, or a series of multiple events; it can occur over a brief time or chronically; the

exposure can occur in childhood or adulthood; and it can be perpetrated by humans or not. Regardless of the aetiology of the PTSD, patients are prone to comorbidities such as mood and substance-abuse disorders. Despite these numerous sources of heterogeneity in PTSD, findings from human studies seem to converge with data from animal research in certain areas discussed below.

Stress, Glucocorticoids/HPA Axis and the Hippocampus in Animals and Humans

Rodent and non-human primate studies have demonstrated neural damage such as apical dendrite atrophy (CA3 neurons), degeneration of CA3 hippocampal neurons, and decreased hippocampal blood flow in response to a variety of chronic stressors (social, restraint, physical) (Uno *et al.*, 1989; Watanabe, Gould and McEwen, 1992; Endo *et al.*, 1999). These effects appear to be a consequence of stress-related excessive glucocorticoid (GC) secretion which in part seems to be mediated by an excess of excitatory amino acids (EAA's) such as glutamate, producing NMDA receptor mediated excitotoxic effects (Sapolsky, Krey and McEwen, 1985; Woolley, Gould and McEwen, 1990; Gould and Tanapat, 1999).

Smaller hippocampal volumes have also been demonstrated in human subjects with PTSD, due to combat as well as childhood sexual abuse (in both sexes, ranging from 5–26%) (see Table XIX-9.1A for structural studies in PTSD) with the exception of one study involving children/adolescent subjects with PTSD (De Bellis *et al.*, 1999). Bremner *et al.* (1995), reported smaller right hippocampal volumes in 26 veterans with PTSD (vs 22 civilian controls without PTSD). In the PTSD subjects, lower percent retention scores on a standard test of verbal memory were associated with lower right hippocampal volumes. Gurvits *et al.* (1996) found smaller bilateral hippocampal volumes in seven Vietnam combat veterans with PTSD (vs seven Vietnam combat veterans without and eight civilians without PTSD). The hippocampal volumes across the 14 veterans were inversely correlated with the extent of combat exposure and PTSD symptom severity. This raises the question of the specificity of the volumetric reductions to the PTSD process. Thus the basis for these volumetric differences is still unclear. Several possibilities exist including volume loss being the effect of the traumatic exposure vs sequelae of the development of the PTSD symptoms, or that people with smaller hippocampi are predisposed to developing PTSD following trauma (Pitman, 2001).

Clinical research has revealed decreased hippocampal volumes and elevated cortisol levels in Cushing's syndrome, which was reversed with treatment (Starkman *et al.*, 1992, 1999). Smaller hippocampal volumes and high cortisol levels have also been reported in major depressive disorder (for review see Brown, Rush and McEwen, 1999). The hippocampus is also involved in the modulation of the hypothalamic-pituitary-adrenal (HPA) axis, and lesions of the hippocampus appear to increase the release of glucocorticoids during stress which in turn may further damage the hippocampus (Feldman and Conforti, 1980; Sapolsky, Krey and McEwen, 1986; Herman *et al.*, 1989). However, hypo and not hypercortisolaemia has been frequently reported in patients with PTSD (Newport and Nemeroff, 2000), which is harder to reconcile with the GC mediated hippocampal volume loss theory. One hypothesis that attempts to explain this apparent disparity suggests GC hypersensitivity in PTSD patients resulting in hypocortisolaemia (due to feedback inhibition) and reduced hippocampal volumes (Yehuda, 1998).

Fear Conditioning, PTSD Symptoms and Functional Neuroimaging Studies in PTSD

The fear-conditioning paradigm seems relevant to the understanding of some PTSD symptomatology. For example, certain aspects of the response to environmental triggers of past trauma strongly resemble the animal fear-conditioning response. However, the indelibility of the trauma-related memory and resistance to extinction of the fear response in PTSD is distinct from normal fear conditioning in animal studies. Simple experimental noxious stimuli currently used in animal research provide a suboptimal comparison to the real life complexity of the human post-traumatic stress response. Thus, one of the challenges in PTSD research is to disentangle pre-existing neurobiological factors that might predispose to developing PTSD in the face of trauma exposure (i.e. risk factors) from fundamental pathophysiological elements of the disease process as well as non-specific consequences of traumatic exposure.

According to the 'amygdalocentric' model of PTSD, hyperresponsivity within the amygdala to threat-related stimuli, in the face of disrupted functional connectivity between the OFC, amygdala and hippocampal regions may explain some of the cognitive, affective and psychomotor manifestations of PTSD (Rauch and Baxter, 1998; Rauch and Shin, 2002). According to this theory the hyperresponsivity of the amygdala results in characteristic symptoms of hyperarousal. Dysfunctional governance of the amygdala by the prefrontal cortex (specifically the affective division of the anterior cingulate cortex) is implicated in the inappropriate assessment of and response to threat and the persistence of this beyond the relevant period (i.e. inflexibility and dysfunctional 'extinction'). Hippocampal dysfunction may contribute to contextual avoidance, difficulty in identifying safe contexts and explicit memory deficits

Table XIX-9.1A Structural neuroimaging studies in PTSD

Authors	Subjects	Methods	Findings
Bremner *et al.*, 1995	26 Vet-PTSD vs 22 healthy civilians	MRI morphometry	R-hippo. volumes 8% ↓ in vets vs controls. ↓ scores on a standard measure of verbal mem. correlated directly with ↓ R-hippo. volumes.
Gurvits *et al.*, 1996	7 Vietnam vets with PTSD; 7 Vets. and 8 nonvet. controls without PTSD	MRI morphometry	Significantly ↓ B/L-hippo. volumes in PTSD vs both control groups. Hippo. volume inversely correlated with extent of exposure (14 vets.)
Bremner *et al.*, 1997a	17 adults with PTSD (childhood abuse) vs 21 non abused controls	MRI morphometry	12% ↓ L-hippocampal volumes.
Stein *et al.*, 1997	21 adults with PTSD vs 21 non abused controls	MRI morphometry	5% smaller L-hippo. volumes in abused subjects. Total hippo. volumes ↓ in abused with ↑ PTSD symptom severity than those with ↓ severity.
DeBellis *et al.*, 1999	44 maltreated children and adolescents vs 61 healthy controls	MRI morphometry	No differences in hippocampal volumes PTSD group had ↓ intracranial and cerebral volumes.

Table XIX-9.1B Functional neuroimaging studies in PTSD

Authors	Subjects	Methods	Findings
Semple *et al.*, 1993	6 combat PTSD and substance abuse vs 7 normal controls	PET-Auditory CPT and word generation	PTSD group exhibited ↑ rCBF in orbitofrontal cortex during auditory CPT and word generation.
Rauch *et al.*, 1996	Mixed gender, 8 PTSD subjects	PET-Symptom provocation with script-driven imagery	Provoked vs control: ↑ rCBF in ACC, R-OFC, insula, Ant. Temp. and visual cortex and right amygdala. ↓ rCBF decreases in L Brocas and L middle temporal cortex. No comparison group.
Shin *et al.*, 1999	8 F with PTSD (childhood sexual abuse) vs 8 trauma controls with out PTSD	PET-Symptom provocation with script-driven imagery	Trauma tic vs neutral contrast: Anterior paralimbic activation in both groups. Group by condition interaction: PTSD group showed significantly > rCBF increases within ant. temp. and OFC. > rCBF increase within ACC in controls than PTSD group.
Bremner *et al.*, 1999a	10 F with PTSD (childhood sexual abuse) vs 12 trauma controls without PTSD	PET-Symptom provocation with script-driven imagery	↓ recruitment of ACC in PTSD group.
Bremner *et al.*, 1999b	10 Vietnam vets. with PTSD vs 10 Vietnam vets. without	PET-Responses to trauma-related pictures and sounds	PTSD group showed ↑ rCBF in medial PFC (subcallosal gyrus) and ACC.
Liberzon *et al.*, 1999	14 Viet. Vets. With PTSD, 11 vet controls, 14 healthy nonvets	SPECT-Study	Combat sound vs. white noise: All three groups showed activation of ACC/MPFC. PTSD group showed activation in L amygdaloid region.
Bremner *et al.*, 1997b	10 combat vets. With PTSD vs 10 nonvets, no PTSD	PET-Yohimbine (Y) challenge	Y administration associated with increased anxiety and panic symptoms and widespread decreased ↓ CBF in PTSD group.
Shin *et al.*, 1997	7 vets. With combat PTSD and 7 matched trauma exposed vets. without	PET-Cognitive activation: neutral, negative, combat pictures perception and imagery	Combat imagery vs control: PTSD group showed ↑ rCBF in R-amygdala and ventral ACG and ↓ rCBF in L-Inf. frontal gyrus (Brocas).
Rauch *et al.*, 2000	8 combat vets. with PTSD vs 8 control combat vets	fMRI-Study Fearful and happy faces masked temporally by neutral faces	> amygdala activation in PTSD group than control. Magnitude of activation correlated with PTSD severity.

(especially for the traumatic event/s). Conscious appreciation of trigger stimuli may not be necessary to trigger symptomatology. Rauch *et al.* (2000) have previously demonstrated that subjects with PTSD have hyperactive amygdalar responses to emotional facial expression even when subjects were unaware of seeing them (using a backward masking technique).

Functional neuroimaging studies in PTSD have involved symptom provocation studies and neurocognitive probes of specific areas implicated in the pathophysiology of PTSD. Upon exposure to reminders of their traumatic event (script-driven imagery or trauma-related pictures and sounds), patients with PTSD have been shown to exhibit abnormal activity in the anterior paralimbic areas (OFC, ACC) as well as subcortical circuitry involving the amygdala (Rauch *et al.*, 1996; Shin *et al.*, 1999; Bremner *et al.*, 1999b; see Table XIX-9.1B for other functional neuroimaging studies in PTSD).

Semple *et al.* (1993) using an auditory continuous performance test (CPT) and word generation task with PET, reported increased rCBF in the orbitofrontal cortex in six veterans with combat PTSD and substance abuse (vs seven healthy controls). Using a Yohimbine challenge PET study Bremner *et al.* (1997b), reported increased anxiety and panic symptoms and widespread decreased rCBF in 10 combat vets with PTSD (vs 10 healthy controls). Rauch *et al.* (2000), probing amygdala function using fearful and happy faces masked by neutral faces, found greater amygdala activity in the PTSD subjects (vs controls) to masked fearful faces. In this study, the magnitude of amygdala activation correlating with PTSD severity.

Thus some of these studies provide positive evidence for the amygdalocentric hypothesis in the genesis/maintenance of PTSD symptoms. Neuroimaging advances provide an attractive method of testing hypotheses regarding other brain structures in PTSD.

PANIC DISORDER (PD)

PD is one of the most extensively studied of the anxiety disorders, in part due to occurrence of discrete characteristic symptoms, familial segregation, response to specific pharmacological agents and demonstration of the panicogenic effects of specific agents. PD is characterized by mostly spontaneous discrete attacks of terrifying anxiety with cognitive, affective and autonomic hyperarousal. In between attacks, the patient often experiences significant anxious apprehension, in anticipation of the next attack. Contextual avoidance completes the picture and contributes to most of the disability. A comprehensive theory of PD should explain the genesis of spontaneous recurrent panic attacks and associated symptom states such as anticipatory anxiety, agoraphobia and avoidance behaviour.

Panic disorder could be viewed as a result of a normal threat-related system being repeatedly inappropriately triggered/governed by a dysfunctional threat detection system, or a threat response system gone awry, responding excessively to minor or innocuous stimuli. An attractive hypothesis of the genesis of spontaneous panic attacks, is the possibility of subconscious processing of threat-related stimuli by a hyperresponsive amygdalar system, resulting in

a full blown panic attack. There is evidence in healthy subjects for amygdalar recruitment while viewing fear-related stimuli (Whalen *et al.*, 1998) and hyperresponsivity to masked fearful stimuli has been demonstrated in subjects with PTSD (Rauch *et al.*, 2000). According to this model, one of the major processes in PD may be hyperresponsivity of the amygdala circuitry to environmental cues processed without consciousness, resulting in the characteristic panic response without an apparent context (Rauch and Shin, 2002). This remains to be demonstrated in panic disorder.

Phenomenological similarities between panic symptoms and fear-conditioned responses in animals have drawn attention to analogous brain pathways in humans. A deficit in the relay and coordination of upstream (cortical) and downstream (brainstem) sensory information is hypothesized in PD (Gorman *et al.*, 2000). This may result in a hypersensitive amygdalar network, which could inappropriately trigger the brainstem and hypothalamic autonomic and stress neuroendocrine centres causing the characteristic acute panic symptoms (akin to the fear-conditioned response in rats). Cognitive misattribution and contextual fear may result from the involvement of the OFC and hippocampus respectively. The therapeutic effects of serotonergic medications may be explained by their general transmission damping effect in the subcortical and brain stem areas while cognitive therapies may work by enhancing the top-down control of the OFC over the amygdala and related subcortical structures (Gorman *et al.*, 1989, 2000).

Neuroimaging research in subjects with PD has employed diverse modalities. In one qualitative MRI study, the frequency of gross structural abnormalities has been reported to be greater in the PD group (40%) than in the control group (10%; Fontaine *et al.*, 1990; see Table XIX-9.2A). Resting state neuroimaging studies in patients with PD (including those vulnerable to lactate-induced panic), have suggested abnormal hippocampal activity (Reiman *et al.*, 1986; and Table XIX-9.2B), One group has reported post clomipramine treatment normalization of abnormally low left/right asymmetry in hippocampal activity in subjects with PD (Nordahl *et al.*, 1990, 1998). Symptom provocation studies (with pharmacological challenges) have revealed reduced activity in widespread cortical regions, including the prefrontal cortex, during symptomatic states (Reiman *et al.*, 1989; and Table XIX-9.2C). Magnetic resonance spectroscopy studies have reported greater brain lactate levels in response to hyperventilation and lactate infusions (Dager *et al.*, 1995, 1999).

Table XIX-9.2A Structural neuroimaging studies in panic disorder

Authors	Subjects	Methods	Findings
Fontaine *et al.*, 1990	31 patients with PD vs 20 matched controls	Qualitative MRI study	Frequency of gross structural abnormalities: PD group (40%) > control group (10%).
Vythilingam *et al.*, 2000	13 with PD vs 14 control subjects	Quantitative volumetric methods	Sig. smaller mean vols. of L and R-temporal lobes in PD vs controls (normal hippo. volumes).

Table XIX-9.2B Functional neuroimaging studies in panic disorder (PD)

Authors	Subjects	Methods	Findings
Reiman *et al.*, 1986	16 patients with PD and 25 controls	PET-Neutral state study	In patients vulnerable to lactate-induced panic ($N = 8$), abnormally low left/right ratios of parahippo. blood flow.
DeCristofaro *et al.*, 1993	7 treatment-naive with PD vs 5 age-matched controls	SPECT-rCBF at rest	PD (vs control) group showed ↑ rCBF in L-occipital cortex, and ↓ rCBF in the B/L-hippo. areas.
Nordahl *et al.*, 1990	12 PD patients vs 30 controls	PET-FDG while engaged in an auditory CPT	PD group exhibited a lower left/right hippo. rCMRGlu ratio.
Nordahl *et al.*, 1998	IMI-treated subjects with panic disorder	PET-FDG Follow-up study (1990): same methods	R-shift in rCMRglu symmetry within hippo. and post. inf. frontal cortex. IMI-treated group showed ↓ rCMRglu in post. OFC.
Bisaga *et al.*, 1998	6 F with PD and 6 matched controls	PET-FDG	PD subjects showed ↓ rCMRglu in the L-hippo. and parahippo. area.

Table XIX-9.2C Symptom provocation studies in panic disorder

Authors	Subjects	Methods	Findings
Stewart *et al.*, 1988	10 with PD vs 5 controls	SPECT-xenon inhalation r CBF during lactate infusion	PD subjects who had lactate-induced panic attacks ($n = 6$) displayed global cortical CBF ↓.
Woods *et al.*, 1988	6 patients with PD vs 6 controls	SPECT-Yohimbine (Y) infusions	PD group: Y administration increased anxiety and decreased rCBF in bilateral frontal cortex.
Reiman *et al.*, 1989	17 patients with PD vs 15 controls	PET-rCBF during lactate infusions	Patients who had lactate-induced panic ($n = 8$) had ↑ rCBF in B/L temporopolar cortex and B/L insula/claustrum/putamen. Controls and PD patients without lactate-induced panic did not display these changes.
Fischer *et al.*, 1998	Case report	Spontaneous panic attack	↓ rCBF R-orbitofrontal, prelimbic (area 25) ant. cingulate, and ant. temp. cortex.

Consistent with prevailing neurobiological models of PD, it is possible that fundamental abnormalities in monoaminergic neurotransmitter systems, originating in the brain stem, underlie the abnormalities of metabolism, haemodynamics, and chemistry found in widespread territories of cortex. Further, regional abnormalities within the medial temporal lobes provide some support for theories regarding hippocampal or amygdala dysfunction in PD.

OBSESSIVE COMPULSIVE DISORDER (OCD)

The cardinal manifestations of OCD are recurrent, intrusive, egodystonic thoughts, images and urges portending adverse consequences leading to feelings of anxious dread. Patients therefore engage in mental and motor compulsions (repetitive ritualized thoughts or acts) in an attempt to 'neutralize' the obsession/adverse consequence. They often spend many hours a day engaged in these symptoms, leading to significant distress and potential disability. OCD is considered a group of heterogeneous neuropsychiatric disorders. Characteristic neuropsychological dysfunction on tests of organizational strategy and memory, purportedly subserved by the OFC, has been described in OCD patients (Savage *et al.*, 2000). This may explain symptoms of doubt as well as cognitive inflexibility especially related to risk assessment often seen in OCD.

Current animal models are unsatisfactory and have proved of limited value in elucidating OCD pathophysiology. Much of our understanding of functional neuroanatomy implicated in OCD comes from neuroimaging evidence (Rauch and Baxter, 1998). The results of several morphometric MRI (mMRI) studies done in OCD have suggested abnormalities in the volume of the caudate nucleus, though findings between studies have been inconsistent (Scarone *et al.*, 1992; see Table XIX-9.3A for mMRI studies in OCD). Neutral state PET and SPECT paradigms have most consistently reported increased activity in the OFC and ACC areas in OCD subjects versus controls (Baxter *et al.*, 1988; Swedo *et al.*, 1989; see Table XIX-9.3B for other neutral state studies in OCD). Importantly, pre/post treatment studies have reported a treatment-related decrease of activity in the OFC, ACC and CN, regardless of treatment modality (i.e. both for pharmacotherapy with serotonergic reuptake inhibitors, and cognitive behaviour therapy) (e.g. Baxter *et al.*, 1992; Schwartz *et al.*, 1996; and Table XIX-9.3B). Studies designed to investigate predictors of treatment response have yielded impressively consistent results: lower activity within OFC at the pretreatment time point is associated with a positive response to treatment with SRIs while higher left OFC activity predicted a better response in the behavioural therapy responders (Swedo *et al.*, 1989; Brody *et al.*, 1998; Saxena *et al.*, 1999). PET and fMRI symptom provocation studies have shown increased activity in the anterior/lateral OFC, ACC and CN during the provoked state (Rauch *et al.*, 1994; Breiter *et al.*, 1996b). More recently investigators have used neurocognitive probes to study brain areas implicated in OCD. One such task used is the serial reaction task, which leads to implicit or procedural learning demonstrated by shortening reaction times without the subjects' conscious knowledge. Implicit/procedural learning has been shown to be subserved by the striatum, while the medial temporal lobes have been demonstrated to be preferentially involved in explicit or conscious learning. Rauch *et al.* (1997) in a PET study of implicit sequence learning, reported failure of OCD subjects to normally recruit striatum like healthy controls, instead recruiting the medial temporal lobes typically associated with explicit processing (see Table XIX-9.3B for summary of the various functional studies in OCD).

On the basis of evidence from neuropsychiatric disorders effecting the basal ganglia, together with brain imaging data, contemporary models of OCD focus on medial orbitofrontal-striatal-thalamo-cortical circuits (Rauch and Baxter, 1998). According to this scheme striatal dysfunction leads to inefficient thalamic gating, resulting in hyperactivity within the OFC and cingulate cortex. This could result in the intrusive cognitive phenomena and the associated anxiety. Extending this line of thought, compulsions could be conceptualized as repetitive mental and motor behaviours performed to recruit the striatum so as to ultimately achieve thalamic gating (ultimately transiently neutralizing the anxiety and thoughts; Rauch and Shin, 2002).

OCD also provides a more direct approach to investigating functional neuroanatomy, through study of patients undergoing specific neurosurgical lesions for refractory OCD. Rauch *et al.*, (2001) reported a hypermetabolic focus in the posterior cingulate (PET-FDG) to predict better response in refractory OCD patients undergoing stereotactic MRI guided cingulotomy. Ongoing Deep Brain Stimulation studies in OCD (Greenberg, personal communication), involving indwelling electrodes in the anterior limb of the internal capsule, may yield crucial functional neuroanatomic information.

SOCIAL PHOBIA (SoP) AND SPECIFIC PHOBIAS (SpP)

The phobic syndromes involve an excessive, irrational fear of relatively innocuous and usually non-threatening stimuli. Two subtypes of social phobia (also known as social anxiety disorder) are generally recognized, namely, the generalized type and performance-related type. In the generalized subtype, patients experience excessive and irrational fear of negative social scrutiny in most unfamiliar social situations. In the performance subtype the

Table XIX-9.3A Structural neuroimaging studies in OCD

Authors	Subjects	Methods	Findings
Scarone *et al.*, 1992	20 patients with OCD (mixed gender) vs 16 matched controls	MRI morphometry	↑ right caudate volume in OCD group.
Robinson *et al.*, 1995	26 patients with OCD (mixed gender) vs 26 matched controls	MRI morphometry	B/L ↓ caudate volumes in the OCD group.
Jenike *et al.*, 1996	10 F patients with OCD vs matched controls	MRI morphometry	Trends toward a R-shift in caudate volume ($p = 0.06$) as well as overall ↓ caudate volume ($p = 0.10$) in OCD group.
Aylward *et al.*, 1996	24 patients with OCD (mixed gender) vs 21 matched controls	MRI morphometry	No significant differences in striatal volumes.
Rosenberg *et al.*, 1997	19 treatment-naive children with OCD vs 19 matched controls	MRI morphometry	↓ striatal volumes in the OCD group, and inverse correlation between striatal volume and OCD symptom severity.

Table XIX-9.3B Functional neuroimaging studies in OCD

Authors	Subjects	Methods	Findings
Neutral State			
Baxter *et al.*, 1987	14 OCD vs 14 controls and 14 MDD	PET-FDG	OCD: metabolic rates were significantly ↓ in the L-orbital gyrus and B/L in the caudate nuclei. ↑ C/H ratio B/L in treatment responders.
Baxter *et al.*, 1988	10 OCD vs 10 age and sex matched controls	PET-FDG	↑ rCMRGlu in whole cerebral hemispheres, heads of caudate, OG and O/H ratio similar to previous study.
Machlin *et al.*, 1991	10 OCD vs 8 controls	SPECT	OCD group had higher medial-frontal/whole cortex ratio, negatively correlated with anxiety.
Nordahl *et al.*, 1989	8 OCD vs 30 controls	PET-FDG	Higher norm. rCMRGlu in R-OFC and L-ant. OFC in OCD vs controls.
Rubin *et al.*, 1992	10 OCD (adult males) vs 10 age-matched controls	SPECT Xe-133 rCBF and 99mTc-HMPAO	No difference in rCBF with Xe-133. OCD group had ↑ 99mTc uptake in B/L parietal and OFC and L-posterofrontal regions.
Swedo *et al.*, 1989	18 adults with OCD (childhood onset) vs matched controls	PET-FDG	OCD group had ↑ rCMRglu in the L-OF, R-sensorimotor, and B/L prefrontal and ACC. Significant correlation between CMRGlu and OCD severity.
Pre/post Rx			
Baxter *et al.*, 1992		PET-FDG	R-C/H ratio significantly ↓ post Rx (Fluoxetine and CBT).
Benkelfat *et al.*, 1990	8 OCD subjects before and on CMI	PET-rCMRGlu	Significant ↓ in L-caudate rCMRglu in responders vs non responders.
Hoehn-Saric *et al.*, 1991	6 drug free OCD subjects before and during fluoxetine	SPECT	Rx. significantly ↓. symptoms and associated with ↓ medial-frontal/whole brain ratio.
Perani *et al.*, 1995	11 OCD subjects before and after SSRI; 15 age-matched controls	PET [18F]FDG	rCMRGlu significantly ↑ in CC, thalamus and basal ganglia. Post Rx YBOCS improvements associated with B/L ↓ rCMRGlu in CC.
Schwartz *et al.*, 1996	9 OCD before and after 10 weeks of ERP	PET-rCMRGlu	Significant ↓ in B/L-caudate rCMRGlu in ERP responders.
Swedo *et al.*, 1992	Repeat scans in 13 OCD subjects (10 on meds. at least $\times 1$ year)	PET-FDG	Significant ↓ in norm. rCMRGlu in B/L-OF areas. R-OFC rCMRGlu partially correlated with symptom improvement.
Predictors of response			
Brody *et al.*, 1998	27 OCD Before and after 10 ± 2 wks. of BT ($n = 18$) or fluoxetine (F) ($n = 9$)	PET-FDG	↑ norm. rCMRGlu in L-OFC predicted better treatment response in BT group but worse outcome in F group.
Saxena *et al.*, 1999	20 OCD before and after 8–12 weeks of paroxetine (P) 40 mg per day^{-1}	PET-FDG	Paroxetine responders had significant ↓ in rCMRGlu in R-ant. Lat. OFC and caudate. Lower B/L-OFC rCMRglu predicted better improvement in OCD Sx.
Swedo *et al.*, 1989	18 adults with OCD (child onset) and matched controls	PET-FDG	OCD group had ↑ rCMRglu in L-OF, R-SM, and B/L PF and ACC. Significant correlation between rCMRGlu and OCD severity.
Symptom provocation			
McGuire *et al.*, 1994	4 OCD patients	PET H2 15 O	Significant correlation between Sx. severity and rCBF in R-IFG, caudate, parietal, globus pallidus and thalamus and L-hippo and PCG. Also neg. correlation in R-sup. PFC and T/P junction.
Rauch *et al.*, 1994	8 OCD	PET O 15 CO_2 rest and provoked	Sig. ↑ in rCBF during OCD state vs rest in R-caudate, B/L-OFC and L-ACC.
Breiter *et al.*, 1996b	10 OCD and 5 controls	fMRI/control and provoked	Most OCD subjects showed activations in OFC, ACC, insula, caudate and amygdala.
Cognitive activation			
Rauch *et al.*, 1997	OCD vs normal	PET-FDG: Serial Reaction Time task	Both groups showed implicit learning. However OCD group recruited B/L medial temporal areas rather than ventral striatum (seen in controls).

R = Right, L = Left, B/L = Bilateral, M = Male, F = Female, OFC = orbitofrontal cortex, mPFC = medial prefrontal cortex, OG = orbital gyri, O/H = orbital/hemispheric ratio, PCG = posterior cingulate gyrus, T/P = Temporo-parietal, C/H = caudate/hemispheric ratio, ACC = anterior cingulate cortex, Hippo. = hippocampus, PET = Positron Emission Tomography, fMRI = functional Magnetic Resonance Imaging, SPECT = Single Photon Emission Computed Tomography, rCBF = regional cerebral blood flow, rCMRglu = regional cerebral metabolic rate (glucose), Norm. rCMRglu = Normalized regional cerebral metabolic rate (glucose).

phobic symptoms are circumscribed around performance in specific social situations such as public speaking. In specific phobias, the feared object could be animate or inanimate (APA, 1994). Symptoms of anticipatory anxious apprehension, anxiety and panic symptoms and avoidance of triggering situations are common to both disorders. The subtype of blood injury phobia, differs in part from the other phobias, due to a vasovagal syncopal response to blood exposure (Marks, 1988).

Currently, there are no cohesive functional neuroanatomically based models for the phobias (Stein, 1998). Phobias could be viewed as learned aversive responses to specific stimuli or situations, resembling fear conditioning in animals. From a

Table XIX-9.4A Neuroimaging studies in social phobia (SoP)

Authors	Subjects	Methods	Findings
Potts *et al.*, 1994	22 SoP subjects and 22 matched controls	MRI morphometry: Total cerebral, caudate, putaminal and thalamic volumes	The groups did not significantly differ on any of these measures.
Stein *et al.*, 1996	SoP subjects and healthy controls	SPECT-study Neutral state	No significant between-group differences in rCBF.
Birbaumer *et al.*, 1998	7 SoP subjects and 5 controls	fMRI: exposure to neutral human faces or aversive odours	Compared to controls, the SoP group exhibited human face specific amygdalar hyperresponsivity.
Schneider *et al.*, 1999	12 SoP subjects and 12 controls	fMRI classical conditioning paradigm: neutral faces (CS) paired with negative odour and odourless air (US) s	In response to conditioned stimuli associated with the negative odour, the SoP group displayed signal ↑ within amygdala and hippo., whereas controls displayed signal ↓ in these regions.

Table XIX-9.4B Neuroimaging studies in specific phobias (SpP)

Authors	Subjects	Methods	Findings
Mountz *et al.*, 1989	7 small-animal phobics vs 8 controls	PET	Phobics exhibited increased HR, RR and subjective anxiety during exposure to phobic stimuli. No changes in rCBF measurements were observed.
Wik *et al.*, 1993	6 patients with snake phobias	PET exposure to neutral, generally aversive, and snake-related scenes (videotapes)	Phobic condition was associated with significantly ↑ rCBF in secondary visual cortex and ↓ rCBF in prefrontal, post. cingulate and ant. temporopolar cortex, and hippocampus.
Rauch *et al.*, 1995	7 subjects with a variety of small-animal phobias	PET *in vivo* exposure	Provoked vs control condition: ↑ rCBF within multiple ant. paralimbic territories (R-ant. cingulate, R-ant. temporal pole, L-post. orbitofrontal and L-insular cortex), L-somatosensory cortex, and L-thalamus.

neuroanatomical perspective, phobias could represent the product of dysregulated threat detection systems for detecting potentially threatening stimuli or situations. Ongoing studies are actively investigating such hypotheses (Rauch and Shin, 2002).

More recently, contribution from animal research on attachment and affiliative behaviour, social subordination stress and environmental rearing have provided a partial yet useful framework to investigate social phobia (for reviews see Stein, 1998; Mathew, Coplan and Gorman, 2001). Surprisingly few neuroimaging studies exist in this area (see Table XIX-9.4A). In the only morphometric study published to date, Potts *et al.* (1994) compared the total cerebral, caudate, putaminal and thalamic volumes in 22 subjects with SoP vs 22 matched controls and found no differences. More recently Birbaumer *et al.* (1998), used fMRI to study seven SoP subjects and five healthy controls while they were exposed to slides of neutral human faces or aversive odours. The SoP group showed human face specific amygdalar hyperresponsivity compared to the control group. In another fMRI study, Schneider *et al.* (1999) presented 12 subjects with SoP and 12 healthy controls with paired conditioned (CS; neutral facial expressions) and unconditioned stimuli (US; negative odour vs unmanipulated air). The SoP group showed signal increases within the amygdalar and hippocampal in response to the CS paired with the negative odour while controls showed signal decreases in these regions. These findings, while in need of replication, suggest that hyperresponsivity of the medial temporal lobes may be one of the candidate neural substrates for SoP.

It is appealing to try to understand specific phobias from the viewpoint of conditioned fear studies in animals. This is supported by the fact that behavioural therapy for the phobias involving exposure and desensitization (akin to 'extinction') has been used to effectively treat many patients. While this perspective clearly does not explain aetiology or pathophysiology, the resemblance of components of these disorders to animal fear conditioning should trigger a closer look at analogous brain circuits. In a PET-symptom provocation study, Rauch *et al.* (1995) reported increased activity in prefrontal, posterior cingulate, anterior temporopolar, hippocampal and secondary visual areas as well as in multiple anterior paralimbic territories in seven subjects with small-animal phobias. These findings may reflect a hypersensitive threat detection and response network (therefore triggered by relatively innocuous stimuli). However, as is evident in Table XIX-9.4B, the few symptom provocation studies in SpP report inconsistent findings and are hard to meaningfully interpret. More work is clearly needed in this area.

GENERALIZED ANXIETY DISORDER (GAD)

GAD is, in some sense, the prototypical anxiety disorder. Patients usually have pervasive anxiety symptoms pertaining to, but not confined to, two or more life areas for at least a 6 month period. However, 'free-floating' anxiety and pervasive anxious dread is harder to characterize objectively. Perhaps consequently, genetic evidence and biological evidence in GAD is less compelling.

Rodent experiments involving the fear potentiated acoustic startle response (fear-like; conditioning dependent, shows 'extinction') vs the light-enhanced startle response (anxiety-like; unconditioned, slow onset and rate of decay, reduced by anxiolytics) show that these processes are subserved by different neural systems. Based on these findings, it is hypothesized that the bed nucleus of the stria terminalis (BNST) may subserve the anxiety-like phenomena, while the CeA subserves fear phenomena and may not contribute directly to anxiety. Stress (fear)-induced phasic release of CRH by

the neurons in the CeA may lead to long-term activation of the BNST (Davis, 1998). Thus by extension, hypersensitivity of the BNST pathway may be manifested by a state of chronic generalized anxiety with generalization to many innocuous stimuli (internal and external). This remains to be tested in humans with GAD. Neuroimaging evidence is scant and preliminary at this stage. In a recent MRI volumetric study in 12 children with GAD (compared with 24 matched controls), De Bellis *et al.* (2000) reported that the right and total amygdala volumes were significantly larger in those with GAD, all other volumes not differing significantly. The implications of these findings are currently unclear.

Thus no coherent neurocircuitry model exists currently for GAD. Chronic hyperactivity of the danger assessment and response circuits may underlie GAD. However, it is not known if this may be common to all anxiety disorders or could be a process specifically related to GAD.

DISCUSSION AND FUTURE DIRECTIONS

Anxiety disorders are common and are associated with considerable comorbidity (Regier *et al.*, 1998). A better understanding of the neural mechanisms of adaptive as well as pathological anxiety and fear promises to enhance the development of more effective preventive and therapeutic strategies. Advances in animal fear research provide a springboard to related research in humans. The advent of sophisticated neuroimaging techniques promises to accelerate progress in this field, by providing a means for probing human functional anatomy non-invasively.

Based on animal research, the subnuclei of the amygdala have emerged as the central component of the early, rapid processing of danger-related stimuli. The rich connections between the subnuclei of the amygdala and the sensory thalamus, the orbitofrontal and sensory cortices, the hypothalamus and brainstem areas as well as the striatum have been shown to subserve the various characteristic components of the fear response. The amygdala is a crucial component in stimulus-reinforcer association learning and possible modulation of other relevant brain areas. The cellular and molecular basis of fear conditioning and emotional learning and memory are being vigorously investigated. Of specific interest are the processes of plasticity, kindling, LTP and at a cellular level, NMDA receptor and voltage gated calcium channel mediated signal transduction (LeDoux, 2000b).

In healthy human volunteers, induced transient states of anxiety and fear are associated with increased activity in anterior paralimbic regions and decreased activity in heteromodal association cortex (Rauch and Shin, 2002). These findings are in need of further refinement given that other experimentally induced emotions (such as anger and guilt) also activate overlapping pathways (Dougherty *et al.*, 1999; Shin *et al.*, 2000). The processes of habituation and extinction of fear responses, explored via novel functional neuroimaging paradigms, are relevant to the understanding of normal and pathological anxiety, as well as therapies that rely on desensitization.

Functional neuroimaging studies, combined with specific human brain lesions, implicate the amygdala and associated structures in the innate processing of fear-related faces. The medial frontal and OFC are implicated in the top-down governance of the amygdala and related structures. The anterior cingulate cortex is important in attention and choice making, the brain stem nuclei and lateral hypothalamus in the control of the somatic reactions to stress and anxiety, and the striatal regions in mediating automated response routines, as well as influencing gating at the level of the thalamus (Rauch and Shin, 2002). The insular cortex, which has rich connections with the amygdala, is commonly activated in anxiety-related studies. However, most work has implicated the insula in functions related to taste, including the perception of disgust (Phillips *et al.*, 1997, 1998). Its role in fear and anxiety is less clear.

The convergence of different modes and fields of inquiry are enabling growth from simple to more complex models befitting the nature of human anxiety/fear mechanisms. This increased sophistication also reflects an integration of data across scales — from the systems level to the molecular level. In the area of PTSD, evidence from animal fear conditioning and stress research, clinical traumatology as well as innovative neuroimaging paradigms in patients have helped to increase our understanding of the PTSD syndrome. Thus, the amygdala in functional connectivity with the OFC, hippocampus, the hypothalamus as well as brainstem regions, are thought to mediate the characteristic symptoms of the disorder. The contemporary neurocircuitry model of OCD has been derived from the neuropsychiatry of the basal ganglia as well as from neuroimaging studies (Rauch and Savage, 1997; Saxena and Rauch, 2000). Abnormal activity in the orbitofrontal-thalamo-striato-cortical circuits has been shown to normalize with clinical response to both drug and behavioural treatments. With the emergence of deep brain stimulation as an experimental treatment modality for OCD, there is the potential for learning much about the implicated circuitry in human subjects. In PD, panic attacks accompanied by anticipatory anxiety and avoidance suggest involvement of the amygdala, the medial temporal lobes (specifically hippocampus) and functional connections with the OFC and brainstem areas (Gorman *et al.*, 2000). Currently, neurocircuitry models for the phobic disorders and GAD are less coherent, although preliminary, contemporary neurocircuitry models of anxiety disorders facilitate hypothesis-driven research in this domain. While delineating the functional neuroanatomy of psychiatric diseases is extremely important, these neural substrates must be understood in the context of interdigitating environmental influences. The interactions between genetic/temperamental predisposition of individual neural systems with environmental events (e.g. trauma during early neurodevelopment, stressful life events) are important in the development and maintenance of anxiety disorders.

Further research across modes of inquiry and across scales is required to understand the neural substrates of normal relevant brain functions as well as the pathophysiology of anxiety disorders. An integrated approach combining animal studies, molecular biology, genetics, clinical psychiatry, behavioural neurology, cognitive neuroscience, and neuroimaging is essential to make meaningful progress in this field. Ultimately, the hope is for better preventive and therapeutic options to help people who might otherwise suffer from anxiety disorders.

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XIX-10

In Vivo Functional Neurochemistry of Anxiety Disorders

Andrea L. Malizia, Brian Martis and Scott L. Rauch

INTRODUCTION

Human anxiety disorders are the most prevalent psychiatric conditions affecting at least two fifths of the population in their lifetime. About one in 20 of the population has enduring or recurrent anxiety disorders and therefore these conditions are responsible for the highest societal global (medical and social) costs of any psychiatric condition. For instance, out of 92 million working days lost in the UK. due to mental illness in 1993 (18% of all lost days), 49% were due to anxiety or stress representing a cost of over £3 billion (approximately ∈/$ 4.5 billion).

In order to understand the aetiology of these disorders, possibly leading to better treatments, research has been carried out on the biological basis of healthy and pathological anxiety. Compared with other emotions and other psychiatric conditions, anxiety and fear (as defined in Martis *et al.*, Chapter XIX-9) are constructs which have a reasonable mapping between animals and man. Therefore, the study of anxiety and fear in preclinical experiments can provide leads for human research. Animal experiments have generated two overlapping sets of information. One set describes the sufficient or necessary neuroanatomical structures underlying the expression of these emotions (whether innate or conditioned) in animals and the other the neurochemical changes which predispose to or accompany the behavioural changes. However, despite the similarities in anxiety expression between man and other animals, data from preclinical experiments are not sufficient to understand the biology of human anxiety or anxiety disorders.

Direct human experimentation has also contributed to increase our knowledge about brain function and anxiety. Yet, the traditional investigation of human brain processes *in vivo* has many constraints related to the inaccessibility of the tissue under study and to functional complexity whereby understanding of individual modules from lesion studies, pharmacological challenges and electrophysiological recordings cannot provide sufficiently comprehensive hypotheses of system architecture. Indeed, up to the late 1980s, the most informative experimental strategies employed in clinical psychopharmacology recorded behavioural, physiological and cognitive responses to pharmacological probes where the aim was to characterize the central neurochemical changes underlying particular processes or diseases based on preclinical knowledge. These challenges were, and are, limited by their intrinsic inability to characterize neural networks in detail and by the fact that ligand binding and neurotransmitter release cannot be quantified *ex vivo* or by microdialysis in man (except recently in very selected samples of neurosurgical patients). These limitations prevent the conduct of any quantitative human research which aims to relate changes in physiology or behaviour to synaptic parameters. Further, many of the probes used to selectively affect one system or one subset of receptors have often subsequently been discovered to be relatively less selective than originally postulated.

Since the late 1980s, human imaging (Table XIX-10.1) has been used to describe the functional anatomy (discussed in Chapter XIX-9), pharmacology and functional neurochemistry (this chapter) of human anxiety and anxiety disorders with macroanatomical (up to about 1 cm) brain resolution. The use of these technological advances is still in its infancy as novel paradigms and analytical methods are developed; however, the vision for the future is that their utilization should lead to a more robust understanding of the brain mechanisms underlying disease and response to treatments.

This chapter has two aims: a brief commentary on the technical issues related to pharmacological imaging and a review of the current human psychopharmacology imaging knowledge regarding anxiety and anxiety disorders, including some preliminary data.

PHARMACOLOGICAL IMAGING

Two strategies can be employed to detect drug effects on the brain: detection of changes in brain metabolism or activation induced by pharmacological agents and radioligand assay of binding to receptors, transporters, enzymes and of tracer kinetics of precursor pools.

Changes in Brain Metabolism or Activation

The paradigms used here depend on the detection of changes in regional brain metabolism or blood flow following the administration of pharmaceuticals. The principles are as follows.

- Changes in local brain metabolism are mostly induced by changes in neuronal activity; while there is debate on the cellular location of the metabolic changes (i.e., neurons or glia), energy is mostly expended at synaptic sites.
- Changes in local metabolism are tightly linked to changes in local blood perfusion, which overcompensates for the increases in oxygen demands by delivering an excess of deoxyhaemoglobin.
- Imaging techniques can measure changes in local metabolism ([^{11}C] glucose PET or ^{18}fluorodeoxyglucose (FDG) PET), in local perfusion ($H_2{}^{15}0$ PET or $C^{15}O_2$ PET or [^{11}C]butanol PET; ^{99}Tc HMPAO SPECT; ASL (arterial spin labelling) or gadolinium MRI), in local deoxyhaemoglobin concentration (fMRI), in oxygen extraction ($^{15}O_2$ PET) or in local blood volume ($C^{15}O$ PET).

One complicating factor in the interpretation of these techniques is the fact that pharmacological manipulations have effects not only on the brain processes of interest but also on other neuronal or

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Table XIX-10.1 Types of human imaging used for investigating *in vivo* neurochemical processes in the human brain. Note that the significant parameters are derived from the primary measures by using mathematical and statistical models

Imaging modality	Technique	Primary measure	Deduction/physiological significance
MRI	Functional MRI	Deoxyhaemoglobin signal	Change in perfusion.
	Spectroscopy	Specific spectral signal	Total concentration of molecule of interest (GABA, lactate) in whole brain.
PET	Water, butanol	Radioactive counts	Perfusion or change in perfusion.
	Fluorodeoxyglucose (FDG)	Radioactive counts	Regional integrated (over time) glucose analogue transport into brain tissue.
	Receptor binding	Radioactive counts	Binding potential or volume of distribution (related to B_{max}/K_d).
	Labelled precursor	Radioactive counts	Local brain transport of precursors. Relationship with transmitter synthesis unproven.
	Enzyme binding	Radioactive counts	Regional enzyme concentration.
	Transporter binding/uptake	Radioactive counts	Regional transporter availability.
SPET	HMPAO	Radioactive counts	Change in perfusion.
	Receptor binding Transporter binding	Radioactive counts	Relative binding potential or volume of distribution. (related to B_{max}/K_d) or transporter availability.

glial processes and on the innervation to the cerebral vasculature (adventitia). Further, the signal associated with pharmacological modulation of specific brain activations is often very small and the statistical techniques needed to detect these changes have to be more sensitive than ones often employed in simple activation experiments. Newer strategies will improve the quality of data generated. These include the following.

- Comprehensive evaluation of the cerebral effects of the drugs under study using more than one imaging modality and detailing a number of the physiological responses. This strategy would measure changes in regional metabolism as well as in perfusion, or measure changes in magnitude and distribution of activation with control tasks as well as with tasks of interest or compare metabolic maps with maps of changes in radioligand binding. In the few occasions where such combinations of data have been acquired in the past, important differences between changes in regional metabolism and regional perfusion have been detected.
- The inclusion of other measured physiological responses in the analysis, thus allowing detection of the relationship between changes in regional brain activity and another physiological parameter of interest such as saccadic eye movement parameters.
- Use of statistical methods such as path or network analysis which are more sensitive to small predicted changes in activity.

Measure of Radioligand Binding

The principle of these studies is that a ligand is administered which is labelled with a radioactive nucleus. The time course of the distribution of radioactivity in the tissue of interest is recorded with SPET or PET cameras and the data is analysed with an appropriate mathematical model (Gunn, Gunn and Cunningham, 2001) in order to obtain the parameters of interest. Since the signal recorded is a tomographic, time dependent measure of radioactivity in a particular image segment or voxel, it is made up of a number of components (Figure XIX-10.1) comprising free and bound ligand and metabolites in the blood and tissue of interest. Thus the identifiability of the measure of interest depends on a number of factors (Table XIX-10.2) mostly related to radioligand properties. These factors explain why there are very few PET/SPET radioligands that can be successfully used to measure brain chemistry *in vivo* in man despite the fact that thousands have been developed.

Radioligand binding can be used to measure a number of processes.

- Receptor density and affinity. Often a composite measure of these is used such as binding potential or volume of distribution. These are proportional to B_{max}/K_d. Composite parameters are used because a measure can be obtained by doing only one scan and without the injection of significant amounts of cold ligand (Mintun *et al.*, 1983). A number of receptors have thus been measured including dopamine D1 and D2, serotonin 1A, 2 and 2A, $GABA_A$ benzodiazepine, substance P (NK1) and opiate.
- Changes in neurotransmitter concentration. This is deduced by measuring the change in binding potential as receptor availability increases or decreases with changes in neurotransmitter release. So far this strategy has only been successful in measuring dopamine and possibly opiate release.
- Precursor pools — this method has been used to measure the concentration of radiolabelled fluoroDOPA and alpha-methyl-L-tryptophan in the brain. The interest in these measures is that they are thought to relate to precursor pool and rate of synthesis of derivative neurotransmitters such as dopamine and serotonin. The demonstration that they are more likely to be measuring local brain uptake than any other process (Shoaf *et al.*, 2000) recommends caution in the interpretation of results obtained when using these techniques.
- Transporter density. This technique has been used successfully to measure dopamine (Laruelle *et al.*, 1997) and to a lesser extent serotonin reuptake sites.
- Enzyme concentrations. So far this technique has been used to measure MAO in the brain.
- Occupancy or inhibition of any of the above.

NEUROCHEMISTRY OF ANXIETY DISORDERS *IN VIVO* IN MAN

Studies in this area have been scarce to date with most of the effort being concentrated on the $GABA_A$ benzodiazepine receptor. In the receptor binding arena, this has been in part due to practical issues related to the availability of good radiotracers. Preclinical work indicates that investigation of the ascending monoaminergic systems (dopamine, serotonin, noradrenaline), of some peptidergic transmitters such as substance P and the NK1 receptor and CCK, of adenosine receptors and of benzodiazepine-GABAergic activity

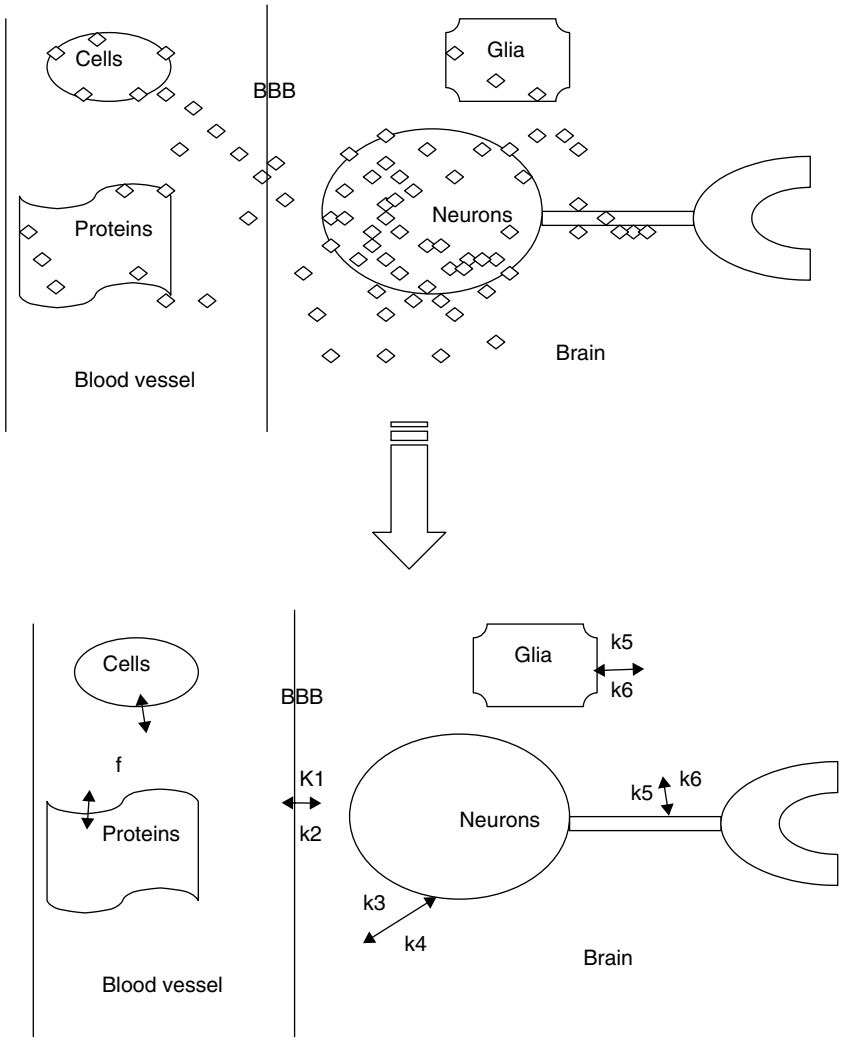

Figure XIX-10.1 (Upper panel) Schematic representation of radioligand (◇) topography. In the vasculature the radioligand is partly bound to cells and proteins. Once it crosses the blood–brain barrier (BBB) it may bind to specific sites on the neurons and sometimes glia and to non-specific sites on neurons and glia. In addition it can exist in the interstitial spaces. (Lower panel) The same scheme with processes as described by common symbols. f is the free fraction in plasma. K1/k2 represents the constants characterizing movement to and from tissue, k3/k4 describes the rate constants for specific binding and k5/k6 the rate constants for non-specific binding

Table XIX-10.2 Necessary characteristics of radioligands for pharmacological imaging in the brain. The essential feature is that the signal recorded is from the whole of the tissue and vasculature *in vivo* and therefore the radioligand characteristics are stringent in order to make it interpretable

Essential and desirable characteristics	Significance
Radioligand crosses blood–brain barrier easily	Allows measurement of total brain concentration of radioligand.
Radioligand specific binding is at least 3× non-specific binding	Allows measurement of specific binding and changes in specific binding.
Radioligand has no radioactive metabolites **OR**	Signal becomes very difficult to model mathematically if radioactive metabolites
Radioactive metabolites do not cross blood–brain barrier	are present in the tissue.
Radioligand has no metabolites which also bind to receptor of interest	*Avoids potential for displacement by metabolite*.
Radioligand has high affinity (low K_d)	*Mean transit time in tissue has to be of same order of magnitude as timing of imaging*.
Radioligand binds to one receptor only **OR**	Resolution of signal specific to one site of specific binding is otherwise not
If to multiple receptors, these have distinct anatomical distribution	possible.
Radioligand has no physiological actions at injected concentrations	Pharmacodynamic effects may affect pharmacokinetic parameters.
Radioligand has high specific (radio) activity	Must inject tracer doses of radioligand (<2% occupancy) for tracer kinetics to apply.
Appropriate reference activity can be obtained from plasma or tissue	Separation of parameters depends on being able to account for signal which is not specific.

would be the most relevant for human anxiety disorders. However, only some of the above can be currently explored in terms of receptor binding. This section of the chapter will examine the extant data, organized by neurotransmitter system and disease.

$GABA_A$ BENZODIAZEPINE

Benzodiazepines, first discovered in the 1950s, were found in the 1970s and 1980s to act by modulating GABA chloride ionophores, thus increasing the effectiveness of local neuronal inhibition. Benzodiazepines and GABA potentiation are known to have an immediate effect on anxiety in man and therefore investigation of their binding in the brain is a field of interest (Nutt and Malizia, 2001). The $GABA_A$ ionophore is a structure made up of five subunits; benzodiazepines in clinical practice bind to receptors expressing $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha5$ subunits. This includes iomazenil and flumazenil, two benzodiazepine antagonists routinely used to image these receptors in man with SPET and PET respectively. Further, other agents that bind at these sites are being developed, which have differential affinity for some of the subtypes (e.g., Ro 15-4513 which has higher affinity for $\alpha5$-containing receptors). In addition, GABA concentrations can now be measured with MR spectroscopy. Three conditions have been investigated: Panic Disorder, Generalized Anxiety Disorder and Post Traumatic Stress Disorder.

Panic Disorder

Benzodiazepine Binding

The separation of Panic Disorder (PD) from other anxiety disorder followed the observation that PD patients did not respond clinically to benzodiazepines but were treated by adequate courses of tricyclic antidepressants. It is now known that higher doses of benzodiazepines or compounds with higher potency at the $GABA_A$ benzodiazepine site are effective at treating PD; however, the original findings were an indication that PD patients have a lower sensitivity to these compounds. Further experimental evidence was produced, showing that PD patients are less sensitive to benzodiazepines on a number of psychophysiological measures, such as saccadic eye movements to target and suppression of noradrenaline appearance rate (Roy Byrne *et al.*, 1989). These findings, coupled with the observation that FG 7142 (a benzodiazepine site exogenous inverse agonist) is panicogenic in man and the discovery of putative endogenous inverse agonists in man (DBI (Elsworth *et al.*, 1986) and tribulin (Corda *et al.*, 1984)) led to theories which postulated that panic attacks were precipitated by the pathological production of a putative endogenous inverse agonist. However, Nutt and colleagues (1990) discovered that flumazenil, a benzodiazepine site antagonist, which has neutral anxiety effects in control subjects, provokes panic attacks in patients with PD. This disproved the putative benzodiazepine receptor inverse agonist theory and two possible explanations were put forward. The first is that fundamental changes at the $GABA_A$ benzodiazepine receptor result in changed receptor binding/function which alter the effects of flumazenil so that it behaves like an inverse agonist. The second is that flumazenil blocks a putative endogenous agonist which is present in a compensatory function in anxiety disorders and that this endogenous ligand is, however, insufficient to prevent the emergence of panic attacks. Following these leads, a number of investigators have sought to determine benzodiazepine receptor density using Iomazenil SPECT or Flumazenil PET. Many of these studies have considerable methodological shortcomings that prevent the formation of robust conclusions. In particular, early scanning after injection of iomazenil (thus contaminating the data with effects due to brain delivery), inappropriate control groups (for example patients with epilepsy who may themselves have alterations at the benzodiazepine site), comorbidity with other affective conditions and the study of patients who have been treated with benzodiazepines (thus inducing changes in regulation of the site) are the most common problems.

Schlegel *et al.* (1994) were the first to report decreased benzodiazepine receptor binding in panic disorder using Iomazenil SPECT comparing, at 90–110 min post-injection, 10 patients with PD with 10 patients with epilepsy on carbamazepine. The decreases were significant in the occipital and frontal lobes and maximal in the temporal lobes. Kaschka, Feistel and Ebert (1995) studied nine medicated patients with PD *and* comorbid depression with a matched group of medicated patients with dysthymia using Iomazenil SPECT (2 h). Decreases in binding were seen in the inferior temporal lobes both medially and laterally and in the inferior frontal lobes. These changes were already detectable at 10 min post-injection reflecting changes dominated by delivery effects. All participants were on antidepressants. Tokunaga *et al.* (1997) published an elegant technical study, having followed a very rigorous scanning methodology. However, their demonstration of reduced benzodiazepine binding in anxiety patients is limited by the uncertainty associated with the fact that he did not use a standard psychiatric classification. On the other hand, Kuikka *et al.* (1995) using two different SPECT cameras (at 90 min post-injection) studied 17 unmedicated patients with PD and 17 healthy age and sex matched controls using Iomazenil and found an increase in Iomazenil signal bilaterally in the temporal cortex and in the right middle/inferior lateral frontal gyrus, while Brandt *et al.* (1998) showed that patients with PD have a significant increase of benzodiazepine receptor binding in the right supraorbital cortex and a trend to an increased uptake in the right temporal cortex. Both these studies are however also likely to be contaminated by ligand delivery issues. Finally, Bremner *et al.* (2000a) showed a relative decrease in measures of benzodiazepine receptor binding in left hippocampus and precuneus in panic disorder patients relative to controls. The group further observed that panic disorder patients who had a panic attack compared with patients who did not have a panic attack at the time of the scan also had a decrease in benzodiazepine receptor binding in prefrontal cortex. They also reported an increase in benzodiazepine binding in the right caudate, in the occipital lobes, in the middle temporal and in the middle frontal cortex. Although patients were drug free at the time of the scan none of them were benzodiazepine naïve.

In summary, most SPECT studies demonstrate decreases in benzodiazepine binding in panic disorder. However, the direction and location of these changes is not consistent across all studies, probably due to methodological issues.

In the only fully quantitative study of benzodiazepine naïve subjects, Malizia *et al.* (1998) employed [^{11}C]flumazenil PET and found a global decrease in binding in benzodiazepine naive, drug-free patients with PD who had no comorbid conditions and did not abuse alcohol (Colour Plate XIX-10.2). These changes were maximal in ventral basal ganglia, orbitofrontal and temporal cortex. Having corrected for the global decrease in binding, PD patients showed a further statistically significant regional decrease in binding in the inferior parietal temporo-occipital areas which was maximal in the lateral posterior temporal lobes as well as decreases in orbitofrontal cortex and ventral striatum. Decreased binding in thalamus, dorso-lateral and medial prefrontal, medial temporal and cerebellar cortex and vermis were accounted for by the global changes. Another PET study (Abadie *et al.*, 1999) compared patients with a number of anxiety disorders and healthy volunteers using flumazenil PET and a tissue reference technique which is semi-quantitative. The variance of the reference areas was such that the data had to be pooled across subjects in order to avoid dealing with negative binding in the pons. No significant differences were

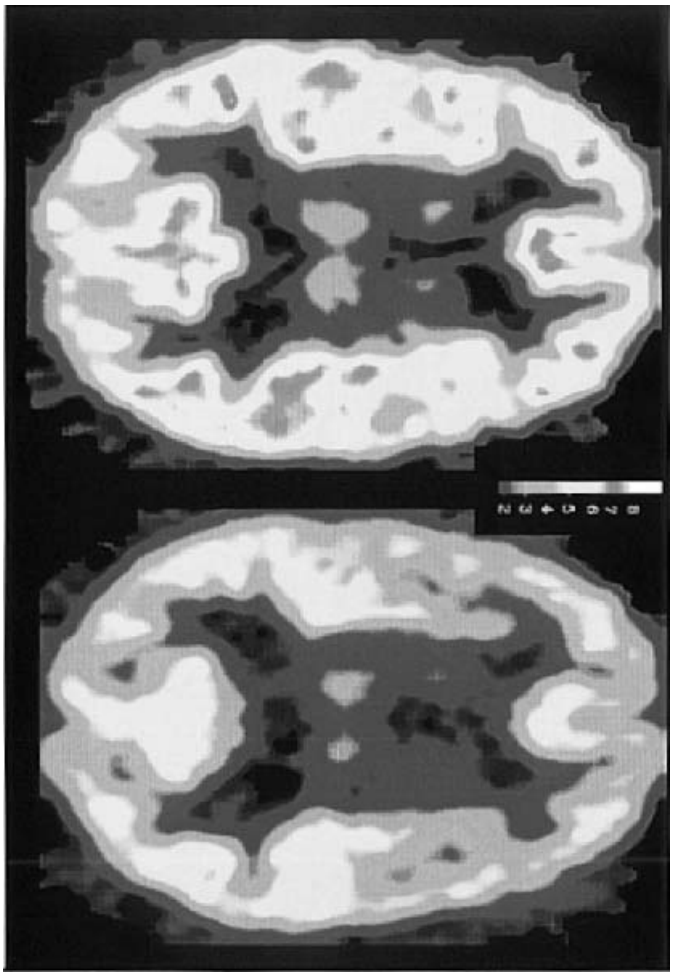

Figure XIX-10.2 A comparison of flumazenil binding between controls and panic disorder patients. The image shows a coloured scale of volume of distribution of flumazenil. The horizontal brain slice is through the middle of the brain showing occipital cortex (left) thalami amd basal ganglia (centre) temporal cortex (middle rim) and frontal cortex (right). A decrease in flumazenil binding is seen throughout the cortex and subcortical structures on the bottom which is the median map for panic disorder patients. The median map for controls is on the top (See Colour Plate XIX-10.2)

found between individual brain areas because of this large variance but for each area examined benzodiazepine receptor density was lower in patients with anxiety disorders.

Decreased benzodiazepine receptor binding is consistent with the idea that panic disorder is due to a deficiency in brain inhibition that leads to, or allows, paroxysmal elevations in anxiety during panic attacks. The peak decreases in benzodiazepine binding are in anatomical areas (e.g., orbitofrontal cortex and insula) thought to be involved in the experience of anxiety in man and could represent a primary pathology. The reduction in binding not only explains some of the known features of benzodiazepine receptor function in panic disorder but is also congruent with animal data showing that chronic stress decreases benzodiazepine binding (Inoue *et al.*, 1985; Weizman *et al.*, 1989, 1990) and that animals with genetically decreased flumazenil binding experience more anxiety (Crestani *et al.*, 1999). It is thus possible that this finding could be the result of experiencing repeated panic attacks or the consequence of one or more of the aetiological factors such as genetic predisposition or life events.

BRAIN GABA CONCENTRATIONS

One of the possible mechanisms by which benzodiazepine $GABA_A$ receptor subunit composition could be altered or downregulated is via a change in brain GABAergic activity. Goddard *et al.* (2001) have demonstrated that patients with panic disorder have a 22% reduction in total occipital cortex GABA concentration (GABA plus homocarnosine) compared with controls. GABA concentration was measured by using MR spectroscopy of occipital cortex *in vivo*; the selection of this area of the brain was dictated by current constraints in data acquisition using this method. This remarkable finding was present in 12 of the 14 patient–control pairs but there were no significant correlations between occipital cortex GABA levels and measures of illness or state anxiety. Clearly, further studies will be needed to determine what the GABA concentration is in areas more germane to the expression of anxiety, but these data provide an interesting model of pathophysiology in panic disorder which may be consistent with reduced flumazenil binding.

Post Traumatic Stress Disorder (PTSD)

Exposure to chronic stress decreases benzodiazepine binding which is maximal in the frontal cortex in some animal experiments (Weizmann *et al.*, 1989). The Yale group (Bremner *et al.*, 2000b) investigated Vietnam veterans with PTSD using Iomazenil SPECT and found that there was a significant decrease in the volume of distribution of benzodiazepine $GABA_A$ receptors in the frontal cortex of these patients in an area which corresponds to Brodmann Area 9. This finding may be of particular significance as this area is involved in extinction of conditioned responses, thus misfunction of local inhibitory circuits could be either a consequence or a predisposition to developing inappropriate responses to trauma.

Generalized Anxiety Disorder

Benzodiazepine binding was also investigated in Generalized Anxiety Disorder using ^{123}I NNC 13-8241 SPECT in Finland where Tiihonen *et al.* (1997) compared 10 patients with 10 age and sex matched healthy volunteers finding decreased benzodiazepine $GABA_A$ binding in the left temporal pole. In addition they found a difference in fractal dimensions of the binding parameters which the authors interpreted as a decrease in variation of cortical receptor density akin to the decrease variability of some heart rate parameters seen in cardiovascular disease. While intriguing, this interpretation is premature as the significance of changes in fractal parameters for binding have not been mapped to cellular or histological differences.

Two papers reported studies of changes in brain metabolism during a vigilance task in patients with generalized anxiety disorders before and after benzodiazepine or placebo administration (Buchsbaum *et al.*, 1987; Wu *et al.*, 1991). Reductions in anxiety could not be mapped to specific changes in cerebral metabolism for the drug group and the usual pattern of benzodiazepine-induced global reductions in metabolism emerged. These types of experiments, however, need to be repeated with more sophisticated analyses as it is likely that detection of the smaller specific modulatory signal is obscured by the global changes.

SEROTONIN (5HT)

Since the discovery of the anxiolytic therapeutic action of $5HT1_A$ agonists and selective serotonin reuptake inhibitors (SSRIs), changes in serotonergic function have been postulated in all anxiety disorders with some authors detailing possible differences in

mechanisms between generalized anxiety and panic (Deakin and Graeff, 1991). Further, polymorphisms at the $5HT1_A$ site have been reported to contribute to variance in human anxiety disorders (Lesch *et al.*, 1992) and other receptor subtypes have been implicated in preclinical anxiety experiments. It may therefore seem surprising that the serotonergic system has been relatively underinvestigated.

Panic Disorder

Two sets of data are available in panic disorder. One relates to $5HT1_A$ binding and one to the effects of SSRI treatment on resting brain metabolism and on activations. $5HT1_A$ receptors are of particular interest as they have functional significance in decreasing raphe firing until functionally downregulated and are thought to mediate the cortical effects of increased serotonergic transmission.

[^{11}C]WAY100635 has been available as a useful PET radioligand to investigate $5HT1_A$ receptors. Following the demonstration of global decreases in benzodiazepine binding, global decreases in cortical and subcortical $5HT1_A$ binding in panic disorder patients when compared with controls have also been demonstrated by the same research group (Sargent *et al.*, 2000). These patients were, however, on active SSRI treatment when scanned and therefore the findings could be a consequence of the therapeutic interventions rather than a correlate of the psychopathology. In this light the findings of an inverse correlation between $5HT1_A$ binding and anxiety trait scores in healthy volunteers (Tauscher *et al.*, 2001) is however intriguing, as it may indicate that low $5HT1_A$ receptor density predisposes to anxiety in man.

The effect of serotonin transporter inhibition on brain metabolism in panic disorder was studied in nine patients successfully treated with imipramine by comparing their resting cerebral metabolism measured via ^{18}FDG PET with healthy volunteers and untreated panic disorder patients (Nordahl *et al.*, 1998). Compared with healthy volunteers, treated PD patients had lower Left/Right hippocampal and prefrontal metabolic ratios (but no difference with untreated PD patients). Lower posterior orbitofrontal metabolism was found in treated patients when compared with healthy volunteers or untreated patients and this effect was ascribed to antidepressant treatment since it was similar to effects observed with the tricyclic imipramine in OCD (Benkelfat *et al.*, 1990). *Post hoc* comparisons revealed that treated patients were no different from controls in the left parietal and left Rolandic areas which were however hypometabolic in untreated panic disorder patients.

Social Phobia

One leading group has employed [^{11}C]5hydroxy-L-tryptophan PET to investigate serotonergic basal metabolism in the brain of patients with social phobia (Marteinsdottir *et al.*, 2001) and has described decreases in signal in the basal ganglia and parts of the limbic system. However, signal with this ligand is closely linked to local transport into the brain (Shoaf *et al.*, 2000) and therefore the significance of these findings will have to be interpreted cautiously.

NORADRENALINE

While the status of noradrenergic manipulation as anxiogenic in preclinical experiments is much debated, there is a considerable body of evidence that $\alpha 2$ antagonists induce anxiety and $\alpha 2$ agonists decrease anxiety in patients with anxiety disorders. This has been mapped as noradrenaline release being anxiogenic while decreases are anxiolytic as activation of the $\alpha 2$ receptors in the locus coeruleus decreases firing. There are no viable noradrenergic ligands to study binding in the human brain *in vivo* but two experiments have been reported which observe brain metabolic changes after the administration of yohimbine (an $\alpha 2$ antagonist) in anxiety disorders.

PTSD

An investigation by the Yale group (Bremner *et al.*, 1997) examined the differences in regional metabolic rate after the administration of yohimbine to patients with PTSD and healthy volunteers. This study demonstrated that yohimbine generated a global small increase in grey matter metabolism in healthy volunteers, while patients with PTSD respond with a moderate global decrease in grey matter metabolism. These effects were maximal in the orbitofrontal cortex and significant in the prefrontal, parietal and temporal cortices and were accompanied by significant behavioural activation in PTSD patients. The authors interpreted this observation as evidence of increased sensitivity to the noradrenergic releasing properties of yohimbine in PTSD, in line with their previous pharmacological observations in this patient group.

Panic Disorder

The same group reported briefly (Woods *et al.*, 1988) on the effects of yohimbine on cerebral blood flow in panic disorder patients. In this study yohimbine provocation of panic resulted in large decreases in HMPAO-SPECT signal in the frontal cortex. The study has however not been described in more detail elsewhere so that a thorough critique is not possible.

DOPAMINE

Dopaminergic manipulation can alter the response to stress in animals and to some extent in man. The evidence for dopaminergic involvement in anxiety disorders is however scanty, both in the treatment and in the aetiology literatures. This may explain why few human imaging studies exist in this arena, despite the fact that there are some very robust dopaminergic radioligands for human work.

Social Phobia

Dopaminergic underactivity and social anxiety have been postulated to be related from animal models, human diseases such as Parkinson's and the effects of dopamine modulating agents (reviewed by Bell, Malizia and Nutt, 1999). Two studies have provided evidence for dopaminergic dysfunction in social phobia by measuring the density of dopamine transporters and D2 (mainly post synaptic) receptors in the basal ganglia of patients with this condition and controls, using SPET. The first study, using ^{123}I β CIT, demonstrated that 11 Finnish Social Phobia patients had decreased binding potential for the dopamine transporter in the striatum (Tiihonen *et al.*, 1997). This was followed by a study by Schneier *et al.* (2000) who demonstrated that 10 New York patients with Social Phobia had lower D2 binding potential than controls. These data suggest three possibilities:

- downregulation of both sites as a trait associated with possible dopaminergic hypofunction;
- increased dopaminergic tonic release, which would decrease the proportion of sites available for radioligand binding;
- mild atrophy of the basal ganglia.

PEPTIDES

CCK 4 and pentagastrin have been demonstrated to be powerful anxiogenic agents in patients with anxiety disorders and in particular with panic disorder. Imaging studies have demonstrated that induction of anxiety in volunteers using these agents activates brain areas thought to be germane to the experience of anxiety (Benkelfat *et al.*, 1995; Javanmard *et al.*, 1999). Further recent data suggests that SSRI treatment of PD patients modifies the brain activation pattern upon infusion of pentagastrin (Boshuisen *et al.*, 2001). These changes are most pronounced in frontal and limbic cortices, but to date it is not clear whether these changes are dominated by the change in anxiety experience after treatment.

SUMMARY AND CONCLUSION

Some human brain imaging techniques are mature and can be used to investigate a number of pharmacological systems in the human brain *in vivo*. While preclinical data have provided useful leads, some of the most striking results have come from investigating hypotheses suggested by observing human disorders. The demonstration of reduced benzodiazepine and $5HT1_A$ binding and reduced GABA concentrations in panic disorder and of dopaminergic dysfunction in the basal ganglia of patients with social phobia are the most notable examples.

It is increasingly important therefore that human imaging should be seen as able to provide leads which can be further validated and explored using preclinical methods. An intriguing example is the demonstration of reduced benzodiazepine binding in panic disorder which was almost immediately complemented by genetic experimentation in mice demonstrating that alterations in $\gamma 2$ and $\alpha 2$ subunits at the benzodiazepine receptors (Crestani *et al.*, 1999; Low *et al.*, 2000) can induce changes such as increased anxiety, decreased sensitivity to benzodiazepine agonists and decreased binding to flumazenil that are also observed in humans with anxiety disorders. Similarly, the demonstration that $5HT1_A$ knockout mice have increased anxiety and decreased flumazenil binding (Sibille *et al.*, 2000) also seems to link the reduced $5HT1_A$ binding seen in panic disorder to benzodiazepine site abnormalities in man and provides a link between the two systems which needs to be further explored.

It is therefore important that these techniques should be increasingly used in biological psychiatry research, as part of a strategy that aims to integrate preclinical and clinical data in a symbiotic cycle of enquiry. The responsibility that human brain imaging researchers face is to use methodology that is robust enough to allow appropriate interpretations of the studies.

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XIX-11

Neurogenetics of Anxiety Disorders

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INTRODUCTION

Family and twin studies show that distinct and/or common genetic factors may play a role in anxiety disorders, although the magnitude of the genetic effect in each of the anxiety disorders is as yet unclear. A genetic liability apparently underlies the phenotypic expression of some of them; however, like other mental disorders, anxiety disorders exhibit a complex inheritance. That is, their transmission most likely requires the interaction of several genes and environmental factors that may predispose individuals to the disorder but do not always lead to its full clinical expression. Anxiety disorders may share a common genetic background with depression, as evidenced by the frequent coexistence of depressive and anxiety symptoms and the response of the anxiety disorders to antidepressant agents. The advances in genetic molecular technology and genetic epidemiology may help researchers to identify the genes contributing to the predisposition to these disorders and to clarify the interaction between genetic and environmental factors. However, in addition to the common difficulties of the molecular genetics of complex diseases, namely, non-Mendelian inheritance patterns, incomplete penetrance, possibility of phenocopies, genetic heterogeneity, and variable expressivity (Lander and Schork, 1994), the major problem in the neurogenetic study of anxiety disorders is the definition of the heritable phenotype (Tsuang, Faraone and Lyons, 1993; Smoller and Tsuang, 1998).

PANIC DISORDER

Family Studies

Family studies have consistently shown that panic disorder (PD), with or without agoraphobia, is a familial phenotype (Tsuang, Faraone and Lyons, 1993; Vieland *et al.*, 1996). First-degree relatives of probands with PD show a 3- to 21-fold higher lifetime risk of the disorder than relatives of unaffected probands. Goldstein *et al.* (1997) found that the risks of PD in adult first-degree relatives of probands was 17-fold higher when the age of onset in the proband was 20 years or less, but only six-fold higher when onset was after age 20 years.

First-degree asymptomatic relatives of patients with PD have a tendency to be more reactive to the CO_2 challenge test (Perna *et al.*, 1996). Furthermore, Perna *et al.* (1996) showed that PD probands with CO_2 hypersensitivity accounted for most of the familial loading. It seems that CO_2 hypersensitivity may be due to a particular genetic dysfunction and individuate a genetically homogeneous subgroup of patients with PD (endophenotype).

Twin Studies

Twin and adoption studies serve as a powerful tool in genetic research. The comparison of concordance rates between monozygotic (MZ) and dizygotic (DZ) twins can help clinicians estimate heritability, which is an index of the contribution of genetic factors to vulnerability to a disorder.

An effect of genetic factors in PD was shown in an early study by Torgersen (1983) who found that PD and agoraphobia with panic attacks were five times as frequent in MZ than in same-sex DZ twins. Ten years later, Kendler *et al.* (1993) assessed 2163 women from a population-based twin registry and noted only a modest familial aggregation of PD on multifactorial-threshold analysis, the best estimates of the heritability of liability ranged from 30% to 40%. In a subsequent study, this team examined the structure of the genetic and environmental risk factors for six major psychiatric disorders (phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism) in an epidemiologic sample of 1030 female–female twin pairs with known zygosity (Kendler *et al.*, 1995). The estimated heritability of PD was 44%. The twin concordancerate for PD was also studied by Bellodi *et al.* (1998) in a sample of 90 same-sex twin pairs. They found that the probandwise concordance rates for PD were significantly higher in the MZ than in the DZ pairs (67% vs 0%), as were the concordance rates for spontaneous panic attacks (71% vs 18%). For CO_2-induced PD, the rates were 55.6% and 12.5%, respectively. These data suggest a relevant role of genetic factors in CO_2-induced panic attacks. The marked differences indicate that the genetic relationship is complex and not simply additive.

Segregation Studies

Segregation analysis of pedigrees determines the mode of transmission of a disorder using mathematical methods. The inclusion of twin pairs or adoptive relatives enables quantification of the degree and nature of environmental effects. Pedigree analyses of PD have suggested that a single major locus contributes to the inheritance of the disorder, although the possibility of polygenic inheritance has not been completely ruled out. Pauls *et al.* (1980) analyzed 19 kindreds of PD patients and found that the disorder is transmitted as a Mendelian autosomal dominant trait with an allele frequency of 0.014, age-dependent penetrance, and average age of onset 21.9 years. Crowe *et al.* (1983), in a preliminary genetic analysis, tested the single major locus and multifactorial polygenic transmission models, and was unable to exclude either one. The best-fitting single-locus model predicted a disorder gene with an allele frequency of 0.05 and a penetrance of 45.5% in women and 24.6% in men. Vieland *et al.* (1996) performed a simple segregation analysis in 126 families of probands

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with DSM-III-R panic disorder and found comparable support for autosomal dominant and recessive models. The best-fitting dominant model predicted a disorder gene with an allele frequency of 0.01, a heterozygote penetrance of 50% and a phenocopy rate of 0.01. The best-fitting recessive model predicted a disorder gene with an allele frequency of 0.20, a homozygote penetrance of 70%, and a phenocopy rate of 0.01 among heterozygotes and normal homozygotes.

Finally, Cavallini and colleagues (1999b) performed a complex segregation analysis on a sample of 165 families of PD probands and on the subgroup homogeneous for CO_2 hypersensitivity. Their results fit a Mendelian hypothesis without distinction between different models of transmission. The analysis of the probands of 134 families found to be hypersensitive to CO_2 supported the existence of a single major locus model with a best fit for a dominant model.

Parent-of-Origin Effect

Since the lifetime risk of developing PD is higher in women than men (2 : 1), researchers have suggested it may be affected by the pattern of maternal vs paternal transmission. Using narrow and broad diagnostic models in a sample of 64 PD pedigrees, Haghighi *et al.* (1999) reported that the proportion of affected children born to a transmitting mother did not differ from the proportion born to a transmitting father, and there was no difference in the frequency of affected offspring from maternal or paternal transmission. However, when a subset of 'pure' pedigrees (including only maternal or paternal transmission of PD) was included under the broad diagnostic model, a significant difference was noted in the proportion of affected females and males from maternal (111 females and 23 males) and paternal (nine females and six males) transmissions. Furthermore, when affected and unaffected children were included in the analysis (excluding individuals with unknown status) the cumulative lifetime risk of PD for offspring of transmitting mothers was significantly higher than that of offspring of transmitting fathers. This finding may indicate that maternal transmission confers an increased susceptibility to PD on female offspring. Apparently, PD has a complex mode of inheritance, though further confirmation of the possibility of genomic imprinting or mitochondrial inheritance is still needed. By contrast, in a study of 38 families unlineally affected with PD and agoraphobia, Battaglia *et al.* (1999) noted no differences in sex distribution between offspring of transmitting mothers and offspring of transmitting fathers, nor was there a significant difference in the anticipation of age at onset of PD. The authors concluded that at least in their sample, there was no evidence of a parent-of-origin effect.

Molecular Genetics

Linkage Studies

As mentioned, some segregation analyses have suggested the involvement of a major gene in the aetiology of PD (Pauls *et al.*, 1980; Crowe *et al.*, 1983; Vieland *et al.*, 1996). These findings have prompted linkage analyses in families with multiple affected individuals. The aim of these studies is to pinpoint disease genes by showing that a genetic marker with a known genetic location tends to be transmitted along with the disease within families (Faraone, Tsuang and Tsuang, 1999).

Crowe *et al.* (1987b) studied 26 families segregating PD for linkage to 29 polymorphic blood group antigens and found a suggestion of linkage to the alpha-haptoglobin locus. However, this locus was later excluded by testing additional families with DNA markers (Crowe *et al.*, 1990). The candidate genes pro-opiomelanocortin (Crowe, Noyes and Persico, 1987a), tyrosine hydroxylase (Mutchler *et al.*, 1990), adrenergic receptor genes ($\alpha 1/\beta$ 2 pair on chromosome 5q32-q34, the $\alpha 2/\beta$ 1 pair on chromosome 10q24-q26, and a second $\alpha 2$ locus on chromosome 4) (Wang, Crowe and Noyes, 1992), Cys311 variant of dopamine receptor 2 (DRD2) (Crawford *et al.*, 1995), DRD4 and dopamine transporter (DAT) (Hamilton *et al.*, 2000a), the GABA-Aâl receptor (Schmidt, Zoega and Crowe, 1993) and GABA α1-5, β1, β3, γ2 (Crowe *et al.*, 1997) and the functional polymorphism in the promoter of the gene for serotonin transporter (5-HTT) (Hamilton *et al.*, 1999) have all been excluded.

Knowles *et al.* (1998) conducted a large-scale study on first-pass genomic screen for PD. They collected 23 multiplex families consisting of 368 individuals, of whom 269 were directly interviewed, and used 540 microsatellite DNA markers for genotyping. The data were analyzed with both a dominant and a recessive model. The authors failed to detect definitive evidence of linkage to PD (no lod scores exceed 2.0 for either dominant or recessive parametric model): they ruled out linkage over more than 95% of the genetic length of the autosomes under the homogeneous dominant model with reduced penetrance, and over 60% under the homogeneous recessive model with reduced penetrance. This team is currently trying to enlarge their sample size, refine the phenotype using an endophenotype (CO_2 sensitivity) (Perna *et al.*, 1996), and look for segregation of comorbid disorders. Since they failed to detect loci that contribute to the predisposition to develop PD in the genome-wide genetic screen, the authors concluded that the genetic structure of the disorder seems to be more complex than the simple autosomal and dominant recessive models. Crowe *et al.* (2001) performed a genome scan of 23 multiplex families of panic disorder, including 90 family members with PD and 23 members who had recurrent, spontaneous panic attacks that did not satisfy the full PD diagnostic criteria. Two-point lod scores were calculated with both a dominant and a recessive model, and maps of lod scores < -2.00, assuming genetic homogeneity, were constructed by using DSM-III-R panic disorder as the affected phenotype. The greatest lod score was 2.23 (theta = 0.15) at the D7S2846 locus, located at 57.8 cM on chromosome 7p.

A 10 cM linkage genome scan, in a set of 20 American pedigrees (153 subjects), ascertained through probands with panic disorder (PD), pointed towards two genomic regions which met criteria for suggestive linkage. One of these regions is on chromosome 1 (LOD score = 2.04) and the other (LOD score = 2.01) is located on chromosome 11p. For agoraphobia, the most promising potential linkage was on chromosome 3. The authors suggested that PD and agoraphobia are complex traits that share some, but not all, of their susceptibility loci (Gelernter *et al.*, 2001).

Association Studies: Candidate Genes

In this approach, candidate genes are selected on the basis of the biology of the disease, and molecular variants are sought. The distribution of different alleles of the candidate genes is compared between affected individuals and a non-related ethnically similar healthy population. If a particular allele is found to occur significantly more often among the affected persons, the researchers assume an association between the disease phenotype and a particular molecular variant of the gene. To overcome the pitfalls of case-control studies, some researchers perform family-based association studies wherein the control group is composed of the parents or siblings of affected individuals. In the haplotype relative risk (HRR) design, the untransmitted alleles of the parents serve as the ethnically matched controls (Stefanos, Dikeos and Papadimitrious, 1996) on the assumption that a variation in the distribution of alleles of the candidate genes can influence the

expression of the genes or its properties. However, it is possible that this variation is not the actual cause of the biological expression but is in linkage disequilibrium with another molecular change (a mutation) which is the primary cause. While one advantage of association studies is their enhanced power to detect genes with small effects, their major disadvantage is the increased likelihood of false-positive results. One way to reduce the probability of first-order errors is to look for replication in an independent sample or verification in family-based internal controls allowing for linkage disequilibrium analysis using the HRR or transmission disequilibrium test (TDT).

Serotonin Transporter and Receptors

The serotonin transporter (5-HTT) regulates the sodium-dependent reuptake of serotonin into the presynaptic neuron. Platelet-binding studies have suggested a disruption in 5-HTT function in PD (Pecknold *et al.*, 1995). The 5-HTT is encoded by a gene (solute carrier six, member four; SLC6A4) on chromosome 17q11.1-q12 and is organized in 14 exons spanning about 13 kb. Recently, a functional deletion/insertion polymorphism was identified within the promoter of the 5-HTT gene (Heils *et al.*, 1996). *In vitro* research has shown that a polymorphism in the 5-HTT promoter region has an effect on gene expression wherein the long (L) allele of 5-HTT produces expression levels three times greater than the short (S) allele. Some researchers have claimed that this polymorphism (the S allele) may play a role in susceptibility to anxiety-related traits and affective disorders (Collier *et al.*, 1996; Lesch *et al.*, 1996). However, when the 5-HTT gene-linked promoter polymorphic region (5-HTTLPR) genotype and allele frequencies were compared between patients with PD and a control group, no differences were observed (Deckert *et al.*, 1997; Ishiguro *et al.*, 1997; Matsushita *et al.*, 1997). In a family-based study, 74 haplotype relative risk 'trios' were genotyped at the polymorphic locus, which consists of a 44 base pair deletion/insertion in the promoter region. There were no significant differences in allele frequencies or occurrence of genotypes within the triads (Hamilton *et al.*, 1999).

A challenge with m-chlorophenylpiperazine (mCPP), a non-selective 5-HT2C receptor agonist, is associated with emergence of panic attacks more frequently in PD patients as compared to normal controls (Germine *et al.*, 1994). Thus, 5-HT2C gene is a candidate genes for association studies in PD. The association between PD and two adjacent polymorphisms [(GT) 12-18 and (CT) 4-5] in the 5′-regulatory region of the X-chromosomal 5-HT2C was studied in German and an Italian sample (combined $n = 211$) of PD patients and compared it with allele frequencies in two ethnically matched control samples (combined $n = 226$) (Deckert *et al.*, 2000). In the German sample, a comparison of female genotypes containing the short polymorphism haplotype vs female genotypes containing only long haplotypes showed a significant difference ($p = 0.01$), However, such a difference could not be replicated in the Italian sample ($p = 0.54$). Thus it seems that these promoter-associated 5-HT2C receptor gene length polymorphisms do not have a major role in the genetics of PD.

Cholecystokinin

Cholecystokinin (CCK) is the most abundant neuropeptide in the mammalian brain and, in humans, it is expressed in significant quantities in all regions of the brain (Bradwejn and Koszychi, 1994a). The CCK receptors have been classified into two subtypes, CCK-A and CCK-B. A line of evidence suggests a possible role of the CCK system in the neurobiology of PD. The CCK receptor agonist CCK-tetrapeptide (CCK-4) has been found to provoke panic attacks in about half of all normal individuals and almost all patients with PD (Bradwejn, Koszycki and Shriqui, 1991). Furthermore, CCK-4-induced panic attacks can be blocked by imipramine (Bradwejn and Koszycki, 1994b), a tricyclic antidepressant which is efficacious in the treatment of PD. Patients with PD also show lower cerebrospinal fluid (CSF) and lymphocyte CCK concentrations than normal controls (Bradwejn, Koszycki and Shriqui, 1991). A search for mutations in the CCK gene in 30 probands of multiplex PD pedigrees was conducted by Wang *et al.* (1998). They identified a C → T transition at position-36 ($CCK_{-36C/T}$) in a GC box, a binding site for transcription factor Sp1, in the promoter region of the gene. However, a study on the function of the $CCK_{-36C/T}$ polymorphism in the human CCK gene promoter (Hansen, Rehfeld and Nielsen, 2000) showed that the C to T polymorphism does not affect CCK transcription or function, and therefore does not play a direct role in the pathogenesis of PD. The putative association of this polymorphism to PD is likely to be the result of co-segregation with a linked mutation. Another study of CCK polymorphism in 99 patients with PD compared to healthy controls matched for gender and ethnicity showed no significant differences in the CCK peptide gene or the CCK-A gene markers (Kennedy *et al.*, 1999). However, there was a significant association of a CCK-B polymorphism with PD, suggesting a role for this gene in the susceptibility to PD. A later study (Yamada *et al.*, 2001) including 91 unrelated Japanese patients and 100 matched controls did not find evidence for an association between PD and four confirmed polymorphic sites in the CCK-B receptor gene, i.e., three at exon 3 : 1550 G → A, 1962 T → C and 1985 G → A and one at intron 4 : 2491 C → A.

In a recent study (Hattori *et al.*, 2001) a polymorphic compound short tandem repeat (STR) stretch located in the 5′-upstream region of the cholecystokinin gene, approximately −2.2 to −1.8 kb from the cap site was identified. This STR was found to be with 10 different allele lengths. Dividing the STR alleles into three classes (Long: L, Medium: M, and Short: S) produced strong genotypic (MM) (nominal $P = 0.0014$) and allelic (M) (nominal $P = 0.0079$) associations with panic disorder. No such association was found with three single nucleotide polymorphisms (SNPs) in the CCK promoter region: −36C > T and −188A > G, and the rare −345G > C. Haplotypic distributions of the STR and SNPs −188 and −36 were significantly different between panic disorder patients and controls ($P = 0.0003$). The authors suggest that the novel STR or a nearby variant may confer susceptibility to the development of panic disorder.

Monoamine Oxidase-A

The monoamine oxidase-A (MAO-A) inhibitor moclobemide, like other antidepressants, has been reported to be effective in the treatment of PD (Tiller, Bouwer and Behnke, 1999). This makes the MAO-A gene, which is localized on chromosome X (Xp21-p11) (Ozelius *et al.*, 1988), a candidate gene for PD.

A possible association between a functional polymorphism in the MAO-A gene promoter and PD was investigated in two independent German ($n = 80$) (Sabol, Hu and Hamer, 1998) and Italian ($n = 129$) (Deckert *et al.*, 1999) samples. Four alleles [3, 3a, 4 and 5 30 base pair (bp) repeats] of the MAO-A gene promoter polymorphism were observed in both samples. The 3a allele contained three repeats plus 18 bp of the repeated motif. An additional rare fifth allele (two 30 bp repeats) was detected in the Italian sample. Functional characterization in a luciferase assay demonstrated that the longer alleles (3a, 4 and 5) were more active than allele 3. In both the German and Italian samples, the longer alleles were significantly more frequent in females with PD compared with female controls; no such significant difference was observed for males. The authors calculated that in females, homozygosity for the long alleles increases the relative risk over heterozygosity as well as homozygosity for the short alleles by factors of 1.4 and 1.8. They suggested that the discrepancy between the males and females may be related to the relatively smaller number of males, so that the

statistical power to display significant differences of the magnitude found in females was very low (~14%). The discrepancy between the male and female patients with PD may also be related to gender differences in the genetic background of PD. The consistency of this finding in two independent samples from different geographic areas makes it unlikely that the association between long alleles of the MAO-A gene promoter polymorphism and PD was a false-positive finding. However, replicative studies as well as verification in haplotype relative risk samples with family-based internal controls are still required. Indeed in one recent family-based study including 620 individuals in 70 multiplex families and 81 triads consisting of proband (62 female and 19 male), mother and father, the authors failed to demonstrate a genetic linkage or association between the same functional promoter polymorphism in the MAO-A gene and PD (Hamilton *et al.*, 2000b). From the pathophysiological and pharmacological points of view, the association between genotype-related high MAO-A activity and PD symptoms (panic attacks, anticipatory anxiety or phobic avoidance) as well as the therapeutic response to MAO-A inhibitors merits further investigation.

α_2 Adrenergic Receptor

Three different genes coding for human α_2 receptor subtypes α_{2A}, α_{2B} and α_{2C} have been cloned and localized on chromosomes 10, 2 and 4, respectively (Bylund *et al.*, 1994). The α_{2A} receptor subtype is expressed in the central nervous system and peripheral tissues, the α_{2B} in the liver and kidneys, and the α_{2C} only in the brain (Lorenz *et al.*, 1990). Yohimbine, an α_2 adrenergic receptor antagonist, has been found to induce marked anxiety and panic attacks in patients with PD (Charney *et al.*, 1987). However, in a study of a polymorphism of the α_{2A} receptor gene in the promoter region at position −1291 in 55 patients with PD and 114 healthy control subjects, Ohara *et al.* (2000) reported no statistically significant difference between the groups in either genotype or allele frequency. These results are in accordance with the linkage study of Wang, Crowe and Noyes, (1992) which revealed no evidence to support the possibility that a genetic mutation at the α_{2A} and α_{2C} receptor loci is responsible for PD.

Adenosine Receptor

The rationale for searching for adenosine receptor gene mutations or polymorphisms in PD is based on the finding that the adenosine receptor antagonist caffeine can provoke panic attacks in patients with PD, and on the similarity between symptoms of caffeine intoxication and anxiety (Boulenger *et al.*, 1984). Deckert *et al.* (1998) screened 38 patients with PD for mutations in the coding sequence of the A_1 and A_{2a} adenosine receptor genes. One silent mutation (716T/G) in the A_1 receptor gene and two silent mutations (432C/T and 1083C/T) in the A_{2a} receptor gene were detected. Thereafter, an association study between the identified DNA sequence variants and PD was performed in an extended sample of 89 patients and matched controls, and it yielded a significant association between PD and the 1083T allele and 1083T/T genotype of the A_{2a} receptor gene. These findings support the hypothesis that the A_{2a} receptor gene, or a locus in linkage disequilibrium with it, confers susceptibility to PD. Yamada *et al.* (2001) in a case-control study in Japanese population did not find evidence for an association between the 1083C → T polymorphism in the A_{2a} receptor gene and PD. Hamilton *et al.* (2001) in a family-based design, employing 596 individuals in 70 panic disorder pedigrees, as well as 77 haplotype relative risk 'triads', examined any linkage or association between the single nucleotide polymorphism in the SP1 site of the CCK gene (in the promoter region) and a simple sequence repeat in the CCK-B receptor, and panic disorder. Employing a variety of diagnostic and genetic models, linkage analysis produced no significant lod scores at either locus. Family-based tests of association, the HRR statistic and the TDT, were likewise non-significant. These two recent studies provide little support for the role of these polymorphisms in CCK and CCK−B receptor in PD.

Dopamine Transporter and D4 Receptor

Challenge studies with dopaminergic agonists such as apomorphine (Pitchot *et al.*, 1992) and epidemiological data associating the use of the dopamine agonist cocaine with PD (Anthony, Tien and Petronis, 1989) provide modest evidence for the possible involvement of a dopaminergic component in PD. A family-based design, with 622 individuals in 70 families, as well as 82 HRR 'triad' families, was employed to detect a possible association between either dopamine transporter (DAT) or dopamine receptor 4 (DRD4) gene polymorphisms and PD (Hamilton *et al.*, 2000a). Three common polymorphisms were studied; two were in DRD4, a 12 bp insertion/deletion in exon 1 and a 48 bp repeat in exon 3, and one was a 40 bp repeat in the three untranslated regions of DAT. Alleles in the triad families were analyzed with the HRR method as well as the TDT statistic. There were no significant differences in allele frequencies or occurrence of genotypes within the triads for any of the three polymorphisms, indicating the absence of involvement of these polymorphisms in susceptibility to PD.

Table XIX-11.1 demonstrates the association studies reported in patients with PD.

PHOBIC DISORDERS

Family Studies

In a direct-interview study of first-degree relatives of probands with social phobia (SP) ($n = 83$) and never mentally ill controls ($n = 231$), Fyer *et al.* (1993) reported that the study group had a significantly increased risk of SP (16% vs 5%, relative risk = 3.12), but not of other anxiety disorders. In a subsequent study, the same authors assessed the rates of phobic disorders in first-degree relatives of four proband groups: simple phobia, SP, agoraphobia with panic attacks, and healthy controls (Fyer *et al.*, 1995). They found a moderate (two- to four-fold increased risk) but specific familial aggregation for each of the three phobic disorders (10% for agoraphobia, 15% for SP and 31% for simple phobia). The relative risk for agoraphobia was 2.7, for SP 3.1, and for simple phobia 3.3. The moderate elevations in familial risk indicate a familial contribution to each of the disorders. The mode of transmission is unknown and seems to be multifactorial complex.

In a direct-interview family study, Stein *et al.* (1998) assessed the familial liability for the discrete (performance-only), non-generalized [performance-only, limited-interactional-only (one or two socially interactive situations), or performance-plus-limited-interactional types of phobia], and generalized subtypes (DSM-IV criteria) of SP in 23 probands with generalized SP and their first-degree family relatives ($n = 106$) and 24 healthy subjects and their relatives ($n = 74$). The generalized subtype of SP was found to be present in 26.4% of the relatives of probands with generalized SP but only 2.7% of the relatives of probands in the comparison group. By contrast, the relative risks for discrete SP and non-generalized SP were not significantly different between the two groups. The authors concluded that only the generalized type (and its probable Axis II counterpart, avoidant personality disorder) occurs more often among the families of probands with generalized SP.

Twin Studies

The genetic epidemiology of phobias was studied in a population-based sample of 2163 female twins (Kendler *et al.*, 1992). The familial aggregation of agoraphobia, SP, situational phobia, and

Table XIX-11.1 Association studies in PD

Candidate gene	Polymorphism	Population	Analysis	Significance	Reference
Serotonin transporter	44-bp insertion/deletion (promoter)	Japanese	Case-control	NS	Ishiguro *et al.* (1997)
		German	Case-control	NS	Deckert *et al.* (1997)
		Japanese	Case-control	NS	Matushita *et al.* (1997)
Cholecystokinin	−36 C/T (promoter)	American	Case-control	$P < 0.05$	Wang *et al.* (1998)
		Canadian	Case-control	NS	Kennedy *et al.* (1999)
CCK-A receptor	New polymorphism	Canadian	Case-control	NS	Kennedy *et al.* (1999)
CCK-B receptor	Single nucleotide polymorphism	Canadian	Case-control	NS	Kennedy *et al.* (1999)
CCK-B receptor	CT repeats polymorphism (promoter)	Canadian	Case-control	$P < 0.004$	Kennedy *et al.* (1999)
Monoamine oxidase-A	30-bp repeats (promoter)	German and Italian	Case-control	Male: NS Female: $P = 0.001$	Deckert *et al.* (1999)
		American	Family-based	NS	Hamilton *et al.* (2000b)
α_{2A} adrenergic receptor	MspI, position — 1291 (promoter)	Japanese	Case-control	NS	Ohara *et al.* (2000)
A_2 adenosine receptor	1083 C/T allele (silent) in exon 2	German	Case-control	$P = 0.01$	Deckert *et al.* (1998)
		Japanese	Case-control	NS	Yamada *et al.* (2001)
Dopamine receptor 4	12-bp insertion/deletion in exon 1	American	Family-based	NS	Hamilton *et al.* (2000a)
	48-bp repeat in exon 3	American	Family-based	NS	Hamilton *et al.* (2000a)
Dopamine transporter	40-bp repeat in the 3 untranslated region	American	Family-based	NS	Hamilton *et al.* (2000a)

bp = base pair; CCK = cholecystokinin

simple phobia appeared to be due to genetic and not familial-environmental factors, with estimates of heritability of liability ranging from 30% to 40%. The authors concluded that the best-fitting multivariate genetic model indicates the existence of genetic and individual-specific environmental aetiologic factors. Nonspecific shared environmental experiences were most important for agoraphobia and SP, and unique environmental experiences for simple phobias. Genetic factors were most important in the predisposition to animal phobia and least important for agoraphobia. Thus, the simple phobias seem to result from the common effect of a modest genetic vulnerability and phobia-specific traumatic event in childhood, whereas agoraphobia and SP result from the combined effect of a more pronounced genetic influence and nonspecific environmental experiences.

Molecular Genetics

Linkage and Association Studies

A line of evidence from molecular genetics studies support the claim that SP, particularly the generalized form, is familial, and is frequently associated with at least one other anxiety disorder and with major depressive disorder. Furthermore, its apparent responsiveness to treatment with selective serotonin reuptake inhibitors (SSRIs) suggest the possible involvement of the serotonergic system in the genetic susceptibility to SP. Stein *et al.* (1998) excluded a possible genetic linkage of generalized SP to the serotonin transporter protein (promoter region) and $5HT_{2A}$ receptor genes. Seventeen multiplex families (122 subjects) were included in the study. In additional studies, the MspI silent T → C 102 polymorphism at the HTR2A gene (Warren *et al.*, 1993) and the insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (Heils *et al.*, 1996) were genotyped, and LOD scores for pairwise analyses were calculated. Linkage studies to other 5-HT receptor subtypes as well as other regions of the genome were not performed as yet.

Gratacòs *et al.* (2001) identified a polymorphic interstitial duplication of human chromosome 15q24-26 (named DUP25), which is significantly associated with panic/agoraphobia/social phobia/joint laxity in families, and with panic disorder in non-familial cases. The authors proposed that DUP25, which is present in 7% control subjects, is a susceptibility factor for a clinical phenotype that includes panic and phobic disorders and joint laxity.

OBSESSIVE–COMPULSIVE DISORDER

The concept of a spectrum of OCD disorders has recently been suggested, including Tourette's syndrome, body dysmorphic disorder hypochondriasis, trichotillomania, pathological gambling, and other impulse control disorders (Hollander *et al.*, 1996). This broader definition of OCD poses problems in defining the phenotype of OCD in family and twin studies as well as in molecular genetic studies. Future research on age of onset, comorbidity, treatment, neuropsychological functioning, personality characteristics and neuroimaging will help to elucidate subsets of endophenotypes of the disorder, and these will broaden our understandings of its neurobiological and genetic mechanisms.

Family Studies

Lenane *et al.* (1990), who interviewed 145 first-degree relatives (89 parents and 56 siblings) of 46 children and adolescents with OCD, found that the familial rate of OCD was higher than the expected rate in the general population (30% of patients had at least one first-degree relative with OCD). Furthermore, there was

a difference in the presenting symptoms of OCD in the probands and their relatives, indicating the possibility of genetic involvement rather than simple social or cultural transmission.

A possible aetiologic relationship between Tourette's disorder and OCD has been shown by several researchers. Leonard *et al.* (1992) re-evaluated 54 children with OCD, of whom 57% also had a lifetime history of tics, 2–7 years after the initial diagnosis of OCD. The presence or absence of tics and Tourette's disorder was also assessed in their first-degree relatives ($n = 171$). At follow-up, 59% ($n = 32$) of the probands were found to have a lifetime history of tics, including eight, all males, who met the criteria for Tourette's disorder. This subgroup had a younger age at onset of OCD. Tourette's disorder was noted in 1.8% of their relatives, and a tic disorder, in 14%. These results indicate that in some cases, OCD and Tourette's disorder may be alternative manifestations of the same underlying illness. In a similar study, Pauls *et al.* (1995) as well as Nestadt *et al.* (2000b) also demonstrated the heterogeneity of OCD, including familial tic-related OCD, familial tic-unrelated OCD, and sporadic OCD.

The familial relationship between OCD and other OCD spectrum disorders was investigated in 80 patients with OCD and their 343 first-degree relatives compared to 73 control probands and their 300 first-degree relatives (Bienvenu *et al.*, 2000). Body dysmorphic disorder, hypochondriasis, eating disorders, nail biting, skin picking, and trichotillomania all occurred more frequently in the case probands and their relatives, whether or not the case probands also had the same diagnosis. These data indicate that these conditions are part of the familial OCD spectrum. However, Black *et al.* (1994), in an earlier study, failed to find significant differences in the lifetime prevalences of anorexia nervosa, bulimia nervosa, or pathologic gambling between first-degree relatives of patients with OCD and first-degree relatives of control subjects.

Twin Studies

Inouye (1965) found an 80% concordance for OCD between pairs of MZ twins ($n = 10$) compared to 20% concordance between pairs of DZ twins ($n = 4$). Higher concordance rates in MZ than DZ twins were also reported by Carey and Gottesman (1981) (87% and 47%, respectively). In addition, the nature of the symptoms and response to treatment were more similar for the MZ twins (Kim, Dysken and Kline, 1990). Unfortunately, all these studies were performed in small samples and did not use blind methodology, so definite conclusions concerning the contribution of genetic factors cannot be made.

Segregation Studies

Cavallini *et al.* (1999a) performed a complex segregation analysis on a sample of 107 Italian families of OCD probands. Based on the hypothesis that OCD shares common underlying genetic factor(s) with Tourette's disorder (Pauls *et al.*, 1986), the authors applied two phenotypic definitions of affected subjects: OCD with and without Tourette's disorder/chronic motor tics. Analysis of the transmission of OCD alone (wherein relatives with Tourette's disorder or chronic motor tics were considered healthy) provided support for a major gene effect. However, when the phenotype was widened, the pattern of inheritance (dominant, recessive or additive) could not be established. Given the phenotypic heterogeneity observed in OCD, Alsobrook *et al.* (1999) studied inheritance patterns using symptom-based factor scores. Analyses limited to families with symmetry and ordering (high factor 3) probands led to a rejection of the polygenic model. Additionally, the relative risk of OCD or subclinical OCD was 1.7 for relatives of probands with a high factor 3 score compared with relatives of probands with a low factor 3 score. In this study patients were defined as having subclinical OCD if they met all DSM-III-R criteria for OCD except one of the following: their obsessions/compulsions consumed less than one hour per day, or they lacked insight into the unreasonable nature of their obsessions and compulsions. The authors suggested that these symptoms may constitute a genetically symptomatic subtype of OCD. In a recent large-scale study with a well-characterized sample, Nestadt *et al.* (2000a) analyzed 80 case families (423 subjects, including adult OCD probands and their first-degree relatives), and 73 control families (373 subjects, including probands and their first-degree relatives) for mode of inheritance. OCD was the only affected phenotype. The results provided further evidence of a Mendelian inheritance of a dominant allele. However, Mendelian factors alone could not fully explain the familial aggregation of this phenotype, and residual familial and sex effects probably play a role in the inheritance of the disorder. Thereafter, polygenic factors may also contribute to the aetiology of OCD. It is of note that this study included both case and control families; thus, the estimated disease frequencies and penetrances were likely less biased.

Molecular Genetics

The family, twin and segregation studies have consistently suggested that the underlying mechanisms of OCD involve genes of major effect. The recent progress in molecular genetic methods has encouraged the search for genes that confer susceptibility to complex diseases, including OCD.

Linkage Studies and Mutation Screening

In a study of a large British kindred multiply affected with Tourette's syndrome, chronic motor tics, and obsessive–compulsive behavior, Brett *et al.* (1995) reported no evidence to support the hypothesis that a genetic variation in the serotonin 5-HT1A receptor and tryptophan oxygenase genes causes susceptibility to Tourette's syndrome and chronic multiple tics.

Interaction between the glutamatergic and serotonergic systems within the striatum suggests the glutamate transporter protein as a functional candidate in OCD. The gene (SLC1A1) for this protein is localized in chromosome 9. Genomic organization of this gene and mutation screening was investigated in families of patients with early-onset OCD (Veenstra-VanderWeele *et al.*, 2001). No evidence was found for a functional mutation and capillary electrophoresis single-stranded conformational polymorphism (SSCP) analysis of a haplotype consisting of two common SNPs within this gene revealed no significant linkage disequilibrium.

Association Studies

The findings of the association studies in OCD are summarized in Table XIX-11.2.

The Serotonergic System

Pharmacological and neurochemical studies point to a dysregulation of the serotonergic system in OCD. SSRIs are effective in the treatment of OCD; exacerbations are common when SSRIs are discontinued (McDougle, Gordman and Price, 1993). Challenge with meta-chlorophenyl-piperazine (mCPP), a mainly serotonin 2C receptor agonist, can induce the emergence of symptoms in patients with OCD (Zohar *et al.*, 1987). Finally, OCD patients have lower platelet serotonin transporter (5-HTT) density than normal controls (Weizman *et al.*, 1986, 1992). It is of note that recent studies have reported an association between a polymorphism in the serotonin transporter gene regulatory region and anxiety-related

Table XIX-11.2 Association studies in OCD

Candidate gene	Polymorphism	Population	Analysis	Significance	Reference
Serotonin transporter	44-bp insertion/deletion (promoter)	European-American	TDT	$P < 0.03$	McDougle *et al.* (1998)
		Caucasian	Case-control	$P = 0.023$	Bengel *et al.* (1999)
		Canadian	Case-control	NS	Billet *et al.* (1997)
		Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
		Afrikaner	Case-control	NS	Kinnear *et al.* (2000)
	VNTR in the 2nd intron	Japanese	Case-control	0.032	Ohara *et al.* (1999)
Tryptophan hydroxylase (TPH)	BfaI	Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
Serotonin receptor 2A	T102C	Mexican	Case-control	NS	Nicolini *et al.* (1996)
	MspI	Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
Serotonin receptor 2C	Cys23Ser mutation	Italian	Case-control	NS	Cavallini *et al.* (1998)
		Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
Serotonin receptor 1Dβ	Silent G861C	Italy	TDT/ sub-TDT	$P < 0.006$	Mundo *et al.* (2000)
Dopamine receptor 2	TaqIA	Canadian	Case-control	NS	Billett *et al.* (1998)
Dopamine receptor 3	Msc I	Italian	Case-control	NS	Catalano *et al.* (1994)
		Canadian	Case-control	NS	Billett *et al.* (1998)
Dopamine receptor 4	48-bp repeat in exon 3	Canadian	Case-control	$P < 0.05$	Billett *et al.* (1998)
		Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
Dopamine transporter	40-bp VNTR	Ashkenazi and Sephardic Jews	Case-control	NS	Frisch *et al.* (2000)
Catechol-0-methyltransferase	G158A	American Caucasians	Case-control	$P = 0.0002$ in male OCD; NS in females	Karayiorgou *et al.* (1997)
		American Caucasians	TDT and HRR	$P = 0.0079$ and $P = 0.0079$ in male OCD; NS in females	Karayiorgou *et al.* (1999)
		American and Canadian	TDT and HRR	NS	Schindler *et al.* (2000)
		Afrikaner	Case-control	$P = 0.0017$	Niehaus *et al.* (2001)
	Promoter region	Afrikaner	Case-control	NS	Kinnear *et al.* (2001)
Monoamine oxidase-A	*Fnu*4H1 polymorphism in exon 8		TDT and HRR	$P = 0.0186$ and $P = 0.0129$ in male OCD; NS in females	Karayiorgou *et al.* (1999)
	EcoRV polymorphism	Mexican	HRR	$P < 0.05$ in female OCD;	Karayiorgou *et al.* (1999)

bp = base pairs; TDT = transmission disequilibrium test; VNTR = variable-number-tandem repeat; HRR = haplotype relative risk.

traits (Lesch *et al.*, 1996) as well as susceptibility to affective disorders (Collier *et al.*, 1996).

Serotonin Transporter

McDougle *et al.* (1998) found evidence of linkage disequilibrium between the serotonin transporter protein gene (SLC6A4) in the promoter region and OCD. The investigators used the TDT design, which examines linkage in the presence of association in affected probands and their biological parents and thereby controls for the stratification effect. The study included 34 European–American family trios, 30 unrelated and four extracted from an extended pedigree. Of the 35 heterozygous parents, 24 transmitted the long (L) allele and 11 transmitted the short (S) allele ($p < 0.03$) to the OCD probands. Separate analysis of the SRI non-responders ($n = 13$) yielded 10 parents who transmitted the L allele and three who transmitted the S allele ($p = 0.052$). Bengel *et al.* (1999) reported an association of a functional polymorphism in the 5-HTTLPR with OCD in a population-based study of 75 Caucasian patients with OCD and 397 ethnically matched individuals. The patients were found to be more likely to carry two copies of the long allele (L) (46.7% vs 32.3%: $\chi^2 = 5.19$, $p = 0.023$). However, this finding did not agree with the study of Billet *et al.* (1997) of 72 patients with OCD and 72 matched controls, which revealed no significant between-group difference in either genotype count or allele frequencies. Furthermore, no significant 5-HTT genetic difference was observed between the patients who responded to SSRIs and those who did not. The possible association of OCD with the insertion/deletion polymorphism in the promoter area of the serotonin transporter gene was also analyzed by Frisch *et al.* (2000) in 77 biologically unrelated OCD patients and ethnically matched controls (Ashkenazi and Sephardic Jews). There was no difference between patients and controls in the allelic distribution of the 5-HTT gene promoter region polymorphism. Similar negative results were reported also in a relatively genetically homogeneous Afrikaner population of South Africa (Kinnear *et al.*, 2000).

A case-control study of a possible association between 5-HTTLPR polymorphism and Tourette syndrome with and without OCD was

carried out by Cavallini *et al.* (2000). The study included 52 patients (53.84% with OCD) and 63 healthy control subjects. No association was found, even when the study sample was divided by the presence or absence of OCD or family history of OCD or tics.

Ohara *et al.* (1999) compared the variable-number-tandem-repeat (VNTR) in the second intron of the 5-HTT gene between 103 patients with anxiety disorders and 106 controls. They found that the frequency of the allele containing 12 copies of the VNTR element (5-HTTin2.12) was significantly higher in the patients with OCD ($n = 15$) and that the presence of the 5-HTTin2.12 allele was significantly associated with the risk of OCD (odds ratio = 10.2, 95% CI 1.34-77.4).

Serotonin Receptors and Tryptophan Hydroxylase

Variants of the genes encoding the serotonin receptors 5-HT2A and 5-HT2C are not associated with OCD. Nicolini *et al.* (1996) studied 5HT2A gene polymorphisms in 67 OCD patients and 54 healthy controls and found no statistically significant between-group differences in either genotype or allele frequencies. Cavallini *et al.* (1998) investigated the role of the Cys23Ser mutation of the 5-HT2C receptor gene in OCD in a case-control study of comparing 109 OCD patients with 107 healthy control subjects. Again, no allelic or genotypic association was revealed. Furthermore, in a subsample of 39 patients with OCD who had previously undergone a challenge test with clomipramine, no association was noted between the 5-HT2C receptor gene mutation and the response to the test. This finding excludes any specific role for the Cys23Ser mutation of the 5-HT2C receptor gene in the aetiology of OCD.

The 5-HT1Dβ is a terminal autoreceptor, involved in the regulation of 5HT release, and it is expressed mostly in the limbic region and in the striatum. It is encoded by an intronless gene located on chromosome 6 (6q14-15) (Demchshyn *et al.* 1992). There are at least three polymorphisms known for this gene: the G861C, the T-261G, and the T371G (Lappalainen *et al.*, 1995; Mundo *et al.*, 2001). Gross *et al.* (1998) reported that acute administration of sumatriptan, a selective agonist of the 5-HT1Dβ receptor, was associated with acute worsening of obsessive–compulsive symptoms in patients with OCD. However, OCD patients resistant to conventional pharmacotherapy have shown improvements with chronic administration of sumatriptan (Stern *et al.*, 1998). Mundo *et al.* (2000) investigated the possibility of a linkage disequilibrium between the 5-HT1Dβ receptor gene G861C polymorphism and OCD using a combination of the TDT, which examines alleles preferentially transmitted from parents to affected offspring, with the sib-TDT, which compares the marker genotypes in affected and unaffected siblings. Sixty-seven OCD probands and their biological parents and siblings participated in the study. After genotyping, 32 families were found suitable for the TDT/sib-TDT procedure. Twenty-two were triads that showed heterozygosity at the marker, and 10 were sibships with one affected and one unaffected sibling. The authors found a significant linkage disequilibrium between the G861C variant of the 5-HT1Dβ receptor gene and OCD, with preferential transmission of the G allele to the affected subjects.

In a case-control study no association was found between OCD and polymorphism in the gene coding tryptophan hydroxylase (TPH), the key enzyme in the synthesis of 5-HT (Frisch *et al.*, 2000).

Dopamine Receptors and Transporter

The involvement of dopamine in the pathophysiology of OCD has been suggested by several pharmacological studies. Addition of dopamine receptor blockers seem to be effective in some SRI-resistant patients with OCD, especially those with comorbid tic disorder or psychotic features (McDougle, Goodman and Price, 1994). Accordingly, cocaine, which blocks the presynaptic dopamine transporter, can aggravate obsessive–compulsive symptoms in patients with OCD and induce such symptoms in subjects with a family history, but not a personal history, of OCD (Satel and McDougle, 1993).

Catalano *et al.* (1994) studied the D3 dopamine receptor gene polymorphisms in 97 patients with OCD and 97 control subjects. No statistically significant differences in allele or genotype frequencies were found. Negative results were reported also by Frisch *et al.* (2000) in a study of the possible association between OCD (75 patients and 172 controls), the D4 dopamine receptor gene and dopamine transporter gene.

In another case-control association study of 100 OCD patients and matched controls, Billett and his group (1998) examined a 40-base-pair repeat in the dopamine transporter gene; the TaqIA polymorphism and the serine/cysteine variation in the D2 dopamine receptor gene; the MscI polymorphism in the D3 dopamine receptor gene; and a 48-base-pair repeat in the D4 dopamine receptor gene. Significant differences in allele frequencies were found between patients and controls only for the D4 receptor gene.

Catechol-O-Methyltransferase

Catechol-O-methyltransferase (COMT) is an Mg^{2+}-dependent enzyme that catalyzes the transfer of methyl groups from S-adenosyl methionine to a hydroxyl group of a catecholic substrate. COMT is widely distributed in the mammalian brain. The gene for COMT, which is involved in the inactivation of catecholamines including the neurotransmitters dopamine and norepinephrine, has been mapped to the 22q11 region (Grossman, Emanuel and Budarf, 1992). In humans, a common functional polymorphism is associated with a three- to four-fold variation in COMT enzyme activity. This variation in activity is due to a GA transition at codon 158 of the COMT gene that results in a valine (high-activity allele, COMT*H)-to-methionine (low-activity allele, COMT*L) substitution (Lachman *et al.*, 1996).

Karayiorgou *et al.* (1997), in a study of 73 Caucasian patients with OCD (42 males and 31 females) and 148 ethnically matched and unrelated control subjects (75 males and 73 females), found that the COMT*L allele was significantly associated in a recessive manner with susceptibility to OCD, particularly in males. Furthermore, the COMT*L/COMT*L genotype was apparently a risk factor for OCD, with an estimated relative risk of 5.91 (95% CI: 2.40-14.53) versus the nonCOMT*L/COMT*L. These findings were later confirmed by the same group in a family-based study (Karayiorgou *et al.*, 1999) wherein the inheritance of the two functional variants of the COMT gene was investigated in 110 nuclear OCD families (affected proband and both biological parents). Both the TDT and HRR analyses revealed a preferential transmission of the low-activity COMT allele from the heterozygous parents of male (but not female) probands. This finding indicates a sexually dimorphic pattern of genetic susceptibility to OCD. Another family-based population study, in a group of 72 North American and Canadian OCD patient/parent trios, using the HRR TDT analyses, did not identify an association between a particular allele and OCD. Furthermore, no evidence was found to support the findings of a gender-based association for COMT. However, a genotype analysis demonstrated a tendency for association between homozygosity at the COMT locus and OCD (Schindler *et al.*, 2000). In contrast, a case-control study in Afrikaner community population found that heterozygosity (COMT*H/COMT*L genotype) was significantly more common than expected in OCD patients ($P = 0.0017$) (Niehaus *et al.*, 2001). No association was found between OCD and a novel polymorphism adjacent to the oestrogen response element (ERE 6) in the promoter region of the COMT gene (Kinnear *et al.*, 2001).

In a recent study by Cavallini *et al.* (2000) of 52 patients with Tourette disorder with or without OCD and 63 healthy control subjects, no association between the val-158-met substitution was noted in the COMT in any of the patients.

Monoamine Oxidase-A

Monoamine oxidases (MAOs) are flavin-containing enzymes that degrade a variety of biogenic amines, including the neurotransmitters norepinephrine, dopamine, and serotonin. Two forms of the enzyme, MAO-A and MAO-B, are encoded by two adjacent genes (Hsu *et al.*, 1989) located at the p11.23-11.4 region of the X chromosome. MAO inhibitors seem to have a beneficial effect in a subset of OCD patients (Liebowitz *et al.*, 1990). Karayiorgou *et al.* (1999), in their sample of 110 nuclear OCD families, found a sexual dimorphic association between OCD and an allele of the *MAO-A* gene (*MAO-A**297CGG allele of the *Fnu*4H1 marker), previously linked to high MAO-A enzymatic activity. This association was detected particularly among male OCD probands with comorbid major depression.

MAO-A/EcoRV polymorphism was examined in a sample of 122 Mexican OCD patients and 124 healthy subjects (Camarena *et al.*, 2001). An excess of allele 1 in OCD females with major depression disorder was confirmed as previously reported (Camarena *et al.*, 1998). This difference was more strongly associated with OCD females than males in the total sample. Additionally, an HRR analysis of the inheritance of the MAO-A variants was performed in a sample of 51 OCD trios. An allelic association between OCD and MAO-A gene was found in the female probands, i.e., 14 out of 19 transmitted the allele 1.

GENERALIZED ANXIETY DISORDER

Family Studies

A study by Noyes *et al.* (1987) demonstrated a higher frequency of GAD among first-degree relatives ($n = 123$) of GAD probands ($n = 20$) compared to first-degree relatives of control subjects ($n = 20$) and of PD ($n = 40$) and agoraphobia probands ($n = 40$). Among the relatives of GAD patients, more women (24.2%) than men (14%) had GAD. The categorization of GAD as a distinct entity was further confirmed by the distribution of anxiety disorders among the families.

Twin Studies

Kendler *et al.* (1995) addressed the possible interrelationship of genetic and environmental risk factors in six major psychiatric disorders (phobia, GAD, PD, bulimia, major depression and alcoholism). The study included 1030 female–female twin pairs with known zygosity, derived from the population-based Virginia Twin Registry. Major depression was found to be the most frequent comorbidity of GAD. On factor analysis, the authors found statistical evidence of two genetic factors: phobia, PD and bulimia loaded heavily on the first factor, and major depression and GAD on the second. A disorder-specific additive gene was present only for GAD and alcoholism. Individual-specific environmental influences on the risk of GAD and major depression were best explained by a single factor and played a strong aetiological role.

In a recent study, the genetic and environmental contributions to GAD and PD were investigated in 6724 MZ and DZ male–male twin pairs taken from the Vietnam Era Twin Registry (Scherrer *et al.*, 2000). The presence of a non-additive genetic factor specific to PD supports a distinction of PD from GAD. The authors suggested that the common genetic and unique environmental influences in GAD and PD may be partially responsible for the high lifetime co-occurrence of these disorders.

Association Studies: Candidate Genes

Serotonin Transporter

Ohara *et al.* (1999) studied the 5-HTTin2.12 polymorphism in 103 patients with anxiety and 106 control subjects. The frequency of the 5-HTTin2.12 allele was significantly higher compared with controls in both the whole group of anxious patients ($p = 0.027$; odds ratio = 2.06, 95% CI 1.09-3.90) and in the GAD patients separately ($p = 0.0123$; odds ratio = 3.61, 95% CI 1.23-10.6).

Serotonin Receptors

Fehr *et al.* (2000a), using a candidate gene approach, genotyped 50 patients with GAD, 209 patients with alcoholism, 108 patients with major depression, 32 patients with PD, 58 patients with narcolepsy, and 74 healthy volunteers for the serotonin HTR1B receptor gene 861G → C polymorphism. This common *Hinc*II polymorphism had been linked to antisocial alcoholism in a Finnish sample and a sample derived from an American-Indian tribe (Lappalainen *et al.*, 1998). A higher frequency of the HTR1B 861G allele was detected among the male alcohol-dependent patients, but not in the patients with other mental disorders, compared to the control subjects. The same group (Fehr *et al.*, 2000b) also genotyped patients with GAD, alcohol dependence, PD without agoraphobia, and narcolepsy and normal healthy volunteers ($n = 173$ females and 298 males) for the 5-HT2C Cys23Ser polymorphism, but no difference in frequencies and genotypes were found between patients and controls. No association was found between GAD and 5-HT2A receptor polymorphism (T102C), as well as the intron 7 TPH (A218C) polymorphism (Fehr *et al.*, 2001).

POST-TRAUMATIC STRESS DISORDER

Family Studies

Reich, Lyons and Cai (1996) investigated familial vulnerability factors to PTSD in male military veterans by examining family history of four proband groups: PTSD, mixed anxiety disorders, coexisting anxiety and depressive disorders, and screened normal controls. The pattern of psychopathology in the families of the PTSD probands most closely resembled that in the families of the coexisting anxiety and depressive disorders probands.

In a family history study of 36 patients with chronic post-traumatic stress disorder, Davidson *et al.* (1985) reported a positive history of familial psychopathology, mostly alcoholism, depression, and anxiety disorders, in 66% of the patients. Subsequently, these authors investigated the relationship between chronic PTSD and family psychiatric morbidity in first-degree relatives ($n = 285$) of 81 female rape survivors with or without lifetime PTSD, 31 major depressive disorder controls, 20 anxiety disorder controls, and 39 healthy controls (Davidson *et al.*, 1998). Information was also available by family history for 639 relatives. An increased risk of depression was noted in family members of PTSD probands with depression but not in relatives of PTSD probands without lifetime depression. The authors concluded that PTSD following rape seems to be associated with familial vulnerability to major depression, which may thus serve as a risk factor for PTSD.

Twin Studies

Male–male veteran MZ twin pairs ($n = 2092$) who were discordant for military service in southeast Asia were investigated to evaluate the impact of military service on PTSD (Goldberg *et al.*, 1990). The prevalence of PTSD was over three-fold higher in the twins who served in southeast Asia than in their co-twins who did not (16.8% vs 5.0%) and nine-fold higher in the twins who experienced high levels of combat (95% CI: 4.8-17.6). The same group (True *et al.*, 1993) also performed a twin study of genetic and environmental contributions to liability for PTSD. The sample included 4042 Vietnam-era veteran MZ and DZ male twin

pairs. Quantitative genetic analysis revealed that inheritance had a substantial influence on liability for symptoms of re-experiencing the trauma (13%–30%), avoidance of stimuli related to the trauma (30%–34%), and increased arousal (28%–32%). The family environment did not have a significant effect on any of the variables. Another twin study by this group (Lyons *et al.*, 1993) on a similar sample ($n = 4029$) studied genetic and nongenetic factors that influence wartime exposure to traumatic events. Specific events examined were volunteering for service in Vietnam, actual service in southeast Asia, a composite index of 18 combat experiences, and awards for combat. Heritability estimates ranged from 35% to 47%. There was no evidence that shared environment had a significant effect on any of the variables.

Association Studies

The Dopaminergic System

Increased levels of 24-hour urine dopamine excretion have been reported in PTSD (Yehuda *et al.*, 1992), and higher than normal levels of 24-hour urine homovanillic acid have been found in sexually abused girls (Debellis *et al.*, 1994; Putnam and Trickett, 1997). Plasma dopamine was also found to be elevated in a small cohort of combat veterans compared to controls (Hamner and Diamond, 1993). Deutch and Young (1995) suggested that PTSD is associated with a dopamine dysregulation which limits the ability of patients to cope with trauma and increases their susceptibility to trauma-related contextual stimuli.

Using a case-control design, Comings *et al.* (1991) investigated the association of PTSD and the DRD2 TaqI polymorphism in 35 European–American patients with PTSD, all with drug or alcohol abuse. The control group was comprised of 314 subjects, 69 of them non-alcoholics. Among the total group of controls, 77 (24.5%) carried the A1 allele, whereas among the non-alcoholic controls, only 10 (14.5%) did so. An increased prevalence of the A1 allele (45.7%) was demonstrated in the PTSD patients compared to the controls. However, after correction for multiple comparisons, the statistical significance was lost. In a subsequent study (Comings, Muhleman and Gysin, 1996) of 56 combat-exposed subjects with ($n = 37$) and without ($n = 19$) PTSD who were hospitalized in an addiction treatment unit, the same authors noted an association between PTSD and the DRD2*A1 allele (59.5% vs 5.3%; $p < 0.0001$). Though this study has an advantage of the inclusion of both PTSD and non-PTSD subjects who were exposed to combat, its small sample size limits the conclusions. Further, a later case-control study conducted by Gelernter *et al.* (1999) failed to demonstrate such an association. The authors noted no allelic association between the DRD2 TaqI 'A1' as well as in the 'B' and 'D' alleles in 52 European-American PTSD patients. Furthermore, the DRD2 haplotype frequencies also did not differ between the patients with PTSD and healthy control subjects. The authors concluded that the DRD2 gene variants do not contribute significantly to the risk of PTSD.

CONCLUDING REMARKS

The pattern and repertoire of behaviors and emotions are determined by gene–environment interactions. Genomic and non-genomic factors influence the risk of emergence of anxiety disorders in individuals and across generations. In addition to the common difficulties in molecular genetics of complex diseases, namely, non-Mendelian inheritance patterns, incomplete penetrance, possibility of phenocopies, genetic heterogeneity, and variable expressivity (Lander and Schork, 1994), the neurogenetic study of anxiety disorders is hampered by the problem of defining the heritable phenotype, including subsets of endophenotypes of the disorders (Tsuang, Faraone and Lyons, 1993; Smoller and Tsuang, 1998). The human genome project, combined with other novel strategies, such as DNA microarrays (Watson *et al.*, 2000), functional magnetic resonance imaging (fMRI) (Rosenberg and Hanna, 2000), and identification of genes and proteins implicated in the brain neurocircuitry of anxiety and fear hold great promise in furthering our understanding of the cellular and molecular mechanisms involved in the pathogenesis of anxiety disorders and developing specific and efficient treatments.

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XIX-12

Gender Differences in Anxiety Disorders

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ANXIETY DISORDERS: INTRODUCTION

Though rarely appreciated, anxiety disorders represent one of the most common psychiatric disorders. Nearly one out of four Americans will meet criteria for an anxiety disorder during their lifetime. Inexplicably, women are much more likely than men to develop anxiety disorders. In fact, lifetime prevalence estimates based on large-scale population surveys conducted within the US suggest that women are two to three times more likely than men to develop panic disorder (7.7% vs 2.9%), agoraphobia (9.0% vs 3.0%), simple phobia (13.9% vs 7.2%), or post-traumatic stress disorder (PTSD) (11.3% vs 6.0%). Lifetime prevalence estimates also suggest that women are 1.5 times more likely than men to develop obsessive–compulsive disorder (OCD) (3.1% vs 2.0%) or social anxiety disorder (16.4% vs 11.2%) (Robins *et al.*, 1984; Bourdon *et al.*, 1988; Breslau, Davis and Andreski, 1990; Regier, Narron and Rae, 1990; Kessler *et al.*, 1994; Leon, Portera and Weissman, 1995; Magee *et al.*, 1996; Yonkers and Ellison, 1996).

Results from the international epidemiological surveys also confirm that anxiety disorders are very common and that women have much higher prevalence rates than men. Although prevalence rates tend to decrease with advancing age, anxiety disorders remain more common in women throughout the life span (Krasucki, Howard and Mann, 1998). There is some evidence that the gender difference for anxiety disorders narrows after the age of 65. This finding, however, may represent an artifact from the combined effects of cumulative, anxiety-related mortality as well as the complex differentiation between anxiety and cognitive impairment. The narrowing of the gender difference may also result from the attenuation of hormonal factors that occurs with advancing age (Krasucki, Howard and Mann, 1998).

The presence of an anxiety disorder has important implications. A lifetime diagnosis of an anxiety disorder is associated with increased functional impairment, diminished educational and occupational opportunities, and elevated morbidity and mortality rates in comparison to the absence of an anxiety disorder. Elevated utilization rates for emergency medical and mental health care services are also linked to the presence of an anxiety disorder. Despite these adverse consequences, very few individuals with an anxiety disorder receive any type of psychiatric treatment (Lindal and Stefansson, 1993; Dick *et al.*, 1994a, 1994b; Kessler *et al.*, 1994, 1997; Weissman *et al.*, 1994; Leon, Portera and Weissman, 1995).

Low recognition rates for anxiety disorders represent a significant obstacle in delaying effective treatments. Somatic symptoms, a cardinal feature of excess anxiety, may effectively obscure their primary psychiatric basis. Most anxiety disorders initially present in a general medical rather than mental health setting. Unfortunately, results from numerous studies confirm that a primary anxiety disorder is unlikely to be considered in a primary care setting until the late stages of a routine diagnostic assessment (Hohmann, 1989; Kennedy and Schwab, 1997; Roy-Byrne and Katon, 1997; Bland, Newman and Orn, 1997b; Fleet *et al.*, 1998). Instead, anxiety is routinely relegated to the position of a diagnosis of exclusion. A number of studies have also suggested that gender differences in the presentation, attribution, and expression of anxiety symptoms may further delay the prompt recognition of anxiety disorders. Results from several studies also suggest that primary care physicians are more likely to attribute anxiety to a mood disorder, even when an anxiety disorder is primary (Rogers *et al.*, 1994).

Anxiety and mood disorders have extensive comorbidity. Lifetime prevalence estimates, in fact, suggest that more than two thirds of anxiety disorder patients will also develop a mood disorder, particularly depression (Kessler *et al.*, 1994). There is also substantial comorbidity between the anxiety disorders. For example, 40% of patients with OCD will also meet criteria for an additional anxiety disorder diagnosis during their lifetime (Rasmussen and Eisen, 1990; Pigott *et al.*, 1994; Hollander *et al.*, 1996a; Antony, Downie and Swinson, 1998). The frequent co-existence of mood and anxiety conditions may also further hinder the prompt and accurate diagnosis of anxiety disorders.

It remains unclear why anxiety disorders are so much more common in women than men. Genetic, biological, developmental, and environmental factors have all been implicated. Since anxiety and depression are both more prevalent in women than men, a shared or similar genetic basis may exist. Results from female twin studies provided some compelling support for a shared genetic diathesis between GAD and depression (Kendler *et al.*, 1992a, 1992b). However, available evidence suggests that the remaining anxiety disorders are characterized by less genetic homogeneity (Kendler *et al.*, 1992c, 1995).

Developmental and environmental factors are also likely to be important in the pathogenesis of anxiety disorders. Histories of childhood trauma or early separation anxiety increase the risk for both sexes that an anxiety disorder will subsequently occur (Young *et al.*, 1997; Stein *et al.*, 1998; Sutherland, Bybee and Sullivan, 1998). There is some intriguing evidence, however, that women may be differentially susceptible to the adverse consequences associated with childhood abuse. Breslau and colleagues have extensively investigated the impact of gender on PTSD. Their results suggest that when exposure rates are similar, women are more likely than men to develop PTSD after the occurrence of trauma. Moreover, a history of childhood trauma is a more reliable predictor in women than men that PTSD will be present as an adult (Breslau *et al.*, 1990, 1997a, 1997b). There is also evidence that gender differences exist in the type of anxiety disorder that may develop in response to chronic environmental stress. Galbaud and colleagues investigated the potential association in psychiatric diagnoses between spouses. They found that a diagnosis of depression, drug addiction, or antisocial personality disorder in one spouse increased the chances that the spouse would meet criteria for an anxiety disorder. However, a different anxiety disorder occurred in the women versus men spouses. That is,

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spouses with drug addiction or antisocial personality disorder were more likely to meet criteria for GAD if men, but PTSD if women (Galbaud-du-Fort *et al.*, 1998).

Although genetic and environmental factors are likely to be important in the development of anxiety disorders, the role of women gonadal hormones may be particularly critical in the pathogenesis of anxiety disorders. Women gonadal steroids, particularly oestrogen and progesterone, can elicit potent biological effects within the central nervous system. In addition, dramatic and cyclical fluctuations in gonadal hormone concentrations routinely occur throughout the female reproductive life span. These unique features may contribute to the finding that women have an increased risk for anxiety disorders. Despite the significant gender differences identified for prevalence rates, remarkably little research has focused on detecting any further gender differences in anxiety disorders. With these issues in mind, this chapter will provide a review of the available data concerning: (a) the potential impact of gender on the epidemiology, phenomenology, and clinical course of anxiety disorders; and (b) the impact of reproductive cycle events on the clinical course of anxiety disorders.

GENERALIZED ANXIETY DISORDER (GAD)

GAD is one of the most common of the anxiety disorders with most estimates suggesting a lifetime prevalence rate of between 5% and 6%. Women are two to three times more likely than men to meet lifetime criteria for GAD. The onset of GAD is typically during late adolescence or early adulthood and most studies suggest that a chronic, persistent course is most common (Boyd *et al.*, 1990; Kessler *et al.*, 1994; Wittchen *et al.*, 1994; Yonkers *et al.*, 1996; Woodman *et al.*, 1999). Although results from most population surveys suggest that anxiety disorders tend to decrease with increasing age, the prevalence rate for GAD remains at a constant rate throughout life (Krasucki, Howard and Mann, 1998).

GAD most commonly presents in the primary care setting (Woodman *et al.*, 1999). A diagnosis of GAD is associated with elevated rates of functional impairment. GAD is also linked to greater levels of medically unexplained symptoms and over-utilization of health care resources. Roy-Byrne and Katon suggest that the increase in health care utilization reported with GAD does not occur by a direct path, but instead by acting as a catalyst that modifies the presentation of other psychiatric disorders that more directly affect health care costs (Roy-Byrne and Katon, 1997). Patients with GAD are also more likely to be prescribed psychotropic medication in comparison to patients without GAD (Wittchen *et al.*, 1994).

In addition to being associated with considerable disability and morbidity, remission rates with GAD are reported to be fairly low. For example, preliminary results from the large, prospective HARP Study indicate that after two years of follow-up only 8% of the patients with GAD were considered symptom-free (Yonkers *et al.*, 1996). A recent systematic comparison of GAD and panic patients revealed similarly dismal results. The GAD patients reported an earlier age of onset and a longer duration of illness. Moreover, significantly lower remission rates were noted in the patients with GAD (18%) than in the panic (45%) patients during the five-year study (Woodman *et al.*, 1999).

Gender Differences in GAD

More than 85% of patients with primary GAD will meet criteria for an additional lifetime psychiatric disorder (Boyd *et al.*, 1990; Kessler *et al.*, 1994; Wittchen *et al.*, 1994). Women with GAD are significantly more likely than men to meet criteria for an additional lifetime psychiatric condition (complicated GAD). Since results from population surveys and prospective studies suggest that complicated GAD has a worse prognosis and reduced chance of remission than GAD alone, this finding may help to explain why women with GAD are reported to have a more chronic course and greater symptom severity than men with GAD (Yonkers *et al.*, 1996). Women with GAD are also more likely than men with GAD to meet criteria for a depressive disorder, especially dysthymia (Wittchen *et al.*, 1994). This finding may also have important consequences since the presence of depression in patients with GAD has been associated with increased functional impairment and a greater risk of suicide in comparison to a diagnosis of GAD alone (Robins *et al.*, 1984; Wittchen *et al.*, 1994; Breslau, Schultz and Peterson, 1995; Bakish, 1999). Preliminary evidence also suggests that women with GAD are more likely to seek treatment with a health care professional than men, especially if comorbid diagnoses are also present (Bland, Newman and Orn, 1997a).

The finding that gender differences exist in GAD may have additional ramifications. The frequent co-occurrence of depression and GAD in women may also provide an important clue in understanding their underlying pathophysiology. That is, their common co-existence may reflect an enhanced vulnerability that is in part mediated by factors distinct to the women gender. While earlier investigations into the preponderance of anxiety disorders in women have tended to emphasize psychosocial and environmental factors, recent research has also investigated the potential role of genetic factors. Kendler and colleagues have investigated these issues by analysing data obtained from bivariate female twins. The female twin pair data provides compelling support for the role of genetic transmission as a primary mediating factor for GAD. The female twin data also suggests that GAD and depression may arise from the same genotype. That is, if a certain genotype is present within an individual, Kendler and colleagues hypothesize that either GAD or depression may develop as determined by a variety of environmental and biological influences (Kendler *et al.*, 1992a, 1992b, 1995).

The pathophysiology of GAD remains undetermined although serotonergic, noradrenergic, and GABAergic dysregulation are commonly implicated (Brawman-Mintzer and Lydiard, 1997). However, a neuroimaging study conducted in GAD patients demonstrated evidence of a more homogeneous distribution of benzodiazepine receptors throughout the cerebral hemispheres in the GAD patients versus control subjects. The reduced heterogeneity in cerebral blood flow associated with a diagnosis of GAD may be similar to the finding of reduced heterogeneity in myocardial blood flow associated with ischaemic heart disease (Tiihonen *et al.*, 1997). Unfortunately, the potential impact of gender on brain function in GAD has not been systematically assessed.

Medications reported to be effective for GAD include buspirone, benzodiazepines, venlafaxine and the SSRI antidepressants (Brawman-Mintzer and Lydiard, 1997). Data derived from animal models of anxiety suggest that response to anxiolytic medication, especially benzodiazepine anxiolytics, may be significantly influenced by gender as well as the phase of the oestrous cycle (Fernandez-Guasti and Picazo, 1990). While these results suggest that important gender differences in treatment response may occur in GAD, systematic data is not available. Unfortunately, the potential impact of gender on brain function in GAD has also not been systematically assessed.

There is some evidence that GAD symptoms become more severe during the premenstrual period in women with GAD and co-existing premenstrual syndrome (PMS). However, no change in symptom severity during the menstrual cycle was detected in the women with GAD who did not have co-existing PMS according to the same report (McLeod *et al.*, 1993). Further information about the impact of the menstrual cycle, pregnancy, or the menopause on the symptoms or course of GAD has not been reported at this time.

These findings indicate that important gender differences exist in the prevalence, clinical features, and comorbid conditions that may complicate GAD. There is some evidence that GAD symptom severity may be influenced by female reproductive hormone cycles but further information, especially about the potential impact of pregnancy and the post-partum period on GAD, is currently absent. Since preliminary evidence suggests that women with GAD have a more chronic course and a worse outcome, systematic data concerning gender differences in underlying pathophysiology and/or treatment response is clearly needed.

PANIC DISORDER

Estimates derived from large-scale community surveys suggest a 1.5%–2.0% lifetime prevalence rate for panic disorder. Women are two to three times more likely than men to meet lifetime criteria for panic disorder (Regier *et al.*, 1988, 1990; Eaton, Dryman and Weissman *et al.*, 1991; Kessler *et al.*, 1994; Dick, Bland and Newman, 1994b). Panic onset is typically during adolescence or early adulthood and is very rare after the age of 40. Men and women have a similar age of onset for panic disorder (Dick, Bland and Newman, 1994b). Despite substantial fluctuations in severity over time, most patients with panic disorder report a chronic clinical course (Joyce *et al.*, 1989; Keller and Hanks, 1993).

A diagnosis of panic disorder has been linked to a number of adverse consequences including increased utilization of medical and mental health services and elevated rates of suicidal behaviour (Weissman *et al.*, 1989; Katerndahl, 1990; Hollifield *et al.*, 1997; Katerndahl and Realini, 1997). Data from the largest prospective study of patients with anxiety disorders to date, the HARP study, confirm that 40% of panic patients will be in treatment at one year and 30%–40% are likely to require long-term, maintenance medication (Keller and Hanks, 1993).

Gender Differences in Panic Disorder

Several significant gender differences have been identified for panic disorder. As previously noted, panic is two to three times more common in women than in men. In addition, the occurrence of panic attacks even in the absence of meeting full criteria for panic disorder may represent a significant development, especially in women. For example, 63% of women and 40% of men that experienced panic attacks subsequently developed an additional psychiatric disorder in one report (Reed and Wittchen, 1998). Gender differences have also been identified in the phenomenology of panic disorder. Women with panic disorder report more individual panic symptoms and greater levels of phobic avoidance in comparison to men with panic disorder (Dick, Bland and Newman, 1994b). They are also more likely to report that leaving home alone or using public transportation triggers their panic attacks. Women with panic disorder are also more likely than men to report that they rely on family members to enter fearful situations. The apparently increased level of dependence on others endorsed by women with panic disorder may help to explain the finding that women with panic have a greater degree of functional impairment in comparison to men with panic (Weissman *et al.*, 1997; Starcevic *et al.*, 1998; Turgeon, Marchand and Dupuis, 1998).

Women with primary panic disorder are more likely than men to meet criteria for an additional lifetime psychiatric disorder (Yonkers *et al.*, 1998). Gender also appears to have a significant impact on the type of comorbid psychiatric diagnosis that will present during the course of panic disorder. Comorbid agoraphobia depression, GAD, simple phobia, and somatization disorder are more likely to complicate panic disorder in women than in men (Katerndahl, 1990; Andrade, Eaton and Chilcoat, 1996; Marshall, 1996; Yonkers *et al.*, 1996, 1998). In contrast, men with panic disorder have greater rates of comorbid alcohol abuse. Alcohol-related disorders are not uncommon in women with panic disorder, however, as illustrated by the finding that women with panic disorder are at an increased risk of developing alcohol abuse or dependence in comparison to women without panic disorder (Otto *et al.*, 1992; Cox *et al.*, 1993; Kessler *et al.*, 1994). Interestingly, preliminary evidence suggests that women with panic disorder may have an elevated rate of relatives with alcohol dependence. Although further research is needed, this and additional findings has resulted in considerable speculation concerning a possible genetic link between panic and alcohol abuse in women (Battaglia *et al.*, 1995; Kendler *et al.*, 1995).

The data suggesting that women with panic disorder have an elevated risk for comorbid psychiatric disorders in general and certain comorbid disorders in particular may have critical implications. The presence of agoraphobia in panic disorder is associated with a less favourable outcome than the presence of panic disorder alone. Since agoraphobia is more common in women than in men with panic disorder, women with panic disorder might be expected to have a worse prognosis. There is evidence supporting this finding. For example, women with panic disorder and agoraphobia report greater phobic avoidance, more catastrophic thoughts, and a heightened awareness of body sensations in comparison to men that have panic disorder with agoraphobia. Women with panic disorder complicated by agoraphobia are also more likely to meet criteria for social anxiety disorder and/or PTSD (Stein *et al.*, 1989; Turgeon, Marchand and Dupuis, 1998). The increased prevalence of agoraphobia in women with panic disorder may also contribute to the consistent finding of a more chronic and severe clinical course for panic disorder in women in comparison to men (Boyd *et al.*, 1990; Hollifield *et al.*, 1997; Katerndahl and Realini, 1997; Joyce *et al.*, 1989; Yonkers *et al.*, 1998). Recent prospective data also suggests that if a period of remission occurs during the course of panic disorder, women have an increased risk for recurrence of panic disorder (Yonkers *et al.*, 1998).

These findings indicate that a diagnosis of panic disorder in women in comparison to men is associated with a more complicated course and an overall poorer outcome. Since the aetiology of panic disorder remains obscure, it is difficult to explain why women have a greater risk and a more malevolent course for panic disorder than men. Numerous neurobiological and psychological theories have been proposed for panic disorder. Most psychological theories emphasize the importance of cognitive misinterpretation and 'false threat alarms' as the basis for the subsequent development of panic disorder (Windmann, 1998). Biological theories, in contrast, implicate altered brain function and an abnormal ventilatory response in the pathophysiology of panic (Klein, 1993; Bell and Nutt, 1998). Neural circuits within the amygdala and its ascending cortical pathways are most often speculated to represent the functional neuroanatomy of panic disorder. Altered dysregulation of the ventilatory response and associated neurovascular instability may also be important factors in the pathophysiology of panic disorder (Coplan and Lydiard, 1998). Both physiological (e.g., CO_2 sensitivity) and psychological (anxiety sensitivity) factors have been suggested as predisposing factors in the development of panic disorder (Coplan and Lydiard, 1998; Stein, Jang and Livesley, 1999). Despite the striking gender differences identified in the prevalence and clinical features associated with panic disorder, evidence of significant gender differences in the purported pathophysiology of panic disorder have not been detected.

Results from covariate female twin studies suggest that genetic or familial factors play a role in panic disorder, although their contribution is estimated to be relatively modest (Kendler *et al.*, 1993, 1995). There is some evidence that genetic factors may be particularly critical in determining risk for panic disorder in

women. That is, the presence of a specific genetic polymorphism in women may convey an increased risk for panic disorder. Women with panic disorder are reported to have a higher occurrence of a novel repeat genetic polymorphism on chromosome X than control subjects. This genetic polymorphism is thought to mediate expression of monoamine oxidase A. Since monoamine-oxidase-inhibiting antidepressants are known to be effective anti-panic agents, this finding has been interpreted as evidence that altered monoamine oxidase A activity may be a risk factor for panic disorder, at least in women (Deckert *et al.*, 1999).

Important gender differences in the presentation, clinical features, and overall course of panic disorder have been identified. Women with panic disorder are more likely to have agoraphobia and other comorbid conditions than men with panic disorder. The elevated risk for comorbid conditions likely contributes to the poorer prognosis reported for women than men with panic disorder. However, the potential contribution of differential avoidance strategies and other clinical characteristics that may influence prognosis in panic disorder requires further study.

Panic Disorder and Female Reproductive Cycles

Oestrogen and progesterone have complex effects on the CNS. Oestrogen is generally considered a facilitator of neurotransmission via its ability to reduce MAO enzyme activity and enhance serotonergic tone. Oestrogen's biological actions are thought to convey some mood-enhancing or antidepressant effects. In contrast, progesterone increases MAO enzyme activity. Pregnanolone, a major metabolite of progesterone, enhances GABA tone via its effects as an allosteric modulator at the GABA-benzodiazepine receptor complex. Since GABA represents one of the major inhibitors of neurotransmission within the CNS, progesterone's biological effects may convey an anxiolytic action (Shear, 1997; Stahl, 1997; Warnock and Bundren, 1997).

The female reproductive cycle (menstruation, menopause, and pregnancy) is characterized by relatively dramatic fluctuations in oestrogen and progesterone concentrations. These hormonal fluctuations may have a role in determining the overall risk for panic disorder and may also have a substantial impact on the clinical course of panic disorder in women. The dramatic decline in oestrogen and progesterone levels that characterizes the mid-luteal phase of the menstrual cycle has been linked to the emergence or worsening of anxiety symptoms in women (Yonkers and Ellison, 1996). Several reports, primarily retrospective in nature, confirm that women with panic disorder report an increase in their anxiety and panic symptoms during the mid-luteal or premenstrual phase of the menstrual cycle (Cook *et al.*, 1990; Griez *et al.*, 1990). This finding, however, has not been replicated in studies conducted on a prospective basis. Instead, prospective studies reported to date have failed to detect any significant association between menstrual cycle phase and ratings of panic or anxiety symptoms in women with panic disorder (Stein *et al.*, 1989; Cook *et al.*, 1990).

In contrast, biological challenge paradigms conducted in women with panic disorder have detected some evidence of changes in anxiety sensitivity across the menstrual cycle. Acute ingestion of carbon dioxide (CO_2) is often used as a provocative challenge to precipitate anxiety and panic attacks in experimental conditions (Griez *et al.*, 1990). Women with panic disorder have been reported to demonstrate evidence of menstrual cycle phase (follicular vs luteal)-dependent changes in anxiety sensitivity in at least one report (Fishman *et al.*, 1994). In this report, women with panic disorder demonstrated elevated anxiety responses during the mid-luteal phase of the menstrual cycle in comparison to the earlier follicular phase when serially administered CO_2. The women control subjects did not demonstrate any significant relationship between CO_2-induced anxiety and phase of the menstrual cycle during the same study (Perna *et al.*, 1995). Interestingly, subsequent treatment of the same group of women with panic disorder with alprazolam was associated with apparent 'normalization' of the CO_2-induced anxiety response in the same report (Fishman *et al.*, 1994).

Precipitous oestrogen withdrawal, whether physiological or induced by medication or surgical intervention, has also been linked to the subsequent emergence of panic disorder. In a large study ($n = 390$) of peri-menopausal women with new-onset but 'ill-defined' psychological and somatic symptoms, 7% were found to meet criteria for panic disorder (Ushiroyama and Sugimoto, 1994). Moreover, results from another report indicate that a diagnosis of panic disorder should be considered in peri-menopausal women with hot flashes that fail to attenuate during hormone replacement therapy (van der Feltz-Cornelis, 1999). Medical interventions that have primary progesterone-like effects, such as birth control pills or Norplant implants, have also been associated with the acute development of panic disorder (Wagner and Berenson, 1994). Ovarian suppressants utilized for the treatment of endometriosis such as leuprolide may also elicit panic attacks and other psychiatric disturbances (Warnock and Bundren, 1997).

Pregnancy and the post-partum period are marked by particularly dramatic fluctuations in gonadal hormone concentrations. Pregnancy is characterized by 2–3-fold increases in oestrogen and a dramatic elevation (80–100 times) in progesterone concentration (Altshuler, Hendrick and Cohen, 1998). Progesterone is a potent stimulant for oxygen drive and as previously noted its metabolite, allopregnanolone, has GABA-enhancing effects. With these actions in mind, women with pre-existing panic disorder might be expected to experience a substantial attenuation or remission in panic during pregnancy. Available data, however, suggests a more variable course for pre-existing panic disorder during pregnancy. Summarizing available data, it appears that about half (40%–45%) will not have a significant change in their panic symptoms, whereas 30%–35% will experience improvement and a substantial worsening in panic will occur in 20%–30% of patients with pre-existing panic disorder during the course of pregnancy (Cohen *et al.*, 1994a, 1994b; Northcott and Stein, 1994). Interestingly, the course of panic disorder during successive pregnancies is often markedly different (Villeponteaux *et al.*, 1992; Cohen *et al.*, 1994a, 1994b; Northcott and Stein, 1994).

Post-partum worsening (35%–63%) is a much more consistent finding demonstrated in women with pre-existing panic disorder (Sholomskas *et al.*, 1993; Cohen *et al.*, 1994a, 1994b; Northcott and Stein, 1994; Beck, 1998). The post-partum period may also be associated with an increased risk for the onset of panic disorder. According to available data, 11%–29% of women with panic disorder report onset during the post-partum period (Sholomskas *et al.*, 1993; Wisner, Peindl and Hanusa, 1996). Since this rate is significantly greater than the expected age-corrected rate for panic onset in women, it is unlikely to represent a coincidental event (Sholomskas *et al.*, 1993). In patients with pre-existing panic disorder, pregnancy does not appear to increase the likelihood that medication for panic can be successfully discontinued (Cohen *et al.*, 1994a, 1994b). Instead, there is some rational for continuing or re-starting pharmacotherapy during the latter part of pregnancy. Cohen and colleagues demonstrated that pregnant women with pre-existing panic disorder who receive anti-panic medication are significantly less likely to experience a post-partum exacerbation than those who did not receive treatment during pregnancy (Cohen *et al.*, 1994b).

Selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), and tricyclic (TCA) antidepressants have all demonstrated efficacy in the treatment of panic disorder (Sheehan, 1999). High-potency benzodiazepine medications such as lorazepam, alprazolam, and clonazepam are also effective anti-panic agents. Each of the anti-panic medications (SSRI, MAOI, TCA, and benzodiazepines) is associated with a similar rate of

improvement (60%–70%) in panic symptomatology. However, buspirone and other medications with primary serotonergic effects do not appear to be more effective than placebo for panic disorder (Bell and Nutt, 1998). Little information is available concerning the potential impact of gender on treatment response in panic disorder. Results from one study (Kalus *et al.*, 1991) suggest that the TCA, desipramine, may be less effective in men than in women with panic disorder.

These findings indicate that important gender differences exist in the prevalence, clinical features, and overall course of panic disorder. Women are more likely to have panic disorder with comorbid conditions, especially agoraphobia. They are also more likely than men to suffer a recurrence of panic symptoms after remission of panic disorder. Available data also suggests that the female reproductive cycle, especially pregnancy and the postpartum period, can have an important impact on the course of panic disorder. Moreover, declining female hormone levels associated with the ageing process or as a consequence of medical or surgical interventions can precipitate or elicit an exacerbation in panic disorder. Although preliminary evidence also suggests that treatment response may be different in men versus women with panic disorder, further studies that focus on this critical issue are clearly needed. The potential contribution of differential avoidance strategies and other clinical characteristics that may influence prognosis in panic disorder also requires further investigation.

SIMPLE PHOBIA

Results from large-scale population surveys confirm that simple phobias represent one of the most common psychiatric disorders. More than 20% of adults are estimated to meet criteria for simple phobia (Eaton, Dryman and Weissman, 1991; Kessler *et al.*, 1994). Simple phobias encompass a wide range of situations and 'feared' objects. Women are twice as likely as men to meet criteria for simple phobia (Bourdon *et al.*, 1988; Boyd *et al.*, 1990; Dick *et al.*, 1994a). Depression, substance abuse, and OCD are frequently comorbid with simple phobias (Bourdon *et al.*, 1988; Regier, Narron and Rae, 1990; Eaton, Dryman and Weissman, 1991; Kessler *et al.*, 1994; Magee *et al.*, 1996). Some authors have suggested that simple phobias can be sub-classified into three primary classes: (a) situational phobias (e.g., claustrophobia, acrophobia); (b) animal phobias (e.g., fear of spiders, insects, snakes, etc.); and (c) health-related phobias (e.g., fear of injections, blood, dental procedures, etc.). Using this schemata for simple phobia, situational phobias are most common, followed by animal and then health-related phobia, respectively. Women reportedly are two to three times more likely than men to meet criteria for situational and animal phobia, whereas health-related phobia appears to occur at a similar rate in women and men (Fredrikson *et al.*, 1996).

In addition to increased prevalence rates, women may also have an earlier age of onset for simple phobia than men (Dick *et al.*, 1994a). Although they tend to have a chronic course, the associated disability is generally reported as minimal and relatively few (20%–25%) people with simple phobias seek treatment (Boyd *et al.*, 1990; Lindal and Stefansson, 1993). Data derived from female twin studies suggest that environmental factors are more critical than genetic factors in the development of simple phobia (Kendler *et al.*, 1992c). Additional data concerning potential gender differences or the possible influence of reproductive cycles on simple phobia is currently lacking.

SOCIAL ANXIETY DISORDER

Although an initial population study reported that it was relatively rare, data from subsequent population surveys estimate that more than 13% of people will meet lifetime criteria for social anxiety disorder (Boyd *et al.*, 1990; Schneier *et al.*, 1992; Kessler *et al.*, 1994; Stein, Walker and Forde, 1994; Stein and Chavira, 1998). Women are slightly (1.5 times) more likely than men to meet criteria for social anxiety disorder (Kessler *et al.*, 1994; Dick *et al.*, 1994a). Two subtypes (generalized and discrete) of social anxiety disorder are generally recognized. The generalized subtype of social anxiety disorder is comprised of individuals with pervasive performance and interact ional fears in a broad range of social activities, whereas the discrete subtype is limited to anxiety in only certain, focal situations. The two subtypes of social anxiety disorder appear remarkably similar in terms of age of onset, family history, and certain socio-demographic correlates (Kessler *et al.*, 1994; Stein, Walker and Forde, 1994; Stein and Chavira, 1998). However, considerable differences have been identified between the subtypes in terms of clinical course and complications. Discrete social anxiety is associated with an episodic course and minimal functional impairment (Stein, Walker and Forde, 1994) In contrast, generalized social anxiety is more likely to be chronic, complicated by comorbid psychiatric conditions, and associated with substantial functional impairment (Kessler, Stein and Berglund, 1998). Despite the considerable disability associated with generalized social anxiety disorder, relatively few (20%–25%) patients enter treatment (Boyd *et al.*, 1990; Kessler *et al.*, 1994; Schneier *et al.*, 1994).

The low treatment rates reported may in part reflect the difficulties encountered in rapidly and accurately diagnosing social anxiety disorder. Most studies suggest that as many as 5% of patients evaluated in primary care meet criteria for social anxiety disorder, but very few are properly identified. Results from a recent primary care study illustrate the enormity of this problem. Their primary care physicians recognized less than half of the patients with social anxiety disorder as having 'a psychiatric illness'. Overall during the study, the physicians failed to identify 85% of the patients who met criteria for social anxiety disorder (Lecrubier and Weiller, 1997; Bisserbee *et al.*, 1996).

Comorbid disorders are extremely common and may help to obscure the primary diagnosis of social anxiety disorder. Since onset frequently occurs during adolescence, comorbid disorders generally develop after the emergence of social anxiety disorder (Schneier *et al.*, 1992; Stein and Chavira, 1998; Kessler, Stein and Berglund, 1998). Agoraphobia probably represents the most common comorbid psychiatric condition (odds ratio 10 : 4) in social anxiety disorder. Comorbid depression (60%–90%) and alcohol abuse (30%–40%) also frequently complicate generalized social anxiety disorder (Kessler *et al.*, 1994, 1998; Dick *et al.*, 1994a; Stein and Chavira, 1998). Patients with generalized social anxiety disorder have elevated rates of drug abuse including an increased risk for prescription drug abuse (Bisserbee *et al.*, 1996; Lecrubier and Weiller, 1997).

A considerable overlap in diagnostic criteria exists between avoidant personality and social anxiety disorder. It is not surprising, therefore, that 70%–90% of patients with generalized social anxiety have co-existing avoidant personality disorder. The co-occurrence of social anxiety disorder and avoidant personality disorder appears to convey an increased risk for depressive disorder as well as a greater degree of functional impairment in comparison to social anxiety alone (Alpert *et al.*, 1997).

Childhood behavioural inhibition is implicated as a non-specific risk factor for the development of an anxiety disorder in adults. However, social anxiety disorder, in particular, is most strongly associated with a history of behavioural inhibition during childhood (Mick and Telch, 1998). Childhood selective mutism may represent a precursor to the development of social anxiety disorder. In fact, 70% of the first-degree relatives of children with selective mutism were reported to have social anxiety disorder in one study (Black and Uhde, 1995).

The neurobiology of social anxiety disorder is poorly understood, although preliminary research has identified evidence of several different biological abnormalities. Challenge paradigms comparing social anxiety disorder patients and control subjects after acute administration of carbon dioxide, cholecystokinin, or caffeine have detected evidence of enhanced sensitivity and potential cardiovascular and adrenergic abnormalities in the social anxiety disorder patients. Serotonergic dysfunction is implicated by pharmacological challenge results as well as the efficacy of the SSRI antidepressants in the treatment of social anxiety disorder (Nutt, Bell and Malizia, 1998; Ballenger *et al.*, 1998; Davidson, 1998). Patients with social anxiety disorder administered serotonergic probes demonstrate evidence of altered neuroendocrine and behavioural responses in comparison to control subjects. Functional neuroimaging studies reveal evidence of altered dopaminergic function in social anxiety subjects. In particular, a significant reduction in striatal dopamine reuptake has been detected in social anxiety disorder patients versus control subjects (Nutt, Bell and Malizia, 1998).

A number of pharmacological treatments, including the MAOIs, reversible inhibitors of MAO-A, beta-blockers, high-potency benzodiazepines, and the SSRIs have all demonstrated efficacy in the treatment of social anxiety disorder (Ballenger *et al.*, 1998; Davidson, 1998; Pollack, 1999). In the largest multicentre, placebo-controlled trial reported to date, paroxetine was more effective than placebo in both moderate and severe social anxiety disorder (Stein *et al.*, 1998). According to the International Consensus Treatment Guidelines for Mood and Anxiety Disorders, SSRI antidepressants are considered first-line therapy for social anxiety disorder. These guidelines also advocate long-term (>12 months) treatment for patients with generalized social anxiety disorder that have: (a) persistent symptoms despite treatment; (b) a comorbid psychiatric condition; (c) a history of relapse after treatment discontinuation; or (d) a very early onset of social anxiety disorder (Ballenger *et al.*, 1998). However, little information is available concerning the potential impact of gender on treatment response in social anxiety disorder.

Gender Differences in SAD

Evidence of significant gender differences has been reported in patients with SAD. As previously noted, women (15.5%) have a slightly elevated risk for SAD than men (11.1%). Interestingly, men with SAD appear more likely to seek treatment than women with SAD (Weinstock, 1999). Turk and colleagues systematically assessed the potential impact of gender on SAD in a recent report. They failed to detect evidence of gender differences in the prevalence of SAD subtype, the occurrence of comorbid disorders such as additional anxiety disorders, mood disorders, or avoidant personality disorder, or in the clinical course of a group of patients with SAD. However, Turk and colleagues did identify some significant gender differences in the phenomenology of SAD. Women with SAD exhibited more severe social fears than men with GAD. In addition, data derived from self-report measures revealed that the SAD women endorsed more severe fear in a wide range of activities including talking to authority figures, acting/performing/speaking/working in front of others or while being observed, being the centre of attention, expressing disagreement or disapproval to people they do not know very well, or giving a party. Women were also more likely than men to report that going to a party was a trigger for SAD symptoms. In contrast, the men with SAD endorsed more severe symptoms in only two distinct situations: urinating in public bathrooms and returning goods to a store. Men more frequently reported fear of urinating in a public restroom than women in the same group of SAD patients (Turk *et al.*, 1998). Although no gender differences in comorbid conditions were detected in the report by Turk and Co-workers, results from two other studies (Dick *et al.*, 1994a; Lecrubier and Weiller, 1997) have that agoraphobia may be more frequent in women than in men with SAD.

Results from bivariate female twin studies and family studies provide support for the importance of genetic factors in the development of generalized SAD (Kendler *et al.*, 1992c; Stein *et al.*, 1998, 1999). Patients with generalized SAD are 10 times more likely to have relatives with SAD than control subjects (Stein *et al.*, 1998). Results from the Stein and Co-workers study (1998) also imparted strong support for the validity of separating SAD into generalized versus non-generalized groups for research purposes. Data derived during the same study failed to support the role of genetic or familial effects as primary factors in the transmission of the non-generalized form of SAD. Little information is available concerning the impact of female reproductive cycle events and SAD. However, since depression, panic, and substance abuse are frequent complications of untreated SAD, further research appears indicated (Weinstock, 1999).

These results suggest that women are slightly more likely to meet criteria for SAD during their lifetime and that the clinical features associated with SAD may be substantially influenced by gender. The presence of comorbid conditions in primary SAD may also be impacted by gender, although conflicting findings have been reported. Probably the largest vacuum in knowledge about SAD exists in the areas of treatment response and in the potential impact of reproductive hormone cycle events on the onset and/or clinical course of SAD in women. Given the significant psychosocial burden associated with a diagnosis of SAD, future research efforts should focus on these important issues.

OCD

Results from the ECA and the subsequent Cross-National OCD Collaborative Group Study confirm that the lifetime prevalence rate for OCD is between 2% and 3% worldwide. These studies also demonstrate that women are 1.5 times more likely than men to meet lifetime criteria for OCD (Karno *et al.*, 1988; Weissman *et al.*, 1994). The onset of OCD is generally during adolescence or young adulthood. Baer has demonstrated that OCD symptoms can be subdivided into three primary symptom factors: 'symmetry/hoarding', 'contamination/cleaning', and 'pure obsessions' (Baer, 1994). Sixty to seventy percent of patients with OCD endorse substantial psychosocial and occupational dysfunction (Koran, Thieneman and Davenport, 1996; Hollander *et al.*, 1998). An OCD diagnosis is also associated with elevated utilization of medical and mental health services (Kennedy and Schwab, 1997; Hollander *et al.*, 1998). The total annual cost of OCD-related impairment is estimated to exceed $8 billion dollars (Dupont *et al.*, 1995). Unfortunately, OCD remains vastly under-diagnosed and persistently under-treated. Moreover, results from a recently published survey of OCD patients suggest that the average time between symptom onset and initial treatment contact in OCD exceeds 10 years (Hollander *et al.*, 1998).

OCD has historically been considered to be chronic in course, but recent data suggests that the course may be more variable than previously appreciated. An episodic clinical course may occur in as much as 1/3 of OCD patients (Perugi *et al.*, 1998; Antony, Downie and Swinson, 1998; Steketee *et al.*, 1997; Thomsen and Mikkelsen, 1995). Factors reported to be associated with an episodic course of OCD include lower rates of checking rituals and an increased risk of relatives with mood disorders (Perugi *et al.*, 1998). Sustained remission, however, appears to be fairly rare in OCD with either an episodic or chronic clinical course (Hollander *et al.*, 1996b; Bland, Newman and Orn, 1997b; Perugi *et al.*, 1998).

Most patients with OCD will have comorbid psychiatric disorders. Major depressive disorder appears to be the most common

comorbid diagnosis with a 60%–80% lifetime prevalence rate. Additional anxiety disorder diagnoses (40%) also frequently co-exist in OCD, especially panic disorder and social anxiety disorder (Rasmussen and Eisen, 1992; Pigott *et al.*, 1994; Weissman *et al.*, 1994; Antony, Downie and Swinson, 1998). Other conditions that frequently occur in OCD include substance abuse, schizophrenia, body dysmorphic disorder, hypochondriasis, Tourette's Syndrome, and anorexia nervosa (Rasmussen and Eisen, 1990, 1992; Pigott *et al.*, 1994; Weissman *et al.*, 1994; Antony, Downie and Swinson, 1998). Interestingly, the presence of comorbid mood or anxiety disorders does not appear to convey any additional burden in terms of the phenomenology, course, or prognosis of OCD (Demal *et al.*, 1993; Steketee *et al.*, 1997). In a systematic assessment of over 300 OCD patients, Perugi and colleagues found that 16% were comorbid for bipolar disorder. The OCD patients with co-existing bipolar disorder had a more gradual onset and a more episodic course. An elevated rate of sexual and religious obsessions was also associated with the presence of bipolar disorder in OCD (Perugi *et al.*, 1998).

Numerous challenge studies with serotonergic (5-HT) probes have revealed evidence of altered behavioural or neuroendocrine responses in OCD patients in comparison to control subjects. In contrast, OCD patients do not appear to exhibit evidence of altered noradrenergic and/or dopaminergic function in comparison to control subjects using various challenge paradigms (Zohar and Insel, 1987; Goodman *et al.*, 1990; Pigott, 1996). The preferential efficacy of serotonin-selective medications for OCD coupled with the evidence of selective serotonergic dysfunction in challenge studies provides fairly compelling evidence for the importance of serotonin in OCD.

Data from epidemiological and familial-genetic studies provide strong support for an important association between OCD and tic disorder/Tourette's syndrome (Karno *et al.*, 1988; Leonard *et al.*, 1992; Rasmussen and Eisen, 1992; Santangelo *et al.*, 1994). Tic disorder occurs in approximately 10%–15% of patients with OCD and patients with tic disorders have extremely high rates of comorbid OCD. In fact, most estimates suggest that 40%–60% of patients with TS or tic disorder will also have OCD. The considerable overlap in occurrence between OCD and tic disorders has elicited considerable interest. Several investigators have explored potential differences between patients with OCD and those with OCD and comorbid tic disorder (Pauls *et al.*, 1995). In terms of phenomenology, OCD patients with comorbid TS are more likely than OCD patients without TS to report obsessions involving non-violent images, excessive concern with appearance, and need for symmetry. Touching, blinking or staring, and counting compulsions are also reported to be more common in OCD patients with comorbid TS than in those with OCD alone. Preliminary evidence also suggests that a childhood history of ADHD is more common in OCD patients with tic disorder than in those without comorbid tic disorder (Petter, Richter and Sandor, 1998). In addition, OCD patients with co-existing TS have higher rates of bipolar disorder, social phobia, body dysmorphic disorder, attention deficit hyperactivity disorder (ADHD), and substance use disorders in comparison to those with OCD alone or TS alone (Coffey *et al.*, 1998). Family studies also provide support for the contention that OCD with comorbid tic disorder represents a distinct subtype of OCD. OCD is much more prevalent than tic disorder in the first-degree relatives of patients with OCD, whereas the risk for tic disorder is two times greater than the risk for OCD in the first-degree relatives of patients with OCD and tics (Yang and Liu, 1998).

These putative differences in clinical features have resulted in considerable speculation concerning potential differences in the underlying pathophysiology of OCD versus OCD with comorbid TS or tic disorder. In particular, OCD without tic disorder may arise primarily from serotonergic abnormalities, whereas dysfunction in the dopaminergic and serotonergic systems may occur in those with OCD comorbid for tic disorder (Petter, Richter and Sandor, 1998). Recent investigations using genetic association techniques have explored dopaminergic (D) function in OCD. Polymorphic variations have been identified in the genes that determine the expression of the D2, D3, and D4 receptors, respectively. Patients with OCD have been reported to have an increased frequency of the allele associated with a polymorphic variant of the D4 receptor; no differences were detected between the OCD and control subjects in the distribution of alleles in the genotypes for the D2 and D3 receptors (Billett *et al.*, 1998). However, results from another recent report suggest that future genetic association studies conducted in OCD should consider the presence of a comorbid tic disorder as a potential confounding factor. Patients with OCD and tic disorder were noted to have an increased frequency of the variant alleles identified for both the D2 and D4 receptor genes when compared to patients with OCD alone (Nicolini *et al.*, 1998). These findings provide further evidence that dopamine function may be altered in OCD, especially in OCD patients with comorbid tic disorder who may represent a genetically distinct subtype of OCD.

The SSRI antidepressants and the TCA, clomipramine, remain the cornerstone of the pharmacological treatment for OCD (Orloff *et al.*, 1994; Jefferson *et al.*, 1995; Stein, Spadaccini and Hollander, 1995). Although these medications effectively reduce OCD symptoms, the average improvement is fairly modest (30%–40%). Patients who developed OCD later in life had a better chance of response than do those who became ill earlier, independent of length of illness (Ackerman *et al.*, 1994). Re-analysis of data from the multicentre, placebo-controlled, fixed-dose trials of fluoxetine in OCD revealed that response rates and overall improvement were greatest for patients with histories of remission, lack of previous drug treatment, and more severe OCD (especially with greater interference and distress from obsessions) (Ackerman, Greenland and Bystritsky, 1998). Non-response to either clomipramine or fluoxetine treatment has been linked to the presence of: (a) concomitant schizotypal personality disorder; (b) prominent compulsions; and (c) a longer illness length (Ravizza *et al.*, 1995).

Gender Differences in OCD

The impact of gender has been extensively investigated in OCD in comparison to the other anxiety disorders. In addition to the gender difference noted in lifetime prevalence rates for OCD, results from community surveys have consistently demonstrated that the mean age of OCD onset is significantly earlier in men (20 years) than in women (25 years). Data from clinical samples also support the finding that OCD onset is earlier in males than in females, although the age of onset in clinical samples is typically during adolescence rather than young adulthood (Rasmussen and Eisen, 1990, 1992).

Children often manifest OCD-like behaviours as part of their normal development. For example, a standardized assessment for OCD symptoms was administered to a large community sample of children aged 9 to 15 years. The girls and the boys endorsed similar amounts of obsessive–compulsive behaviours during the study. The boys were more likely to admit to checking behaviours and cleaning behaviours were more common in the girls. OCD-like behaviours were more common in the 9- to 12-year-olds than in the 13–15-year-old children. Elevated anxiety levels were reported in the few subjects that continued to have persistent OCD behaviours during adolescence (Zohar and Bruno, 1997). These results suggest that OCD-like behaviours are common in elementary aged children, but persistence into adolescence is rare and may represent the initial symptoms of clinical OCD.

Childhood-onset OCD appears to have some unique features in comparison to adult-onset OCD. As previously noted, boys are two to three times more likely than girls to have OCD during childhood. Comorbid psychiatric disorders, especially ADHD and other developmental disorders, are very common in childhood-onset

OCD. Neuropsychological deficits and familial loading for OCD are also common in childhood-onset OCD. Long-term studies indicate that childhood-onset OCD may also be associated with a graver prognosis. Most reports suggest that patients with childhood-onset OCD rarely experience remission (Demal *et al.*, 1993; Thomsen and Mikkelsen, 1995; Hantouche and Lancrenon, 1996; Steketee *et al.*, 1997). In fact, Leonard and colleagues conducted one of the largest ($n = 54$) and longest (up to seven years) prospective follow-up study of children and adolescents with OCD. They reported that after an average of three years of follow-up, 81% of the patients treated with clomipramine were significantly improved, but only 6% of the subjects were considered to be in remission. Predictors of worse outcome for the childhood-onset OCD patients were: (a) more severe OCD symptoms score after 5 weeks of clomipramine therapy; (b) a lifetime history of a tic disorder; and (c) the presence of a parental Axis I psychiatric diagnosis (Leonard *et al.*, 1992).

As previously noted, men typically have an earlier onset of OCD than women. In fact, three times as many boys as girls meet diagnostic criteria for OCD. Recent investigations have identified a potential variant of OCD designated as early onset OCD. This variant is characterized by the onset of OCD before the age of 10, occurs predominantly in boys, and is strongly associated with tic disorder and a positive family history (Leonard *et al.*, 1992; Pauls *et al.*, 1995). The data concerning the potential link between OCD and tic disorder has already been reviewed; however, it is important to note that men are much more likely to have OCD with a comorbid tic disorder as well as an earlier onset of illness. This finding may also suggest that gender differences may exist in the pathophysiological mechanisms that underlie OCD. Dopamine dysregulation may be more prominent in men with OCD, whereas women gonadal steroid hormones and their complex interactions with serotonin may be more critical to the development of OCD in women. The dramatic shift that occurs in gender prevalence rates for OCD after the onset of puberty provides support for the importance of female reproductive hormones. Women begin to develop OCD at a much greater rate than men after menarche; the increase is sufficiently robust that the overall prevalence rate in OCD is greater for women (1.5 : 1.0) than men (Weissman *et al.*, 1994; Karno *et al.*, 1988).

Gender differences have also been identified in the phenomenology and clinical course of OCD. Aggressive obsessions and cleaning compulsions may occur more frequently in women with OCD (Noshirvani *et al.*, 1991; Castle, Deale and Marks, 1995; Lensi *et al.*, 1996). In a systematic assessment of adolescents with OCD, females endorsed a greater amount of compulsive rituals, whereas obsessions were more common in the males with OCD (Valleni-Basile *et al.*, 1994). Women with OCD may also have a more episodic clinical course and less severe symptoms (Thomsen and Mikkelsen, 1995; Hantouche and Lancrenon, 1996).

Women with OCD may also have a greater risk of certain comorbid conditions than men with OCD. Comorbid panic disorder and eating disorders such as anorexia nervosa or bulimia nervosa are reported to occur more frequently in women than men with OCD (Noshirvani *et al.*, 1991; Rubenstein *et al.*, 1992; Tamburrino, Kaufman and Hertzer, 1994; Castle, Deale and Marks, 1995; Kendler *et al.*, 1995; Yaryura-Tobias, Neziroglu and Kaplan, 1995; Lensi *et al.*, 1996). Since anorexia and bulimia nervosa are associated with marked alterations in female reproductive hormone function, the frequent association of OCD with eating disorders also provides indirect evidence for the importance of the female reproductive cycle in the course of OCD.

Gender differences may also exist in response to serotonergic probes during challenge studies conducted in OCD patients. Women with OCD administered the serotonergic probe fenfluramine have an attenuated cortisol response in comparison to men with OCD and control subjects (Monteleone *et al.*, 1997). Acute intravenous administration of clomipramine is also used as a probe of 5-HT function. Results from clomipramine challenge studies conducted in OCD also suggest gender differences in response. Men with OCD experienced an increase in symptoms during the clomipramine challenge, whereas no substantial change was detected in the OCD women. The OCD women also demonstrated a better antiobsessional response during a subsequent 10-week trial with either clomipramine or fluvoxamine. Interestingly, the gender difference detected in antiobsessional response was more pronounced after clomipramine than fluvoxamine treatment (Mundo *et al.*, 1999). Potential gender differences in treatment response have been explored from the data derived from the multicentre, placebo-controlled treatment trials of clomipramine and fluoxetine, respectively. Both analyses failed to detect any evidence of gender differences in medication response in OCD (Ackerman *et al.*, 1994, 1998). Given the differences highlighted between OCD and OCD with tic disorder, future studies should explore the potential relationship between distinct OCD subgroups and medication response.

OCD and Female Reproductive Cycle Events

There are numerous reports suggesting that the female reproductive cycle may have a substantial influence on OCD. The striking increase in prevalence rates for OCD that occurs in women after the onset of puberty has already been reviewed. Several reports suggest a relationship between menstrual cycle and OCD. Undergraduate women without demonstrable OCD have been reported to engage in more OCD-like behaviours such as 'excessive cleaning or cleaning of things not usually cleaned' during the luteal phase than at any other time during the menstrual cycle (Dillon and Brooks, 1992), Moreover, the premenstrual (late-luteal phase) period may be associated with an exacerbation in symptoms in women with OCD (Yaryura-Tobias, Neziroglu and Kaplan, 1995; Williams and Koran, 1997). In the largest study to date, the impact of the menstrual cycle on the course of their OCD symptoms was retrospectively examined in 57 women with OCD. Nearly half (42%) of the women reported premenstrual worsening in their OCD symptoms and a substantial number (21%) also noted premenstrual dysphoria (Williams and Koran, 1997).

Several case series suggest that a substantial portion (13%–36%) of women with OCD report the onset of their illness during pregnancy or the post-partum period (Buttolph and Holland, 1990; Sichel *et al.*, 1993; Neziroglu *et al.*, 1994; Williams and Koran, 1997; Altshuler, Hendrick and Cohen, 1998). A number of case reports suggest that women with pre-existing OCD will experience a worsening in symptomatology during pregnancy (Brandt and Mackenzie, 1987; Buttolph and Holland, 1990; Stein *et al.*, 1993; Neziroglu *et al.*, 1994; Weiss *et al.*, 1995; Chelmow and Halfin, 1997; Altshuler, Hendrick and Cohen, 1998). However, in the largest reported study to date concerning the impact of pregnancy on OCD, most of the women with pre-existing OCD (69%) reported no significant change in symptoms during pregnancy. Relatively few of the OCD patients reported a significant worsening (17%) or a substantial improvement (14%) in OCD symptoms during pregnancy. Relatively few (13%) of the women reported the onset of OCD during pregnancy. Substantial changes in OCD symptoms, however, were likely to occur during the post-partum period. Post-partum worsening of OCD (29%) and post-partum depression (37%) was a frequent finding in the OCD women (Williams and Koran, 1997). The exacerbation noted during the post-partum period is a fairly consistent finding in women with pre-existing OCD. Most studies have reported that 20%–30% of women with OCD will experience a significant post-partum worsening in OCD symptoms (Buttolph and Holland, 1990; Sichel *et al.*, 1993; Williams and Koran, 1997; Altshuler, Hendrick and Cohen, 1998).

These results provide further evidence that changes in female reproductive hormone concentrations can substantially influence the severity and course of OCD. Pregnancy and the post-partum period may represent a time of increased vulnerability for the initial emergence of OCD or for significant worsening in women with pre-existing OCD.

POST-TRAUMATIC STRESS DISORDER (PTSD)

Although many individuals are exposed to trauma, only one out of four will develop PTSD (Breslau, Davis and Andreski, 1990). Community surveys consistently report that the lifetime prevalence rate for PTSD is two times greater in women (12.5%) than men (6.2%) (Drummond, 1993). The most common cause of PTSD in men is combat exposure. In contrast, women are most likely to develop PTSD as a consequence of sexual assault, sexual molestation, or childhood physical abuse (Kessler *et al.*, 1995). PTSD is often complicated by comorbid conditions such as depression and alcohol abuse (Breslau *et al.*, 1997b). PTSD can occur at any age, but certain traumatic events are associated with an especially high risk of subsequent PTSD development. For example, Foa reported that 95% of rape victims and 75% of victims of non-sexual assaults develop PTSD symptoms within 2 weeks of the traumatic event (Foa, 1997).

Although exposure to life-threatening traumatic events is fairly common, a number of studies confirm that PTSD is poorly recognized in a variety of clinical settings. For example, Davidson *et al.* (1998) found that 81% of new patient referrals to an outpatient psychiatric clinic had a positive history of significant trauma. Although almost 30% of the patients with exposure to trauma met criteria for PTSD, only 8% were correctly diagnosed with PTSD. Unfortunately the low rates of recognition typically associated with PTSD can have catastrophic consequences. Comorbid conditions are extremely common in PTSD, especially mood and substance use disorders. In fact, the presence of PTSD has been associated with an elevated risk for major depression, dysthymia, and mania. According to the National Comorbidity Survey, comorbid alcohol and substance abuse occur twice as often in the presence of PTSD in comparison to patients without PTSD (Kessler *et al.*, 1995; Breslau *et al.*, 1997b).

Cumulative evidence indicates that the SSRI antidepressants constitute first-line pharmacotherapy for PTSD (Nagy *et al.*, 1993; Rothbaum, Ninan and Thomas, 1996; Marshall *et al.*, 1998; Davidson and Connor, 1999). TCA and MAOI antidepressants are also effective for PTSD and should be considered for patients with PTSD who fail to respond to SSRI treatment. The SSRI antidepressants appear to have a broad spectrum of activity in PTSD. Common PTSD symptoms such as anxiety, insomnia, and an exaggerated startle response are reported to improve during SSRI treatment. Moreover, SSRI treatment may also ameliorate PTSD symptoms such as intrusive trauma-related recollections, feelings of emotional numbing, and avoidance behaviours (Davidson and Connor, 1999). Pharmacotherapy for PTSD should be initiated at a relatively low dose and maintained for at least 12 months before discontinuation is considered.

Gender Differences in PTSD

The gender difference in prevalence rate for PTSD has been linked to a differential rate of exposure to trauma. However, this assumption appears to be incorrect. In a sample of over 1000 young adults, Breslau and colleagues found similar rates of exposure to traumatic events, but substantially more women than men met criteria for PTSD (Breslau *et al.*, 1997b). Potential confounding factors such as the increased prevalence of pre-existing anxiety or major depressive disorders in women were examined, but failed to account for the observed gender difference noted in the prevalence of PTSD. Instead, women appeared to have a markedly increased susceptibility for PTSD development, especially if the trauma occurred prior to age 15 (Breslau *et al.*, 1990, 1995, 1997b; Kessler *et al.*, 1994). Results from the National Comorbidity Survey also implicate multiple factors in the elevated risk of PTSD in women. However, there was substantial overlap between factors that predicted an increased risk for trauma exposure and development of PTSD. Once the overlapping risk factors are excluded, only one risk factor (history of affective disorder) predicted PTSD in women, whereas two (history of anxiety disorder and parental mental disorder) factors were associated with an increased risk of PTSD in men (Bromet, Sonnega and Kessler, 1998). These findings suggest that most variables identified as predictive of PTSD are actually more indicative of trauma exposure than PTSD.

Major depression and generalized anxiety disorder are common comorbid disorders in PTSD regardless of gender, whereas co-existing somatoform pain disorder is more common only in women with PTSD. Pre-existing depression appears to convey an increased risk for subsequent exposure to traumatic events as well as the development of PTSD once trauma occurs (Breslau *et al.*, 1997a, 1997b). Certain traumatic experiences are more likely to precipitate PTSD. Women who are victims of sexual assault have an extremely high risk of subsequent development of PTSD. For example, Foa and colleagues found that 3 months after the trauma, women rape victims were twice as likely as women victimized by non-sexual crimes (48% vs 25%) to have PTSD (Foa, 1997). Women may be particularly susceptible to the long-term complications of abuse that occurs during childhood. In fact, women with histories of childhood abuse are reported to have a level of functional impairment that is commensurate with that of women with recent abuse (McCauley *et al.*, 1997). Women victimized by domestic violence are more likely to develop anxiety symptoms as well as PTSD, whereas male victims of domestic violence are at greater risk of developing substance use disorders. An elevated risk of depression and an increased number of physical and psychological health problems have also been reported in women exposed to ongoing domestic violence. This finding appears to be independent of the severity of domestic violence or the presence of injuries sustained. That is, women who sustain severe injuries do not appear more likely to develop psychiatric symptoms or PTSD (Sutherland, Bybee and Sullivan, 1998). While this finding may seem counter-intuitive, it likely reflects the importance of 'perceived threat' in the formation of PTSD. That is, a victim's perception of danger or possibility of death during an assault or exposure to trauma may be more important subsequent risk for PTSD as compared to more objective or realistic assessments of life-threatening events. These findings suggest that sexual assault, childhood abuse, and individual assessment of threat during the occurrence of trauma may represent the strongest predictors of subsequent PTSD development in women.

These findings suggest that sexual assault and childhood sexual abuse represent significant risk factors for the development of PTSD in women. They also confirm that childhood abuse has particularly devastating complications that are likely to persist into adulthood in women. Certain factors appear to substantially increase the risk of development of PTSD and other complications in women. These factors include: (a) exposure to sexually related trauma or aggression; (b) occurrence of abuse or severe trauma during childhood or prior to the age of 15; and (c) perception within the victim that the traumatic event is life threatening or escape is unlikely. With these issues in mind, future research efforts should focus on identifying the biological correlates associated with childhood trauma and/or sexual abuse/assault.

Gender differences have also been identified in the biological alterations associated with PTSD. An elevated norepinephrine-to-cortisol ratio has been reported in men with PTSD, whereas women with PTSD have demonstrated significantly elevated levels of urinary norepinephrine, epinephrine, dopamine, and cortisol on a daily basis (Lemieux and Coe, 1995). The HPA axis has strong, multi-level inhibitory effects on the female reproductive hormones. Since HPA axis alterations are implicated in PTSD, the marked fluctuations in oestrogen and progesterone levels that characterize the female reproductive cycle may well have a significant impact on the course of PTSD. The relative hypercortisolism that occurs during the third trimester of pregnancy is speculated to cause a transient suppression of the adrenals during the post-partum period (Chrousos, Torpy and Gold, 1998). This finding suggests that women with pre-existing PTSD may experience substantial changes in symptomatology during pregnancy or the post-partum period. Unfortunately, very little systematic information is available concerning the potential impact of the reproductive cycle on PTSD.

SUMMARY

Women are much more likely than men to meet lifetime criteria for GAD, panic disorder, simple phobia, SAD, OCD, and/or PTSD. Potentially important gender differences have also been identified in the phenomenology and clinical course for each of the anxiety disorders. Women with GAD are more likely to meet criteria for a comorbid psychiatric disorder, seek treatment, and experience a more severe clinical course in comparison to men with GAD. Phobic avoidance, an elevated risk for comorbid conditions, and a less favourable clinical outcome are associated with female gender in patients with panic disorder. Women, in comparison to men, with simple phobia are more likely to have an earlier age of onset and a situational or animal-related simple phobia; men and women have a similar risk for developing health-related simple phobia. Men are more likely to seek treatment for SAD than women, but women with SAD endorse a wider range of activities that elicit severe anxiety and/or avoidance behaviours. Numerous gender differences have been identified in OCD including an earlier age of onset in males, gender-related symptom manifestations (cleaning compulsions more common in women, obsessions more common in men) and comorbid conditions (eating disorders common in women with OCD, tics more common in males with OCD), and a more episodic clinical course for women with OCD. Women appear to be more susceptible to the development of PTSD after the occurrence of severe trauma such as sexual assault or childhood abuse, whereas men are more likely to develop PTSD as a consequence of combat exposure.

The gender differences that have been identified are likely to arise from a variety of factors including genetic, environmental, and neurobiological influences. However, increasing evidence also suggests that the female reproductive hormone cycle may also have a substantial influence in the development and/or perpetuation of anxiety disorders in women. Despite the fact that the majority of people afflicted by anxiety disorders are women of childbearing potential, relatively little data is available concerning the impact of female reproductive hormone cycle events on the onset and/or clinical course of anxiety disorders. Much of the available information focuses on women with either panic disorder or OCD. Information concerning the impact of the menstrual cycle on panic disorder is somewhat mixed, but there is mounting evidence suggesting that the post-partum period, and perhaps the peri-menopause, are associated with exacerbations in panic symptomatology. Menarche appears to increase the prevalence of OCD in females and the post-partum period is often associated with an increased risk of OCD onset and/or symptom worsening in women with pre-existing OCD. While these results are intriguing, further research is clearly needed to further clarify these important relationships. Lastly, there is some evidence suggesting that gender differences may exist in the neurobiological and/or genetic basis of anxiety disorders. While preliminary in nature, these findings suggest that further research efforts should focus on delineating these potential gender differences as well as their subsequent impact on treatment response and functional outcome. Hopefully, such investigations will lead to improved prevention strategies and/or treatments that will eventually result in eliminating the finding that women afflicted with anxiety disorders often have a more severe clinical course and a worse overall outcome than men with anxiety disorders.

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XIX-13

Therapeutic Armamentarium in Anxiety Disorders

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INTRODUCTION

For decades benzodiazepines have been the mainstay in the treatment of anxiety disorders. It is only during the last decade that this situation has changed. With the introduction of serotonin reuptake inhibitors (SSRI's), 5-HT receptor-specific drugs like 5-HT_{1A} agonists and recently, dual action antidepressants, new possibilities have been created for the treatment of panic disorder, obsessive compulsive disorder, social phobia, generalized anxiety disorder and post-traumatic stress disorder. In this chapter the pharmacotherapeutic options for the treatment of these anxiety disorders will be reviewed. We will focus on new developments and not reiterate all the studies performed with old tricyclic antidepressants (TCAs) such as imipramine, as these studies have been mentioned and reviewed in many other textbooks and papers.

PANIC DISORDER

Panic disorder (PD) is a chronic and recurring syndrome which requires long-term treatment. The disorder encompasses five essential domains: the panic attacks themselves, the associated anticipatory anxiety, phobic avoidance, and the resultant functional impairment and effects on quality of life. However, the complete resolution of panic attacks, the signal feature of panic disorder, is clearly important to patients. Modern studies on the efficacy in PD should measure symptoms domains outlined above (Shear and Maser, 1994).

Originally, tricyclic antidepressants (TCAs) like imipramine and clomipramine were the first found to be beneficial in panic disorder. Imipramine originally played a role in the sixties in the pharmacological dissection of panic and anxiety, as it was discovered that in patients suffering from anxiety neurosis, panic attacks disappeared during treatment with imipramine. The efficacy of imipramine has been the subject of many studies and this drug was used as a reference drug for years. TCAs, in spite of their proven efficacy, have many drawbacks, including high rates of non-compliance due to anticholinergic side-effects, weight gain, daytime sedation, orthostatic hypotension, lethality in overdose and withdrawal reactions (Wolfe, 1997; Bennett *et al.*, 1998; den Boer, Bosker and Slaap, 2000). After six months treatment with imipramine dry mouth, constipation, sweating was still present as a substantial burden to patients (Mavissakalian and Perel, 2000). It is questionable whether by current standards, TCAs would be marketed if they were introduced today. Many studies have also been performed with BDZs in PD and there is a large database indicating that high potency BDZs such as clonazepam and alprazolam are efficacious in PD. Due to developments in psychopharmacology during the last decade it has, however, been questioned whether BDZs should be the first choice in the treatment of PD in view of their serious side-effects and withdrawal reactions.

Benzodiazepines in Panic Disorder

There are several placebo-controlled studies suggesting that clonazepam has therapeutic effects in PD. In one of the largest studies with clonazepam conducted so far, Rosenbaum and co-workers (1997) included 413 patients with PD in a fixed-dose placebo-controlled study in a 6-week study followed by a 7-week discontinuation phase. They used dosages ranging from 0.5 mg to 4.0 mg clonazepam daily and found that dosages of clonazepam of 1.0 mg were equally effective in reducing the number of panic attacks. During the discontinuation phase most patients worsened and reported increases in the number of panic attacks. Recently, Valenca *et al.* (2000) undertook a 6-week placebo-controlled study in 24 PD patients. They found that 11% of the placebo-treated patients were panic free after six weeks, whereas 62% of the clonazepam treated patients were panic free. Because treatment with BDZs has been associated with severe withdrawal reactions, Moroz and Rosenbaum (1999) treated PD patients with 0.25 up to 4.0 mg per day clonazepam for 6 weeks in a double-blind placebo controlled design, followed by a 7-week discontinuation phase during which the doses were gradually tapered. They observed no symptoms suggestive of withdrawal syndrome, nor evidence for rebound during the gradual tapering of clonazepam, but some patients did show worsening of symptomatology, most notably a recurrence of panic attacks. These data show that in the short-term clonazepam is efficacious but treatment in this study was probably too short to achieve full remission of panic attacks. On the other hand, a naturalistic follow-up study for 2 years in 204 patients showed that improvement in global severity of PD did not change during treatment with stable dosages of clonazepam, indicating that there was no development of tolerance (Worthington *et al.*, 1998). In spite of the fact that clonazepam has been marketed in the US for PD, the two pivotal studies did not fulfil all the criteria that were set forth by Shear and Maser (1994), moreover, there is evidence that the higher dosages that are required in many patients are associated with withdrawal symptoms (Davidson *et al.*, 1998).

Two large studies investigating the anxiolytic effects of alprazolam have been conducted in more than 1600 patients suffering from PD. The Cross-National Collaborative Panic Study and the Philadelphia study, which was a maintenance study (Ballenger *et al.*, 1988; Noyes *et al.*, 1988; Pecknold *et al.*, 1988; Rickels *et al.*, 1993; Schweizer *et al.*, 1993). The results of these studies suggested that alprazolam was an effective drug in reducing the number of panic attacks. In spite of these results, these studies were criticized for several points; there was a very high placebo drop-out rate and the average dose was high (mean dose at week 8: 5.6 to 5.8 mg per day). These are important issues as even lower dosages have been shown to lead to impaired recall and other cognitive disturbances (Pomara *et al.*, 1998). In addition, cessation of alprazolam treatment induces

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severe withdrawal reactions, for which PD patients appear to be very vulnerable compared to patients suffering from generalized anxiety disorder (Klein *et al.*, 1994). Cognitive-behavioural therapy (CBT), prescription of drugs such as trazodone, valproate and carbamazepine have all been shown to facilitate alprazolam discontinuation (Rickels *et al.*, 1999; Bruce, Spiegel and Hegel, 1999). In addition, there is a relapse rate of 44% to 56% after discontinuation of alprazolam (Spiegel, 1998).

One placebo-controlled study compared alprazolam with exposure and relaxation, either alone or in combination (Marks *et al.*, 1993). In this study, alprazolam plus exposure was more effective than alprazolam plus relaxation, although the number of panic-free patients did not differ between placebo (plus either relaxation of exposure) and alprazolam plus relaxation. There is evidence from placebo-controlled studies that diazepam could also be effective in the treatment of PD. However, in most studies conducted with diazepam very high dosages have been used. In a placebo-controlled 8-week study comparing diazepam with alprazolam, the mean dose of diazepam was 40 mg per day, which according to European standards is considered extremely high (Noyes *et al.*, 1996). Diazepam was found to be as effective as alprazolam in reducing the number of panic attacks, but throughout the study sedation was more severe for patients taking diazepam or alprazolam than for those taking placebo.

In sum, there is evidence that the high-potency BDZs clonazepam and alprazolam are effective in the treatment of PD. The onset of efficacy on BDZs in rapid, but due to the high number of side-effects and withdrawal reactions we would not consider them as first-line treatment of PD.

Table XIX-13.1 Percent of patients free from panic attacks at endpoint in placebo-controlled studies of SSRIs and clomipramine

Study	Duration (weeks)	Percent panic-free			
		N	SSRI	Clomipramine	Placebo
Paroxetine					
Oehrberg *et al.*, 1995[†]	12	120	36%*	—	16%
Lecrubier *et al.*, 1997a	12	367	51%*	37%~	32%
Lecrubier *et al.*, 1997b	36	176	85%*	72%~	59%
Ballenger *et al.*, 1998	10	278	86%*	—	50%
Bakker *et al.*, 1999	12	131	75%	59%"	37%
Fluvoxamine					
Hoehn-Saric *et al.*, 1993	8	50	61%*	—	22%
Black *et al.*, 1993	8	75	73%*	—	25%
Citalopram					
Wade *et al.*, 1997	8	475	43–58%*	50%*	32%
Sertraline					
Pohl *et al.*, 1998	10	168	62%*	—	46%
Pollack *et al.*, 1998	10	176	57%~	—	47%
Londborg *et al.*, 1998	12	178	57%~	—	41%

*Significantly different from placebo; [†]one or zero panic attacks in a three week period; ~not significantly different from placebo; CT = cognitive therapy.

SELECTIVE SEROTONIN REUPTAKE INHIBITORS

Resolution of Panic Attacks

The SSRIs are effective in the treatment of panic disorder for which they are increasingly considered as first-line treatment. Comprehensive data has been published for SSRIs in particular, and the proportion of patients achieving panic-free rates have been reported in patients treated with paroxetine, fluvoxamine, citalopram, and sertraline in placebo-controlled studies (Table XIX-13.1).

Paroxetine

There is an extensive clinical database available for paroxetine — the largest data set among the SSRIs — which comprises data for over 700 patients with panic disorder treated with paroxetine for periods ranging from 10 to 36 weeks (Oehrberg *et al.*, 1995; Lecrubier and Judge, 1997; Lecrubier *et al.*, 1997; Ballenger *et al.*, 1998a). In a 12-week, double-blind, placebo-controlled comparison of paroxetine and clomipramine in 367 patients with panic disorder, paroxetine reduced the number of panic attacks to zero in 51% of patients, compared with 37% of patients treated with clomipramine and 32% of those on placebo (Lecrubier and Judge, 1997). In a two-centre study, Bakker *et al.* (1999) included 131 patients in a placebo-controlled double-blind study comparing paroxetine, clomipramine and cognitive therapy. They found that 37% of placebo treated patients were free from panic attacks in the last three week interval of this 12 weeks study. In the clomipramine and cognitive therapy group, this number was 17% and 54% and respectively. Interestingly, they reported a very low response-rate to clomipramine, in contrast to other studies. The highest response was found in the paroxetine treated patients: 75% from the patients were panic-free from week 10–12 or earlier. In this study, cognitive therapy did not differ from placebo on the pivotal measures.

Fluvoxamine

Fluvoxamine has been shown to reduce the number of panic attacks to zero in two, 8-week, placebo-controlled studies (125 patients in total): up to 73% of patients treated with fluvoxamine were panic free by the end of study, compared with approximately 25% of patients on placebo (Black, Uhde and Tancer, 1992; Hoehn-Saric *et al.*, 1993).

Citalopram

Citalopram has been compared with clomipramine in an 8-week, placebo-controlled study in 475 patients with panic disorder, with or without agoraphobia (Wade *et al.*, 1997). At the most effective citalopram dose (20–30 mg per day), approximately 58% of patients were panic-free compared with 50% of patients receiving clomipramine and 32% of placebo patients.

Sertraline

The results from a 12-week, double-blind, placebo-controlled, study of sertraline in 178 outpatients with panic disorder have recently been published (Londborg *et al.*, 1998). At study endpoint, 57% of the pooled sertraline sample were panic free compared with 41% of the placebo subjects; a non-significant difference. A large increase in the placebo response from week 9 onwards may have masked the treatment response. In a study with a similar design Pohl and associates (1998) found that significantly more sertraline-treated patients (62%) than placebo-treated patients were free of panic attacks at the end of treatment. From the analysis of the pooled results from these two studies it was concluded that the mean frequency of panic attacks was very high in this sample

(10 panic attacks per week), but this did not preclude superiority of sertraline over placebo, which was present in all dosages used (50 mg, 100 mg and 200 mg). In addition, analysis of the pooled data set showed that illness severity, chronicity or the presence of clinically relevant depressive symptomatology did not influence treatment results (Sheikh *et al.*, 2000). As yet there are no published comparative data for sertraline against other active treatments.

Fluoxetine

Fluoxetine was for a long time the least well-studied of the SSRIs in panic disorder, but a large multisite double-blind placebo-controlled study including 243 patients suffering from PD has now been published (Michelson *et al.*, 1998). In this study 10 and 20 mg per day were used in an acute 10 weeks treatment phase and responders to the acute phase were randomly assigned to a 24-week continuation-phase using the same double-blind design. Fluoxetine 20 mg appeared to be slightly superior to 10 mg, but both dosages led to statistically significant superior effects compared to placebo on the number of panic attacks, overall anxiety (20 mg better), phobic symptoms, depression and overall impairment.

Long-Term Efficacy

Of the SSRIs, only paroxetine, fluvoxamine and citalopram have demonstrated long-term efficacy in the treatment of panic disorder. A 9-month extension of an acute treatment study with paroxetine in 176 patients with panic disorder showed that, not only was the efficacy of paroxetine maintained, but the proportion of patients who became free of panic attacks continued to increase: after 9 months 85% of patients were panic-free compared with 72% of patients on clomipramine (Lecrubier *et al.*, 1997). Similarly, in a second study, 80 patients with panic disorder who responded to three months' maintenance treatment with paroxetine were randomized to paroxetine or placebo for a further 3 months (Judge and Steiner, 1996). Only 5% of those who continued to take paroxetine experienced a relapse compared with 30% of patients switched to placebo. Seventy-three patients with panic disorder underwent 12 months' further treatment with fluvoxamine in an open extension of two placebo-controlled studies (Holland *et al.*, 1994). Patients who had been transferred from placebo and those continuing on fluvoxamine continued to improve indicating that the effect of fluvoxamine in panic disorder is probably sustained in the long term. In a double-blind placebo-controlled long-term study lasting one year comparing citalopram (fixed dose ranges: 10 or 15 mg per day; 20 or 30 mg per day; 40 or 60 mg per day) vs clomipramine, it was found that all drug-treatment groups treatment outcome was better than placebo. Only the lowest dose of citalopram was less effective. In almost all patients remaining in the study ($N = 279$) panic attacks tended to disappear during active treatment (Lepola *et al.*, 1998).

Anxiety Levels

Reductions in global anxiety ratings with paroxetine, fluvoxamine, and citalopram have been reported in treatment studies in panic disorder (den Boer *et al.*, 1987, 1988; Black *et al.*, 1992; Hoehn-Saric *et al.*, 1993; Oehrberg *et al.*, 1995; Lecrubier and Judge, 1997; Lecrubier *et al.*, 1997; Wade *et al.*, 1997; Ballenger *et al.*, 1998a). All three SSRIs demonstrated comparable efficacy to clomipramine with respect to this endpoint measure (den Boer *et al.*, 1987; Lecrubier and Judge, 1997; Wade *et al.*, 1997).

Sertraline significantly reduced the time spent with anticipatory anxiety compared with placebo in a multicentre comparison in outpatients (Londborg *et al.*, 1998; Pohl, Wolkow and Clary, 1998), but in the study of Pollack *et al.* (1998) sertraline was not significantly different from placebo on measures for anticipatory anxiety. Limited data are available for the reduction of global anxiety in panic disorder with fluoxetine. It has been compared with desipramine in one small 10-week study where it was found to reduce anxiety levels (Bystritsky *et al.*, 1994). In the study by Michelson and co-workers (1998) it was found that fluoxetine 20 mg per day (but not 10 mg per day) significantly reduced global anxiety.

Phobic Avoidance

Paroxetine

Paroxetine produced a significant reduction in overall phobic score compared with placebo during a 12-week, placebo-controlled study in 367 patients (Lecrubier and Judge, 1997). The reduction was similar to that observed in patients on clomipramine. Further improvements in phobic avoidance were evident during the long-term extension phase of this study (Lecrubier *et al.*, 1997).

Fluvoxamine

Fluvoxamine was also found to be efficacious in the reduction of phobic avoidance in patients with panic disorder in several studies (den Boer *et al.*, 1987, 1988, 1990; van Vliet, Westenberg and den Boer, 1996). In all studies, although phobic avoidance was reduced, patients on fluvoxamine were still exhibiting signs of avoidance at study endpoint.

Fluoxetine and Citalopram

Fluoxetine reduced phobic avoidance in a small comparative study with desipramine (Bystrisky *et al.*, 1994). In the large double-blind study by Michelson *et al.* (1998) fluoxetine reduced phobic avoidance better than placebo. In the 12 months extension follow-up period of the study by Lepola *et al.* (1998) in which citalopram was compared to clomipramine and placebo, it was found that the reduction in total phobia score as assessed with the Phobia Scale was significantly greater in all patients receiving citalopram by month 9 and 12 compared to the group receiving placebo. Using the same scale, the group treated with clomipramine did not differ from placebo at month 12 (Leinonen *et al.*, 2000). In this study, however, no mention was made of the number of panic attacks, so that it is unclear whether clomipramine and citalopram showed comparable efficacy on this measure.

Overall Impairment

The effect of SSRIs on overall impairment in patients with panic disorder has been assessed in placebo-controlled clinical trials conducted with paroxetine (Lecrubier and Judge, 1997; Lecrubier *et al.*, 1997), fluvoxamine (Black, Uhde and Tancer, 1992; Hoehn-Saric *et al.*, 1993) sertraline (Pohl, Wolkow and Clary, 1998; Pollack *et al.*, 1998) and fluoxetine (Michelson *et al.*, 1998). Reductions in overall impairment, as assessed by the Sheehan Disability Scale in work, social and family life items, have been observed with both paroxetine, fluvoxamine and fluoxetine, whereas sertraline treated patients did significantly better than placebo on the Quality of Life Enjoyment and Satisfaction Questionnaire.

SSRI's Compared with Cognitive-Behavioural Therapy

There is limited data about the combination of cognitive therapy and pharmacotherapy in the short- and long-term treatment of PD. In order to evaluate whether drug therapy or psychosocial therapies are efficacious for PD, Barlow and co-workers conducted

a double-blind placebo controlled study in 312 patients suffering from PD (Barlow *et al.*, 2000). Patients received imipramine (up to 300 mg per day), CBT only, placebo only, CBT plus imipramine, or CBT plus placebo. Patients were treated for 12 weeks, responders were seen monthly for 6 months. Both imipramine and CBT were found to be significantly superior to placebo in the acute phase (12 weeks) and during the 6 months continuation phase. Combining CBT with imipramine did not increase response rates in the acute phase, but by the end of maintenance (6 months) the combination led to more substantial advantage compared to either condition alone.

Two studies have compared the efficacy of fluvoxamine and cognitive-behavioural therapy (CBT) in PD (Black, Uhde and Tancer, 1992; Sharp, Power and Simpson, 1996). In dosages up to 300 mg per day for 8 weeks, fluvoxamine reduced anxiety levels significantly more effectively than CBT or placebo alone (Black, Uhde and Tancer, 1992). The percentage of patients who were panic free at the end of study was higher in patients receiving fluvoxamine than CBT of placebo alone. In usual dose-levels of 150 mg per day, fluvoxamine was not better than CBT, whereas an additive effect was seen when the two treatments were combined (Sharp, Power and Simpson, 1996). Although the number of studies combining exposure *in vivo* with pharmacotherapy was limited, van Balkom *et al.* (1997) concluded from a meta-analysis from studies pertaining to different treatment conditions, that in the short-term treatment of PD the combination is superior. De Beurs and co-workers (1995) compared fluvoxamine (150 mg per day) plus exposure therapy, placebo plus exposure, panic management plus exposure or exposure alone in a 12 week, double-blind study. They found that fluvoxamine plus exposure therapy reduced agoraphobic behaviour more than the other three treatment conditions. Also after a two-year follow-up study of patients from this study, it was found that the beneficial effects of combination therapy was maintained long term, although the differences between the treatment condìtions were no longer significant (de Beurs *et al.*, 1999).

Dual Action Antidepressants in Panic Disorder

Although the aetiology of PD is unclear, it has been suggested that besides serotonergic dysfunction noradrenergic dysfunction is also associated with PD. Although SSRI's appear to be consistently effective in relieving the symptoms of PD the results of studies with selective noradrenergic agents are generally negative. Maprotiline (a selective noradrenergic agent) for example was found to be ineffective in PD (den Boer and Westenberg, 1988). On the other hand a small anxiolytic effect was seen during the use of the selective noradrenalin reuptake inhibitor reboxetine (Phillips *et al.*, 2000). In addition, a recent pilot study conducted in SSRI-resistant panic disorder found reboxetine to be efficacious in reducing the number of panic attacks (Dannon, Iancu and Grunhaus, 2001).

New drugs have been developed to combine noradrenergic and serotonergic sites of action. Mirtazapine is one of these drugs. It is an antidepressant with a different mode of action than the SSRI's and thus with a different side effect profile. The mechanism of action is enhancement of both noradrenergic and serotonergic neurotransmission without reuptake inhibition (den Boer *et al.*, 1994, 1995).

A number of placebo controlled studies have proven the efficacy of mirtazapine in major depressive disorder (Gorman, 1999; Thompson, 1999; Kent, 2000). Efficacy in moderate to severe depression is equivalent to that of TCAs and SSRIs. The tolerability and the safety profile of mirtazapine are more favourable than that of TCAs. The efficacy of mirtazapine in the treatment of anxiety symptoms has been described (Fawcett *et al.*, 1998; Connor *et al.*, 1999a, 1999b; Goodnick *et al.*, 1999; Nutt, 1998). So far, one open label study was published showing efficacy for mirtazapine in PD (Carpenter *et al.*, 1999). We recently completed a larger open-label study in 23 patients with PD according to DSM-IV (Boshuisen *et al.*, 2001). Patients were treated for 15 weeks including a three-week placebo run-in period. As primary efficacy measures we studied the decrease in the number of panic attacks and the number of patients being panic free in the last three weeks of the study. 73.6% were considered responders (decrease of at least 50% in panic attack frequency). The results of this open label study in panic disorder suggest that mirtazapine seems to be a fast and effective treatment in panic disorder with a different side effect profile from SSRI's. Double-blind studies with mirtazapine in PD are ongoing.

Experience with venlafaxine in PD is limited. Two open-label study reported complete cessation of panic attacks in patients suffering from PD (Geracioti, 1995; Papp *et al.*, 1998). In the only double-blind placebo-controlled study published so far, Pollack *et al.* (1996) evaluated the efficacy of immediate-release venlafaxine for the treatment of PD in an 8-week trial. In this small-scale study 25 outpatients suffering from PD were included. Based on endpoint analysis venlafaxine-treated patients showed significantly greater improvement than the placebo group on the CGI, whereas the difference in the mean number of panic attacks was not significant between the two treatment conditions.

5-HT RECEPTOR AGONISTS

Evidence regarding the efficacy of the partial 5-HT_{1A} receptor partial agonists, buspirone and gepirone in panic disorder has been conflicting. Buspirone has shown antipanic activity in two placebo-controlled trials (Robinson *et al.*, 1989; Sheehan *et al.*, 1993), but has not demonstrated superior efficacy to placebo on all outcome measures. Similarly, an 8-week placebo-controlled study of buspirone and imipramine in 52 patients with panic disorder showed that buspirone was not significantly superior to placebo in its anxiolytic effects (Sheehan *et al.*, 1990). There have been no reports supporting the ability of buspirone to reduce the number of panic attacks to zero in a population of patients with panic disorder. In contrast, 59% of patients ($n = 21$) on gepirone reached panic-free status by the end of a 6-week open trial (Pecknold *et al.*, 1993). However, the potent and selective 5-HT_{1A} agonist, flesinoxan did not reduce the number of panic attacks in two small pilot studies (20 patients in total) (van Vliet, Westenberg and den Boer, 1996). Furthermore, treatment with flesinoxan produced a profound increase in symptoms of anxiety in these patients.

Buspirone in combination with cognitive-behavioural therapy (CBT), has demonstrated greater efficacy than CBT and placebo, suggesting that buspirone may be an effective adjunct to CBT (Bouvard *et al.*, 1997), however, this effect was not maintained in the long term.

5-HT RECEPTOR ANTAGONISTS

Electrophysiological experiments in anaesthetized animals have highlighted the role of somatodendritic 5-HT_{1A} autoreceptors in the delayed onset of action of antidepressants (Blier, de Montigny and Chaput, 1987). Microdialysis experiments in conscious animals have shown that the increase in extracellular 5-HT following reuptake inhibition by SSRIs is counteracted by activation of these autoreceptors (Invernizzi, Belli and Samanin, 1992; Hjorth and Auerbach, 1994). Repeated administration of SSRIs is thought to desensitize the somatodendritic autoreceptors, resulting in an enhanced availability of serotonin (Chaput, de Montigny and Blier, 1991). Arguably, this desensitization process can be mimicked instantaneously by blockade of the autoreceptors with 5-HT_{1A}

receptor antagonists. Consequently, coadministration of SSRIs with 5-HT_{1A} antagonists would hasten the therapeutic effect of SSRIs (Artigas, 1993). Several studies have indeed reported beneficial effects of this augmentation strategy with pindolol (e.g. Artigas, Perez and Alvarez, 1994; Blier and Bergeron, 1995) in major depression.

A preliminary report suggested that augmentation with pindolol is also effective in treatment resistant panic disorder (Dannon *et al.*, 1997). However, an augmentation study by van Vliet *et al.* (2001) with panic disorder patients was negative. In this open-label study patients were treated with either fluvoxamine and pindolol (7.5 mg daily) or fluvoxamine alone. The results of this study show that pindolol as add-on medication to fluvoxamine does not hasten the anxiolytic or antipanic effects of fluvoxamine. In a recent study 25 therapy-resistant PD patients received fluoxetine (20 mg per day) and additionally either placebo or pindolol (2.5 mg t.i.d.). Patients treated with the combination of pindolol and fluoxetine showed a significantly better treatment response over patients treated with fluoxetine plus placebo (Hirschmann *et al.*, 2000). Although preliminary in nature, this study is at odds with the findings with fluvoxamine. It is possible that pharmacological differences among the SSRI's could explain these differences, on the other hand it should be noted that there were differences in the inclusion criteria among the studies: in the study by van Vliet and co-workers treatment resistance was not a prerequisite for inclusion, whereas the other two studies did include only treatment resistant patients.

Preliminary open studies with the 5-HT_2 antagonists ritanserin and trazadone suggested that both agents may have antipanic and antiphobic activity (Griez, Pols and Lousberg, 1988; Charney *et al.*, 1986). However, placebo-controlled studies with a larger patient sample in which ritanserin was compared with fluvoxamine (den Boer and Westenberg, 1990), and trazodone with imipramine and alprazolam (Charney *et al.*, 1986) showed no reduction in panic attack frequency for either agent. Preliminary evidence has been reported for the efficacy of nefazodone in a small open, 8-week study of 14 patients with panic disorder and comorbid depression (DeMartinis, Schweizer and Rickels, 1996). Another open-label study reported beneficial effects of nefazodone in nine out of 10 patients (Bystritsky *et al.*, 1999). In spite of these promising open-label studies, these results have not been confirmed in well designed placebo controlled studies.

The 5-HT_3 antagonist ondansetron demonstrated only limited efficacy in a small pilot study of 31 patients with panic disorder and agoraphobia (Schneier *et al.*, 1996). The response rate of 48% was not beyond the upper limit of placebo response rates and, in the absence of a placebo control group, it was not possible to attribute the response rate to an improvement in panic attacks. As a result, the use of ondansetron in panic disorder is no longer being actively pursued.

Inositol

Inositol is a natural isomer of glucose and a precursor for the second-messenger phosphatidyl–inositol system. There is one recent double-blind comparative study versus fluvoxamine in which inositol has been investigated in panic disorder. In this study 20 patients were treated with inositol up to 18 mg per day for one month and the clinical outcome was compared to patients treated with fluvoxamine up to 150 mg per day (Palatnik *et al.*, 2001). The results of this preliminary study suggest similar efficacy for inositol and fluvoxamine in reducing the number of panic attacks. When replicated in larger studies treatment with inositol might offer an alternative for patients who are sensitive to the side-effects of SSRI's, as inositol is almost devoid of side-effects.

Conclusion

The only serotonergic agents which have clearly demonstrated efficacy as antipanic agents in both short and long-term investigations are the SSRIs. The SSRIs produce improvements in anticipatory anxiety and phobic avoidance. In addition, the SSRIs proven ability to enable patients with panic disorder to achieve panic-free status indicates that, as a first-line treatment in panic disorder, the SSRIs are a good therapeutic choice. In contrast, the 5-HT agonists and antagonists have demonstrated equivocal efficacy in panic disorder. Most of our judgements about the therapeutic efficacy of SSRIs (and other antidepressants) are based upon double-blind placebo controlled studies. To what extent the results of randomized clinical trials apply to everyday care cannot be judged without regular measurement of outcomes in daily practice. In a three-year naturalistic prospective study in PD, Toni *et al.* (2000) described the evolution of panic and agoraphoba in 326 DSM-IIIR PD patients. The main antidepressants used were paroxetine, imipramine and clomipramine. Although efficacy did not show major differences during the entire follow-up period, paroxetine-treated patients suffered significantly less from jitteriness and anticholinergic side-effects, thus reducing barriers to compliance for the SSRI treated patients. Preliminary results with inositol may appear promising, although the evidence for its efficacy is still circumstantial.

OBSESSIVE COMPULSIVE DISORDER

Introduction

The 'Serotonin Hypothesis of OCD' evolved from the speculation that the unique efficacy of the TCA, clomipramine, in OCD was due to its effects in facilitating 5-HT neurotransmission (Insel *et al.*, 1985; Zohar and Insel, 1987; Jenike *et al.*, 1989; Goodman *et al.*, 1990; Murphy and Pigott, 1990; Rasmussen, Elsen and Pato, 1993; Zohar *et al.*, 2000). Clomipramine was the first drug reported to be effective in OCD (Fernandez and Lopez-Ibor, 1967). The efficacy of clomipramine has been reviewed in many publications and will not be repeated here. OCD patients respond specifically to drugs that influence the serotonergic system; noradrenergic drugs like nortryptiline or drugs with weaker 5-HT reuptake inhibiting properties like imipramine and amitryptiline appear to be without effect on OCD symptomatology (Leonard *et al.*, 1989, 1991). The potential role of 5-HT in the pathogenesis of OCD has led to the investigation of other agents, in particular the SSRIs.

SSRIs in the Treatment of OCD

Paroxetine, fluvoxamine, sertraline and fluoxetine have all been investigated in large placebo-controlled trials and their efficacy as anti-obsessional agents is well established. A 12-week, fixed-dose study of paroxetine versus placebo in 263 patients with OCD demonstrated that paroxetine (40 and 60 mg per day) was significantly more effective than placebo in reducing OCD symptoms (mean reduction as assessed with the Yale–Brown Obsessive Compulsive Inventory (Y-BOCS), 25% and 29% versus 13% respectively) (Wheadon, Bushnell and Steiner, 1993).

Similarly, fluvoxamine was reported to be more effective than placebo in reducing OCD symptoms in a number of randomized double-blind studies (for review see Figgett and McClellan, 2000; Vythilingum, Cartwright and Hollander, 2000). In four placebo-controlled studies fluvoxamine at dosages of 100 to 300 mg per day was found to significantly reduce symptoms of OCD compared to placebo (Mallya, White and Waternaux, 1992; Greist, Jenike and Robinson, 1995a; Goodman *et al.*, 1996; Mundo, Bianchi and Bellodi, 1997). In most of these studies the response rate as

measured with the Y-BOCS was in the range of 40–52%. As with the other SSRI's, the average dose for fluvoxamine in OCD is higher than in other anxiety disorders and depression and is in the range of 150–300 mg per day.

Greist and colleagues (1995b) reported the results of a 12-week, placebo-controlled, fixed-dose study of sertraline in 324 patients with OCD; the active treatment (50 and 200 mg per day) was significantly more effective than placebo in reducing OCD symptoms (mean Y-BOCS reduction, 24% and 28% versus 15%). In a follow-up study of 40 weeks duration Greist and colleagues (1995b) reported continued improvement of sertraline over time. Interestingly, the occurrence of side-effects subsided throughout the year of treatment. A recent double-blind placebo-controlled study using a flexible dose schedule of sertraline (50–200 mg per day) in 167 patients with OCD confirmed the anti-obsessional/compulsive effects of this SSRI (Kronig *et al.*, 1999). Also, in patients suffering from OCD who also met the criteria for major depression, sertraline was found to be more effective in reducing both OCD and depressed symptomatology compared to the noradrenaline reuptake inhibitor desipramine (Hoehn-Saric *et al.*, 2000).

In a double-blind, fixed-dose study 217 OCD patients were treated with fluoxetine (20, 40 or 60 mg) or placebo for 8 weeks (Montgomery *et al.*, 1993). Only the 40 and 60 mg dosages were found to be statistically superior to placebo, indicating that also with fluoxetine higher dosages are required. In a 13-week, multicentre, placebo-controlled, fixed-dose study of 355 patients with OCD, fluoxetine (20, 40 and 60 mg per day) was significantly more effective than placebo (mean Y-BOCS reduction, 20%, 22%, and 27% versus 3%) in reducing OCD symptoms (Tollefson *et al.*, 1994a). The trend may suggest greater efficacy with increasing dosage.

The efficacy of citalopram has been less well-studied in OCD than the other SSRIs. Recently, however, citalopram has also demonstrated efficacy in OCD in two open studies (Mundo, Bianchi and Bellodi, 1997; Koponen *et al.*, 1997). In one of these studies, a 24-week pilot study in 29 patients with OCD, 76% of patients showed alleviation of symptoms in comparison with baseline (Koponen *et al.*, 1997).

SSRIs vs Clomipramine

The efficacy of the SSRIs in the treatment of OCD has been compared with clomipramine, which for a long time was considered the gold-standard treatment for this anxiety disorder. Paroxetine, fluoxetine and fluvoxamine have been shown to be at least as effective as clomipramine in the treatment of patients with OCD, but better tolerated (Wheadon, Bushnell and Steiner, 1993; Zohar and Judge, 1996; Goodman *et al.*, 1997; Koran, Mueller and Maloney, 1996). The first multicentre, placebo-controlled study of an SSRI versus clomipramine, in 399 patients with OCD, was performed with paroxetine (Zohar and Judge, 1996). Both paroxetine and clomipramine were associated with similar efficacy at weeks 6, 8, and 12. Furthermore, during a multicentre, flexible dose, 10-week study in 66 patients with OCD, significant and similar reductions in OCD symptoms were seen for fluvoxamine and clomipramine (mean Y-BOCS reduction, 33% and 31%) (Freeman *et al.*, 1994). Similar results were obtained in a recent double-blind comparison of fluvoxamine and clomipramine in 133 patients suffering from OCD. In terms of efficacy as measured with the Y-BOCS, no statistically significant differences were found between the two treatment conditions but the side-effect profile was more favourable in the fluvoxamine treated patients (Mundo, Maina and Uslenghi, 2000).

In a 16-week comparative study of sertraline and clomipramine in 86 patients with OCD a significantly greater reduction in OCD symptoms was found for patients on sertraline compared with clomipramine (mean Y-BOCS reduction, 51% versus 43%; $p < 0.05$) (Bisserbe *et al.*, 1997). However, a fair comparison may have been precluded because of the greater number of clomipramine subjects who withdrew early in the study (18 clomipramine versus three sertraline within 28 days). When compared with clomipramine in a 26-week, double-blind, crossover study in 11 patients with OCD, fluoxetine was associated with significant reductions from baseline in OCD symptoms which were comparable with clomipramine (Pigott *et al.*, 1990). Similar results were observed in an 8-week double-blind comparative study of fluoxetine vs clomipramine in 55 OCD patients (Lopez-Ibor *et al.*, 1996). Interestingly, a 10-week, double-blind study comparing fluvoxamine, paroxetine and citalopram demonstrated that there were no significant differences between the three treatments in terms of efficacy (Mundo, Bianchi and Bellodi, 1997).

Despite the chronicity of OCD and the recommendation that pharmacotherapy should exceed 9–12 months' duration, there are comparatively few published data on the efficacy of SSRIs or clomipramine for periods longer than 20 weeks (Jenike *et al.*, 1990a, b; Rasmussen, Elsen and Pato, 1993). It has been demonstrated that OCD symptoms continued to improve for up to 6 months of paroxetine therapy. Furthermore, patients on placebo relapsed three times faster than those on paroxetine during the extension phase of one placebo-controlled study.

In terms of long-term efficacy, a two-year, open-label follow-up of responders to clomipramine and fluvoxamine, found both maintenance treatments to be significantly superior to placebo in preventing relapse in patients with OCD (Ravizza *et al.*, 1996). Seventy-six patients with OCD who responded to treatment with fluoxetine during an acute treatment study maintained symptom improvement during a 24-week continuation study (Tollefson *et al.*, 1994b). Similarly, in a two-year, open-label follow-up of responders to clomipramine and fluoxetine, both maintenance treatments were found to be significantly superior to placebo in preventing relapse (Ravizza *et al.*, 1996). There is no data available on the long-term efficacy of citalopram or sertraline in OCD.

Several meta-analyses have addressed the question of the differential efficacy of clompiramine and SSRI's in OCD (e.g. Picinelli *et al.*, 1995; Greist *et al.*, 1995c). The main conclusion of these meta-analyses was that clompiramine had a somewhat better effect on OCD-symptomatology than the SSRI's, although the side-effect profile of the SSRI's was superior to clomipramine. These findings stand in sharp contrast with the direct head-to-head comparative studies vs clomipramine reviewed above: in these studies similar efficacy of the SSRI's was found. We should, however, remain aware of the methodological problems encountered in meta-analyses. Variables such as study design, time period, chronology and site-specific factors, which may have impact on the results, cannot be considered in meta-analyses.

In sum, there is substantial evidence from head-to-head comparisons of paroxetine, fluoxetine, sertraline and fluvoxamine with clomipramine to conclude a similar efficacy and a lower incidence of side-effects with the SSRI (see also Pigott and Seay, 1999).

Anti-compulsive or Antidepressant Action?

Symptoms of depression often co-occur with OCD raising the possibility that non-specific antidepressant and anxiolytic effects may determine the success of SSRI's in OCD (e.g. Marks *et al.*, 1980). In subsequent studies discussed above it has been shown that initial depression was not predictive, nor related in any way to the degree of improvement in OCD (e.g. Price *et al.*, 1987). Moreover, if the anti-obsessional and anti-compulsive effects were in fact related to antidepressant efficacy, that other antidepressants such as nortryptiline should also be effective in the treatment of OCD, and this clearly is not the case.

5-HT RECEPTOR AGONISTS

The 5-HT_{1A} receptor agonist buspirone has demonstrated equivocal results in efficacy studies in patients with OCD. Buspirone was ineffective in a small, 8-week, open study in 14 patients with OCD (Jenike and Baer, 1988). However, Pato and colleagues (1991) demonstrated that buspirone and clomipramine produced significant improvements in symptoms in 18 patients with OCD; no differences were noted between the active treatments (Pato *et al.*, 1991). There is no published evidence of the efficacy of gepirone or ipsapirone in OCD.

Evidence for the augmenting effect of buspirone in SSRI treatment-refractory patients with OCD is also equivocal. In a 20-week, open study with fluoxetine, clinical response was enhanced in 50% of patients by addition of buspirone during the last 8 weeks of the study (Jenike, Baer and Greist, 1991). In contrast, however, in a double-blind study in which 13 fluoxetine-treated patients with OCD were given adjuvant buspirone or placebo for 4 weeks, there were no significant differences in improvement of OCD symptoms between the two treatment groups (Grady *et al.*, 1993).

5-HT RECEPTOR ANTAGONISTS AND TREATMENT REFRACTORY OCD

There is a paucity of data with respect to the treatment options in treatment refractory OCD. There is circumstantial evidence from open-label studies that adding another SSRI to ongoing treatment with clomipramine could lead to further improvement, but the evidence is not compelling (Pallanti *et al.*, 1999).

A small number of patients with SSRI treatment refractory OCD have been given open trials of risperidone (an atypical antipsychotic agent with potent dopaminergic and serotonergic antagonist activity) (Saxena *et al.*, 1996; Jacobsen, 1995). In the largest of these studies, 21 patients were treated openly with the combination of an SSRI (paroxetine, fluvoxamine, sertraline or fluoxetine) and risperidone (Saxena *et al.*, 1996). Sixteen patients tolerated the combined treatment and, of these, 87% had substantial reductions in OCD symptoms within 3 weeks. Similar results were reported in a recent open label study with risperidone as add-on to ongoing treatment with SSRIs (Pfanner *et al.*, 2000). In a recent double-blind placebo-controlled study it was found that adding risperidone for 6 weeks to ongoing therapy with SSRIs in treatment refractory patients led to a 50% response rate, which was not present in the placebo treated patients (McDougle *et al.*, 2000). There has also been a preliminary report that the 5-$HT_{2A/2C}$ receptor antagonist ritanserin was effective as an adjunct to fluoxetine treatment in OCD patients with psychotic features (Bach, Aigner and Lenz, 1997).

Experience with other atypical antipsychotics in treatment refractory OCD is limited and studies are ongoing. There is one open-label study in which olanzapine (5 mg per day) was added to treatment with fluvoxamine (300 mg per day). It was found that this augmentation led to a significant decrease in the Y-BOCS (Bogetto *et al.*, 2000).

Open studies suggest that pindolol has shown some merit as an adjuvant to SSRIs (paroxetine, fluvoxamine, sertraline or fluoxetine) in OCD (Blier and Bergeron, 1995). In a recent study 14 patients who were nonresponders to previous treatment with two SSRIs and were currently unresponsive to treatment with paroxetine (60 mg per day) were included in a double-blind placebo-controlled pindolol (2.5 mg t.i.d.) augmentation study (Dannon *et al.*, 2000). As measured with the Y-BOCS, significant differences were noted in favour of pindolol compared to placebo, whereas no differences were present between placebo and pindolol on measures of anxiety and depression.

There is anecdotal evidence that treatment-refractory patients might respond to treatment with the 5-HT_{1D} agonist sumatriptan (Stern *et al.*, 1998). In a recent study, however, we were not able to show involvement of the 5-HT_{1D} receptor in OCD in a double-blind placebo controlled challenge study using the selective 5-$HT_{1D/1B}$ receptor agonist zolmitriptan and therefore treating patients with 5-HT_{1D} agonists should not be encouraged before further clinical studies have been performed (Boshuisen and den Boer, 2000).

Finally, inositol augmentation to SSRI treatment did not lead to significant improvement in 10 OCD patients in an open-label study (Seedat and Stein, 1999).

THE LAST RESORT: PSYCHOSURGERY

In a small number of cases, OCD is refractory to irrespective which conventional treatment, and uncontrolled evidence suggests that such cases may respond to different types of psychosurgery. In spite of the fact that only a limited number of operations are performed, a variety of surgical techniques are used. They involve: capsulotomy, cingulotomy, subcaudate tractotomy, combined orbitofrontal/cingulate lesions.

During capsulotomy stereotactic lesions are produced in the anterior limb of the internal capsule. Cingulotomy involves stereotactic lesions of the anterior cingulate cortex. Subcaudate tractotomy involves placement of radioactive rods beneath and in front of the caudate nucleus.

All surgical treatments appear to be effective in approximately 30–40% of the patients, but no reliable clinical studies have been performed based upon which predictive indicators for good outcome can be defined. In addition, the site and size of the lesion remains to be established (for review, see Jenike, 1998). Cummings and co-workers (1995) studied 17 patients with OCD using a neuropsychological test battery after psychosurgery. The psychosurgery group appeared to perform more poorly only on the Wisconsin Card Sorting Test (WCST), indicating the impact of frontal lobe lesions on abilities mediating the formation and shifting of response sets. In another study 18 patients with intractable OCD who underwent cingulotomy participated in a follow-up study. In this small sample, 28% were responders, whereas 17% were partial responders (Baer *et al.*, 1995).

In one of the very rare studies investigating the effects of specific lesion sites in OCD, Irle *et al.* (1998) conducted a long-term follow-up of patients who underwent ventromedial frontal leukotomy during the seventies. Eight out of 11 subjects with lesions of the ventral striatum had developed substance dependence. Subjects with frontostriatal lesions showed most clinical improvement. In all patients, neuropsychological testing only revealed abnormalities on the WCST, whereas subjects with lesions of the dorsolateral frontal convexity showed other cognitive impairments such as attentional problems and memory problems.

In countries where psychosurgery is applied it is advised that an independent multidisciplinary legally constituted review board selects patients who apply for psychosurgery (Rosenfield and Lloyd, 1999).

Conclusion

The SSRIs have demonstrated efficacy in the treatment of OCD while the 5-HT agonists have shown equivocal results. Data from studies of the 5-HT antagonists in patients with OCD suggest that these agents may be useful as augmenting agents in treatment-refractory patients. However, drugs such as risperidone and olanzapine have mixed serotonergic and dopaminergic functions and the efficacy of these agents in OCD may be correlated with their action on more than one neurotransmitter.

Until recently, the placebo response in many studies using antidepressants in OCD has been very low (5–10%). In more recent studies, higher placebo responses were observed (up to 30%). The reason for this could be that patients who participated in the original clomipramine studies could distinguish placebo from clomipramine because of the severe side-effects. It is possible that the newer drugs can be less clearly distinguished from placebo thus yielding a greater placebo response. Lastly, it is conceivable that due to competing studies investigators may be inclined to include patients with mild OCD.

GENERALIZED ANXIETY DISORDER (GAD)

Introduction

Since 1968, the diagnostic and statistical manual, second edition (DSM-II) delineated anxiety neurosis. This category was abolished in the DSM-III from 1980, in which generalized anxiety disorder (GAD) and panic disorder were delineated. In the DSM-III GAD was considered a 'residual' mental disorder which was diagnosed by exclusion. In the DSM-III-R it was defined by the presence of excessive/unrealistic worry as the core symptoms plus additional associated anxiety symptoms. In the DSM-IV, GAD was redefined to include the A-criterion symptoms of excessive uncontrolled anxiety and worry for at least 6 months plus three or more additional somatic and psychological anxiety symptoms (which is consistent with ICD-10 criteria). These include among others: muscle tension, restlessness, fatigue, difficulty in concentrating, irritability, and sleep disturbance.

Usually GAD is considered to be a chronic disorder. The study by Yonkers *et al.* (1996) of 166 GAD patients reported a man duration of GAD of more than 20 years and a likelihood of GAD symptom remission of only 15% and 25% at 1 and 2 years, respectively. This chronicity is consistent with a previous study of a large group of anxiety disorder patients with different diagnoses which reported that GAD patients were symptomatic for 56% of their lives after onset compared with much lower proportions in anxiety disorders traditionally thought to be more severe (Roy-Byrne, 1996) (16% for panic and 29% for agoraphobia with panic).

Differences in Cognitions between GAD and PD: the Role of Worrying

McNally (1994) considered that the distinction between GAD and panic disorder in DSM-III-R was blurred in two ways. Firstly, eliminating the diagnostic hierarchies of DSM-III allowed for very high rates of comorbidity, and revealed that GAD was rarely diagnosed as a single disorder. Secondly, unexpected panic attacks often occur in GAD patients, but few of these patients suffer from panic disorder (Sanderson and Barlow, 1990). The most important worry for PD patients is focused on fear of having another panic attack, as opposed to any other worry (Adler *et al.*, 1989; McNally, 1992, 1994). Worry in GAD patients is usually associated with exaggerated predictions of external catastrophic events (Borkovec and Roemer, 1995; Lydiard, 2000).

In view of the high degree of comorbidity associated with GAD, it has been argued that GAD could be a trait-like 'platform' that sets the stage and confers the vulnerability for the development of major depression, panic and other anxiety disorders. Recent evidence, however, challenged this notion and suggests that initial concerns about extremely high degrees of comorbidity among patients with GAD are misplaced. Wittchen and co-workers showed that comorbidity is a high predictor of help-seeking behaviour in patients with GAD indicating that in earlier studies this sample was over-represented and constitutes a selection bias (Wittchen *et al.*, 1994). In a recent review Kessler (2000) concluded that GAD does not stand out in any particular way with respect to other disorders in predicting other anxiety or depressive disorders (see also Wittchen *et al.*, 2000). Therefore there is no reason to question the validity of GAD as a separate disorder, because this doubt should also apply to any other anxiety or mood disorder.

Benzodiazepines in GAD

Benzodiazepines (BDZs) are frequently prescribed for the treatment of GAD by general practioners. They are widely accepted by both patients and general practitioners because of their rapid onset of action and good tolerability. However, withdrawal symptoms hamper successful discontinuation after prolonged treatment and thus BDZs should not be recommended as first-line monotherapy for the long-term management of anxiety disorders. Interestingly, several studies have shown that BDZs are effective on specific GAD symptoms such as somatic/autonomic symptoms in contrast to the psychic symptoms cluster including apprehensive worry and irritability (for review see Connor and Davidson, 1998). Side-effects of BDZs include sedation, memory difficulties, additive effects on alcohol and severe withdrawal reactions. Several treatment strategies such as cognitive therapy (Otto *et al.*, 1993) and concomitant prescribing of pharmacological agents like carbamazepine (Schweizer *et al.*, 1991) have been employed to assist patients to overcome their severe withdrawal syndrome induced by tapering off BDZs. In a recent study Rickels *et al.* (2000a) studied 107 patients with GAD who were long-term BDZs users. They were enrolled in a BDZs withdrawal programme assessing the efficacy of concomitant imipramine (mean dose: 180 mg per day) vs buspirone (mean dose: 38 mg per day) in a double-blind placebo controlled study. The success of the taper was significantly higher for patients treated with imipramine (82.6%), compared to either buspirone (success rates of taper 67.9%) and placebo (success rate taper: 37.5%). There is also evidence that prior treatment with BDZs is a negative predictor for therapeutic effects of subsequent treatment with buspirone. In a recent study DeMartinis and co-workers (2000) found that clinical improvement with buspirone was similar to BDZs treatment in GAD if patients had no prior treatment of BDZs. When patients were recently treated with BDZs, the effects of buspirone could not be distinguished from placebo.

During the last few years many studies have been performed using SSRIs, dual action antidepressants, 5-HT_{1A} receptor agonists and mixed SSRI/$5HT_{2A/C}$ receptor antagonists like nefazodone in the treatment of GAD. Older antidepressants like imipramine have also been shown to reduce anxiety in patients with GAD, although few clinicians would favour imipramine due to its severe side-effect profile compared to modern antidepressants. Based upon a large review of the published randomized double-blind studies up to 1997, Casacalenda and Boulenger (1998) concluded that imipramine, trazodone and paroxetine displayed similar efficacy in the treatment of GAD. At that time there was, however, a paucity of data of the effects of SSRIs and dual action antidepressants in GAD.

In a recent study it was found that clinical improvement with imipramine in GAD was present in patients with the lowest plasma levels of desipramine. In addition, there was a strong relationship with desipramine and anticholinergic levels, indicating that imipramine may have properties that result in physiological states counteracting its therapeutic effects (McLeod *et al.*, 2000). In addition, there are tolerability limitations using TCAs like imipramine and clomipramine in the treatment of GAD (Wingerson, Nguyen and Roy-Byrne, 1992).

Selective Serotonin Reuptake Inhibitors

There have been few studies to date on the use of the SSRIs in the treatment of GAD. Rocca and colleagues investigated the

efficacy of paroxetine, imipramine and 2′-chlordes-methyldiazepam in 81 patients with GAD (Rocca *et al.*, 1997). Both paroxetine and imipramine treatment resulted in more improvement than 2′-chlordes-methyldiazepam by the fourth week of treatment as measured by HAM-A and COVI Anxiety Rating Scale (CARS). At the end of the study, 68% of patients in the paroxetine group, 72% of those in the imipramine group, and 55% of those in the 2′-chlordes-methyldiazepam group showed a decrease of 50% or more on the HAM-A score. The efficacy of paroxetine in GAD (defined according to DSM-IV criteria) was evaluated in three other placebo-controlled 8-week studies and a long-term (32 week) relapse prevention study. In a multicentre study in 331 patients suffering from GAD paroxetine was found to be statistically superior to placebo on the HAM-A ($p < 0.01$). In addition, impairments in family and social life were beneficially influenced by paroxetine as assessed with the Sheehan disability Scale (McCafferty *et al.*, 2000). In a study in 566 GAD patients the results of treatment with placebo or paroxetine on the HAM-A and health related quality of life was investigated using the EuroQol (EQ)-5D questionnaire. This group reported that patients treated with 20 and 40 mg paroxetine not only showed significantly larger reductions in the HAM-A ($p < 0.001$), but also that improvement in GAD symptomatology is accompanied by improvement in health-related quality of life (Bellew *et al.*, 2000a, 2000b). The final multicentre study in 372 GAD patients treated with paroxetine or placebo provides supportive data for the efficacy of paroxetine in the treatment of GAD (SKB, data on file).

Stocchi and associates (2001) also studied the long-term efficacy of paroxetine (20–50 mg) in 652 patients with GAD. Patients received paroxetine (20–50 mg) for 8 weeks. Patients whose CGI scores had decreased by at least two points (to a score of three or less) at the week 8 visit were then randomized to double-blind treatment with either paroxetine ($n = 278$) or placebo ($n = 288$) for a period of 24 weeks. The results of this study showed that significantly fewer paroxetine- than placebo-treated patients (10.9% vs 39.9%, respectively, $p < 0.001$) relapsed during the 24 weeks of double-blind therapy indicating that anxiolytic efficacy in GAD is maintained during long-term treatment.

Dual Action Antidepressants in GAD

Venlafaxine

The therapeutic potential of venlafaxine extended release (XR) has been investigated in GAD and venlafaxine XR is the first antidepressant which has been registered for this indication. Three large placebo-controlled multicentre studies have been performed using venlafaxine XR in the treatment of GAD (for review see Hackett, 2000). A summary of these studies is shown in Table XIX-13.2.

The main conclusion of the 6-month flexible-dose study of Gelenberg and co-workers (2000) is that venlafaxine XR was significantly more effective than placebo on the main efficacy measures. Hackett, Parks and Salinas (1999a) performed a 24-weeks fixed-dose study of venlafaxine XR and found significant reductions of the HAM-A which were apparent after two weeks of treatment (for dosages and other details, see Table XIX-13.2). In a study with a similar design but a shorter study period Rickels *et al.* (2000b) found beneficial effects of venlafaxine XR in GAD. In addition there are two comparative studies with buspirone and diazepam as comparators (Davidson *et al.* 1999; Hackett, Desmet and Salinas, 1999b [the latter study is only available as a poster-presentation]).

In an 8-week randomized double-blind placebo-controlled multicentre study, venlafaxine XR (75 or 150 mg) was compared to buspirone 30 mg per day. At endpoint, there was a higher mean reduction in the main efficacy variable (HAM-A) in the venlafaxine-treated patients, but the difference with buspirone was not statistically significant (for detailed discussion, see Barman Balfourand and Jarvis, 2001). Reductions in psychic anxiety scores and score for anxious mood and tension were significantly lower for venlafaxine XR treated patients compared to placebo. The effects of buspirone on these measures, however, were not statistically different from placebo.

In sum, venlafaxine is the first antidepressant to be approved by the Food and Drug Administration of the USA and authorities in Europe for the treatment of GAD. It appears to be consistently better than placebo in the treatment for GAD in terms of inducing reductions of psychometric scales measuring severity of anxiety symptoms. There is substantial evidence from long-term studies that this improvement sustained over 6 months of treatment. Further studies are warranted to establish whether this improvement remains in time and contributes to improvements in the quality of life of these patients.

Mirtazapine

There is only one study in which mirtazapine was studied in GAD. In this open-label study patients with a DSM-IV diagnosis of major depressive disorder with comorbid GAD were prescribed mirtazapine for 8 weeks. In addition to its antidepressant effects, mirtazapine led to a significant reduction on the Hamilton Anxiety Rating Scale, indicating that mirtazapine could have potential value in the treatment of GAD (Goodnick *et al.*, 1999).

5-HT Agonists

Buspirone has been shown to be effective in the treatment of GAD in the short term. A 4-week single-blind study of 23 patients with GAD showed that, when titrated to a daily dose of 30 mg, buspirone provided effective anti-anxiety therapy as assessed by standard psychometric rating scales (Cohn, Wilcox and Meltzer, 1986). It needs to emphasized, however, that several of the studies of buspirone were conducted before proper criteria for GAD were defined, and therefore it cannot be stated that buspirone is effective in GAD according to DSM-IV criteria, because there simply are no studies of buspirone treatment in GAD according to DSM-IV criteria.

Concomitant depression occurs in at least 50% of patients with a primary diagnosis of GAD. A composite analysis of five placebo-controlled studies with buspirone in 382 patients with major depression and associated GAD showed significant improvements in anxiety (Robinson *et al.*, 1990). Interestingly, a 6-week, placebo-controlled efficacy study with buspirone in 121 patients with GAD and mild depression reported a significant reduction from baseline in anxiety and depression for patients on buspirone in comparison to those on placebo (Sramek *et al.*, 1996). In addition, a double-blind study in patients with GAD and depression showed that buspirone was equally effective as diazepam in reducing both anxiety and depression (Feighner *et al.*, 1982). These results suggest that buspirone may be suitable for the treatment of patients with GAD and comorbid depression. Furthermore, a meta-analysis of eight placebo-controlled studies in 520 patients with GAD demonstrated significant improvements over baseline for anxiety and depression for patients on buspirone in comparison with those on placebo (Gammans *et al.*, 1992). Similar results were also obtained when the efficacy of buspirone was assessed in comparison with clorazepate (Goldberg and Finnerty, 1982) and alprazolam (Enkelmann, 1991).

Gepirone is also effective in the treatment of GAD, however it has been suggested that the anxiolytic response to gepirone may be delayed: an 8-week, double-blind study in 198 patients

Table XIX-13.2 Efficacy of once-daily venlafaxine XR (VEN) in patients with generalized anxiety disorder without comorbid major depressive disorder in multicentre, randomized, double-blind, placebo-controlled studies

Treatment mg per day	Duration (wk)	No. of pts	Baseline scores		Results at endpoint (mean change from baseline)[a]					Response rate[b] (% of pts)	Discontinuations for unsatisfactory response(% of pts)
			HAM-A total	HAM-A psychic anxiety	HAM-A total	HAM-A psychic anxiety	CGI-I	CGI-S	HAD		
Gelenberg *et al.*, 2000 (flexible dose)											
VEN 75–225[c]	28	115	25	14	↓13.4***	↓7.4***	2.2***	↓2.1***		≥69***	8**
PL		123	25	14	↓8.7	↓4.2	3	↓1.1		42–46	22
Hackett *et al.*, 1999[d] (fixed dose)											
VEN 37.5	24	138	26.6	11.9	↓13.8*	↓7.6*			↓5.2*	61*[e]	17
VEN 75		130	26.3	11.6	↓15.5*	↓8.5*			↓6.3*	69**[e]	10
VEN 150		131	26.3	11.7	↓16.4*	↓9.2*[†]			↓7.1*[†]	75**[e]	2
PL		130	26.7	12.1	↓11.0	↓5.6			↓3.1	46[e]	21
Rickels *et al.* 2000 (fixed dose)											
VEN 75[f]	8	86	24.7	13.9	↓11.2	↓6.7	2.3	↓1.5	↓6.0*		5
VEN 150[f]		81	24.5	14.0	↓12.4	↓7.4*	2.3	↓1.7	↓6.2**		0
VEN 225[f]		86	23.6	13.4	↓11.5*	↓6.9**	2.2*	↓1.6**	↓6.2***		2
PL		96	24.1	13.9	↓9.5	↓5.6	2.6	↓1.3	↓4.2		5

[a]Last observation was carried forward for patients who withdrew from the study.
[b]Response rates were defined as 40 (Gelenberg *et al.*, 2000) or 50% (Hackett *et al.*, 1999) reductions from baseline in HAM-A total scores or a CGI-I score of 1 or 2 (Gelenberg *et al.*, 2000). Values presented from Gelenberg *et al.* (2000) pertain to weeks 6 through 28 of treatment.
[c]VEN dosage was started at 75 mg per day and increased at days 8 and 15 (to 150 and 225 mg per day, respectively) if required.
[d]The results of this study have not been published in full, but are available in a poster (Hackett *et al.*, 1999).
[e]Values estimated from a graph.
[f]VEN dosage was started at 75 mg per day and increased by 75 mg per day per week to the targetted dosage level.
CGI-I = Clinical Global Impression-Global Improvement; **CGI-S** = Clinical Global Impression-Severity of Illness; **HAD** = Hospital Anxiety and Depression Scale (anxiety subscale); **HAM-A** = Hamilton Rating Scale for Anxiety; **PL** = placebo; **pts** = patients; **XR** = extended release; ↓= decrease; *$p \leq 0.05$; **$p \leq 0.01$; ***$p < 0.001$ vs PL;
[†]$p \leq 0.017$ vs VEN 37.5 mg per day
Reprinted with permission from Balfour and Jarvis (2000), *CNS Drugs (Adis International)* **15**(6), 494.

with GAD reported that clinical improvement was significant from week 6 for patients on gepirone compared with significant relief from week 1 onwards for patients on diazepam (Rickels *et al.*, 1997). A 5-week, placebo-controlled, dose-finding efficacy study in 267 patients with GAD has also suggested that ipsapirone represents a viable treatment option for GAD (Cutler, Hesselink and Sramek, 1994).

In a recent study weak anxiolytic effects were observed of the 5-HT_{1A} receptor agonist lesopitron in a double-blind placebo-controlled study vs lorazepam (Sramek *et al.*, 1996; Fresquet *et al.*, 2000). These findings need replication in larger trials.

In terms of long-term therapy, buspirone has been demonstrated to be effective as maintenance therapy: a 12-month, open study in 700 patients with GAD reported a significant reduction in anxiety in patients treated with buspirone (Feighner, 1987).

5-HT Antagonists

Early evidence with the 5-$HT_{2A/2C}$ receptor antagonist ritanserin indicated that it may be an effective anxiolytic agent: 24 patients with GAD treated for 6 weeks with ritanserin or lorazepam showed comparable improvements in symptoms (Bressa, Marini and Gregori, 1987). The efficacy of nefazodone was studied in an 8-week open trial in 21 patients with GAD. Fifteen of the patients completed the study, and of these 80% were rated as 'much' or 'very much' improved as measured by the CGI scale (Hedges *et al.*, 1996). Although promising, these results have not yet been substantiated in placebo-controlled, double-blind trials. Similarly, controlled data with the 5-HT_3 antagonist ondansetron are not yet available.

Conclusion

The 5-HT_{1A} agonists are the most studied of the serotonergic drugs in the treatment of GAD. It is questionable whether newly developed 5-HT1A receptor agonists like lepisetron will be able to conquer a niche in the treatment of GAD in view of the fact that several studies indicate that the efficacy of venlafaxine is better than buspirone. Evidence is also emerging for the efficacy of paroxetine in GAD, but further investigation is warranted for the SSRI drug class. Similarly, only preliminary data are available with the 5-$HT_{2/3}$ receptor antagonists.

SOCIAL ANXIETY DISORDER

Introduction

The majority of individuals will admit to recognizing an element of anticipatory anxiety on specific occasions when they are under public scrutiny, such as prior to giving a speech or playing a musical instrument; this has been called 'normal' social discomfort (Rosenbaum *et al.*, 1994). However, social anxiety disorder is the excessive fear harboured by some people that their performance or social interaction will be viewed as inadequate, to the point of causing them embarrassment or humiliation. These people experience extreme distress in, or will completely avoid, the feared social setting.

Social anxiety disorder is a common mental disorder, yet it is one of the least investigated and most misunderstood (Judd, 1994). Therefore, we will devote more attention in this introduction to diagnostic and epidemiological issues before we discuss the pharmacotherapeutic options. The avoidance of everyday social situations by patients with social anxiety disorder can cause considerable disruption to patients' work, relationships and normal functioning (Schneier *et al.*, 1992; den Boer, 1997; Lépine and Lellouch, 1995). Many patients with social anxiety disorder are single, divorced or separated (Magee *et al.*, 1996). In addition, they may have a lower educational attainment and a lower socioeconomic status; in an epidemiologic study, over 50% of patients with social anxiety disorder did not complete secondary school, more than 70% were in the lowest two quartiles in terms of socioeconomic status, and approximately 22% were receiving welfare payments, suggesting that they were unable to work (Schneier *et al.*, 1992). This level of functional disability imposes an economic burden on society, not only because of sufferers' financial dependency and lack of gainful productivity, but also because of the increased risk of suicide attempts, particularly if there is a comorbid disorder (Schneier *et al.*, 1992). This disabling condition therefore necessitates early recognition and treatment.

Defining and Diagnosing Social Anxiety Disorder

The updated DSM (DSM-IV) (APA, 1994) and the ICD-10 criteria can be used to define social anxiety disorder. Both criteria state that social anxiety disorder is a distinct disorder involving a marked fear or anxiety of behaving in an embarrassing or humiliating manner while under the gaze of other people, which then leads to avoidance of the situations that stimulate this fear (Lépine and Lellouch, 1995; den Boer, 1997). Two distinct subtypes of social anxiety disorder are recognized: non-generalized and generalized. Non-generalized social anxiety disorder involves one or two social or performance situations, such as public speaking, whereas individuals with generalized social anxiety disorder fear a multitude of social and performance situations (Heimberg *et al.*, 1990).

Other conditions from which social anxiety disorder should be distinguished are panic disorder, separation anxiety (in children), and atypical depression, although fear or avoidance of social or performance situations in a public setting should identify the patient with social anxiety disorder. Diagnoses of social anxiety disorder and avoidant personality disorder (APD) have arisen from different historical sources; however, the difference between these conditions has become indistinct with the introduction of the DSM-III-R criteria. Schneier *et al.* (1991) found that most (89%) of the patients with generalized social anxiety disorder were also diagnosed with APD. This co-occurrence of social anxiety disorder and APD may indicate a more severe form of social anxiety disorder (Holt, Heimberg and Hope, 1992).

Management Options

As the need for treatment of social anxiety disorder has been recognized only recently, optimum treatment strategies have not yet been defined. The SSRIs have been recommended as the pharmacologic treatment of choice (Ballenger *et al.*, 1998b) but the role of combined or sequential psychotherapeutic and pharmacologic treatment has still to be clarified (Heimberg, 1993). Drug treatment may relieve the symptoms of social anxiety disorder but habitual patterns of avoidant behaviour may take longer to respond; therefore, a combined strategy may be more effective than either alone (Marshall, 1992).

Pharmacological Approaches

Pharmacological treatment of social anxiety disorder has become established only in the past decade. The most recently investigated drug class is the SSRIs, for which there is growing support (Jefferson, 1995; van Ameringen, Mancini and Oakman, 1999a). Other explored drug classes include tricyclic antidepressants, monoamine oxidase inhibitors (MAOIs), benzodiazepines, and β-blockers (Davidson, 1998).

Table XIX-13.3 Summary of studies of SSRIs in patients with social anxiety disorder

Drug	Type of study	No. Patients	Outcome	Reference
Paroxetine	12 week, double-blind, placebo-controlled study	384	Relative to baseline, paroxetine produced significantly greater improvements than placebo in endpoint Leibowitz Social Anxiety Scale (LSAS) total scores ($p < 0.001$) for the 20 mg dose with similar degrees of improvement exhibited by the 40 mg and 60 mg doses. The proportion of responders CGI-I (score 1 or 2) was higher for paroxetine (20 mg 45%, 40 mg 47%, 60 mg 43%) than placebo (28%) with a statistically significant difference at the 40 mg dose.	Baldwin, 2000
Paroxetine	12-week, double-blind, placebo-controlled study	187	Relative to baseline, paroxetine produced significantly greater improvements than placebo in endpoint Liebowitz Social Anxiety Scale (LSAS) total scores ($p < 0.001$), subscale scores ($p < 0.001$), Social and Anxiety Distress Scale scores (SADS; $p < 0.001$) and Sheehan Disability Inventory social and work item scores (SDI; $p < 0.001$ and $p < 0.05$, respectively). The proportion of responders with paroxetine (55%) was significantly higher than with placebo (24%; $p < 0.001$) based on a CGI global improvement score of 1 or 2.	Stein *et al.*, 1998
	12-week, double-blind, placebo-controlled study	290	Patients receiving paroxetine experienced a significantly greater reduction in LSAS total score compared with placebo ($p \leq 0.001$). The proportion of responders (based on a CGI global improvement score of 1 or 2) in the paroxetine group was significantly greater than in the placebo group (65.7% vs 32.4%; $p < 0.001$). Paroxetine also produced significant improvements in social avoidance (as measured by the SADS) ($p < 0.032$) and in patients' family ($p < 0.001$), social ($p < 0.05$), and work ($p < 0.001$) lives (as measured by the SDI) compared with placebo.	Baldwin *et al.*, 1999
	Randomized, double-blind, placebo-controlled, 12-week trial	99	At week 12, 70.5% of paroxetine-treated patients and 8.3% of placebo-treated patients were responders ($p = 0.0001$). Paroxetine produced significantly greater improvements than placebo in endpoint LSAS total scores ($p = 0.0001$). Paroxetine was also significantly superior to placebo in terms of improvements in Brief Social Phobia Scale ($p = 0.0001$), Fear of Negative Evaluation Scale ($p = 0.0003$), and SDI work ($p = 0.0065$), social ($p = 0.0048$), and family life ($p = 0.0092$) scores.	Allgulander, 1999
	11-week, open study	36	23/30 patients (77%) were 'much' or 'very much' improved on the Clinical Global Impression scale. Baseline/Week 11 reductions on the Duke Social Phobia Scale and Liebowitz Social Phobia Scale were 36/20 ($p < 0.0005$) and 75/37 ($p < 0.0005$), respectively.	Stein *et al.*, 1996
	12-week, open study	18	Paroxetine produced an 83% response assessed by a moderate-to-marked symptomatic improvement on the Liebowitz Panic and Social Phobia disorders rating Scale.	Mancini and Ameringen, 1996
	12 week single-blind; 24 week double-blind	437	A significantly greater proportion of patients in the paroxetine group were 'much improved' or 'very much improved' (as assessed with the CGI) at the end of the study, and that significantly fewer patients relapsed with paroxetine (14%) compared to placebo (39%, $p < 0.001$) during the 24-week follow-up.	Hair *et al.*, 2000; Stein *et al.*, 2001
Sertraline	6-week, open study	24	After 4 and 6 weeks, sertraline produced significant improvements compared with baseline ($p < 0.05$) on the Davidson Brief Social Phobia Rating Scale and Clinical Global Impression Severity and Change scales. 58% responded to treatment.	Martins *et al.*, 1994
	12-week, open study	22	16/20 responded to treatment. All measures of social anxiety and functioning and depression significantly improved compared with baseline.	van Ameringen *et al.*, 1994
	20-week, randomized, double-blind, crossover study	12	Scores on the Liebowitz Social Anxiety Scale significantly improved with sertraline ($p = 0.001$) but not with placebo. 6/12 moderately or markedly improved while taking sertraline compared with 1/12 while taking placebo.	Katzelnick *et al.*, 1995
	12-week, open study	11	5/7 completers substantially responded to treatment.	Munjack *et al.*, 1994/95
Citalopram	12-week, open study	22	19 out of 22 were responders to treatment.	Bouwer and Stein, 1998

Table XIX-13.3 (*continued*)

Drug	Type of study	No. Patients	Outcome	Reference
Fluoxetine	12-week, open study	13	10 patients had a moderate-to-marked improvement independent of changes on the Beck Depression Inventory.	van Ameringen *et al.*, 1993
	6–40 weeks, open study	14	10 patients moderately or markedly improved.	Black *et al.*, 1992
	12 week, open study	20	13 out of 20 patients were responders.	Perugi *et al.*, 1995
Fluvoxamine	12-week, double-blind, placebo-controlled study with 12-week follow-up	30	Fluvoxamine significantly better than placebo on Symptom Checklist 90 ($p < 0.05$) and superior to placebo on social anxiety item of Social Phobia Scale. Further improvements observed during follow-up period.	van Vliet *et al.*, 1994
	Double-blind, randomized, placebo-controlled, 12-week trial	92	At week 12, 53.3% of the fluvoxamine group and 23.5% of the placebo group were responders ($p = 0.01$). Compared with placebo, fluvoxamine produced significantly greater improvements on the BSPS ($p < 0.05$), the Social Phobia Inventory (SPIN; $p < 0.05$), LSAS ($p < 0.05$), and the work and family life items of the SDI ($p < 0.05$).	Stein *et al.*, 1999

Serotonin-Selective Reuptake Inhibitors

Four large, double-blind, placebo-controlled 12-week trials and one long-term relapse prevention study have recently reported a significant improvement of patients with social anxiety disorder prescribed paroxetine compared with those given placebo (Allugander, 1999; Baldwin *et al.*, 1999; Stein *et al.*, 1999; Baldwin, 2000; Stein *et al.*, 2001). Two double-blind placebo-controlled trials of fluvoxamine have also demonstrated the efficacy of this SSRI against the symptoms of social anxiety disorder (Stein *et al.*, 1999; DeVane *et al.*, 1999). Data for other SSRIs are limited to mainly open and small scale trials. The results of open and double-blind studies using SSRIs to treat patients with social anxiety disorder are shown in Table XIX-13.3.

SSRIs also provide effective and well tolerated treatment for depression and panic disorder, both of which may be comorbid with social anxiety disorder. In a recent meta-analysis conducted along the methods set forth by the Cochrane Collaboration, van der Linden and co-workers (2000) concluded that SSRIs as a group were effective for the treatment of social phobia. In addition, SSRIs had a larger response-rate and effect-size compared to the reversible monoamine oxidase inhibitors. There is a paucity of data on the long-term pharmacotherapy of SAD. In order to study the effectiveness of long-term paroxetine treatment in the prevention of relapse in patients with SAD, Stein and co-workers (2001) studied 437 patients in a placebo-controlled, multicentre study. The study comprised a 12 week single-blind treatment phase (paroxetine 20–50 mg) with responders ($n = 323$) being randomized to double-blind treatment with paroxetine or placebo for a further 24 weeks. Stein and co-workers found that a significantly greater proportion of patients in the paroxetine group were 'much improved' or 'very much improved' (as assessed with the CGI) at the end of the study, and that significantly fewer patients relapsed with paroxetine (14%) compared to placebo (39%, $p < 0.001$) during the 24-week follow-up. This study lends support to the widely held belief that patients should at least be treated for a period of one year.

One study has recently been published on the efficacy of sertraline in SAD. In this study 204 patients suffering from SAD were randomly allocated to treatment with either placebo or sertraline (50–200 mg per day; van Ameringen *et al.*, 2001). As assessed with the social phobia subscale of the Marks Fear Questionnaire, 32.6% of the patients using sertraline and 10.8% using placebo were considered responders. The mean reductions of the score on the Brief Social Phobia Scale yielded statistical superiority of sertraline over placebo (34.3% and 18.6% respectively), indicating that also sertraline is an effective treatment for SAD.

There is hardly any information about the efficacy of dual action antidepressants in social phobia. In a 12-week open-label study in which the effects of mirtazapine were evaluated in 16 patients van Vliet and co-workers (2000) found significant reductions in social anxiety and avoidance. Further placebo-controlled studies are necessary to confirm these preliminary findings.

Tricyclic Agents

Studies published more than 20 years ago reported positive results for clomipramine in the treatment of phobic disorders (Beaumont, 1977; Gringras, 1977). However, these were open studies and the patient population was not well defined. A further small study found that clomipramine significantly improved some aspects of social anxiety disorder and agoraphobia compared with diazepam but the patient population was again ill-defined (Allsopp, Cooper and Poole, 1984). A recent small open trial of imipramine did not support its efficacy as a treatment for social anxiety disorder (Simpson *et al.*, 1998).

Monoamine Oxidase Inhibitors

Double-blind, placebo-controlled trials have shown that patients with social anxiety disorder respond better to phenelzine, an irreversible MAOI) than with alprazolam (Gelernter *et al.*, 1991) atenolol (Liebowitz *et al.*, 1992) or placebo (Gelernter *et al.*, 1991; Liebowitz *et al.*, 1992). Earlier studies also suggested that phenelzine was more effective than placebo but these studies suffered from methodologic problems, making interpretation of the results difficult (den Boer, van Vliet and Westenberg, 1995). However, the use of phenelzine is limited due to the potentiation of the tyramine pressor effect, which can lead to hypertensive crises.

Conflicting results have been obtained with reversible MAOIs, such as brofaromine and moclobemide, which do not exhibit the tyramine pressor effect. Brofaromine appeared to significantly improve the symptoms of social anxiety disorder compared with placebo (van Vliet, den Boer and Westenberg, 1992; Lott *et al.*, 1997) but is now no longer in development. Moclobemide also appeared to be statistically significantly more effective than placebo after 8 weeks' treatment in a small double-blind study (Versiani *et al.*, 1992) and a large ($n = 578$), 12-week trial in patients with social anxiety disorder (The International Multicenter Clinical Trial Group on Moclobemide in Social Phobia, 1997). However, a further large ($n = 583$), 12-week, double-blind, placebo-controlled

trial in patients with social anxiety disorder failed to find a significant difference between moclobemide and placebo (Noyes *et al.*, 1997).

Benzodiazepines

Clonazepam and alprazolam have been evaluated in a few open studies and found to be effective in social anxiety disorder (Davidson, Tupler and Potts, 1994). Bromazepam was significantly superior to placebo in a 12-week double-blind study (Versiani *et al.*, 1997). In small controlled trials, clonazepam was significantly superior to placebo (Davidson *et al.*, 1993) while alprazolam was also superior to placebo, but inferior to phenelzine (Gelernter *et al.*, 1991). However, the putative efficacy of phenelzine in social anxiety disorder may partly be due to its sedative effect rather than due to true anxiolysis. Furthermore, since alcohol abuse is a common comorbid condition in social anxiety disorder, benzodiazepines should be avoided due to the risk of excessive sedation. Long-term use may also lead to dependency.

Beta-Blockers

Beta-blockers may alleviate anxiety as a secondary consequence of the reduction in autonomic symptoms (tremors, palpitations). These drugs have been effective in the short-term for performance anxiety (Laverdure and Boulenger, 1991; den Boer, van Vliet and Westenberg, 1994; Jefferson, 1996). Despite promising early work with atenolol, subsequent investigations have not established efficacy for β-blockers in generalized social anxiety disorder (den Boer, van Vliet and Westenberg, 1994).

Other Drugs

A recent randomized placebo-controlled trial involving 69 patients demonstrated the efficacy of the anticonvulsant, gabapentin, in the treatment of social anxiety disorder (Pande *et al.*, 1999). After 14 weeks of treatment, patients receiving gabapentin experienced a significant reduction in the symptoms of social anxiety disorder compared with those receiving placebo. In an open trial of nefazodone, 69% of patients ($n = 23$) showed moderate or marked improvement after 12 weeks of treatment (van Ameringen, Mancini and Oakman, 1999b). Venlafaxine has also produced improvements in social anxiety disorder symptoms in a small open study involving 12 patients (Altamura *et al.*, 1999). Larger placebo-controlled trials are required to fully determine the efficacy of these agents.

Non-Pharmacologic Approaches

Cognitive-Behavioural Therapy

Cognitive-behavioural treatment aims to help people to overcome anxiety reactions in social and performance situations and to alter the beliefs and responses that maintain this behaviour.

One type of treatment, cognitive-behavioural group therapy, is given in 12 weekly sessions, each lasting about two and a half hours. It has six elements: cognitive-behavioural explanation of social anxiety disorder; structured exercises to recognize maladaptive thinking; exposure to simulations of situations that provoke anxiety; cognitive restructuring sessions to teach patients to control maladaptive thoughts; homework assignments in preparation for real social situations; and a self-administered cognitive restructuring routine (Heimberg and Juster, 1994).

Cognitive-behavioural group therapy was associated with long-term benefit in moderately impaired patients, and compared well with pharmacologic treatment (phenelzine, alprazolam) (Heimberg, 1993). Group therapy has been shown to be an effective treatment of social anxiety disorder in comparison with control groups or pill placebo (Heimberg and Juster, 1994; Donohue, van Hasselt and Hersen, 1994).

Impact of Comorbidity on Treatment Strategies

Comorbidity may reflect a more severe psychopathology, with more disability and impaired functioning than in the absence of comorbidity. This may create the expectation of a more difficult treatment course and a less favourable outcome, resistance to treatment, the need to treat each disorder effectively, and extended or long-term maintenance treatment to prevent relapse (Rosenbaum and Pollock, 1994).

Empirically, there are four general principles for dealing with comorbid disorders: tailor treatment to individual patients; use monotherapy in preference to polypharmacy, provided that the chosen drug is effective in both disorders and the comorbid disorder is secondary to the social anxiety disorder; consider compatible drugs for some patients; and administer a combination of psychotherapy and pharmacotherapy (Rosenbaum and Pollock, 1994). Social anxiety disorder comorbid with alcoholism is a special case because of the risk of excessive sedation with concomitant medication (benzodiazepines, for example), and patients should be carefully questioned about their use of alcohol, as well as the amount and the pattern of intake (Marshall, 1994). Comorbidity affects the choice of treatment, as well as its efficacy. A treatment scheme for comorbid conditions is outlined in Table XIX-13.4 (Jefferson, 1995).

Table XIX-13.4 Treatment scheme for comorbid conditions

Disorder	Suggested treatment		
Social anxiety disorder and major depression	SSRI or MAOI		
Social anxiety disorder and panic disorder	SSRI, MAOI or benzodiazepine		
	One drug/both disorders	*One drug per disorder*	*Combined approach*
Social anxiety disorder and obsessive–compulsive disorder (OCD)	SSRI or MAOI	Clonazepam for social anxiety disorder, clomipramine for OCD	SSRI, clomipramine or MAOI and behaviour therapy
Social anxiety disorder and alcoholism	—	SSRI for social anxiety disorder, disulfiram for alcohol abuse	SSRI, disulfiram and Alcoholics Anonymous
Social anxiety disorder with *no* comorbidity	—	—	SSRI, MAOI or benzodiazepine (with or without cognitive-behavioural therapy)

Based on Rosenbaum and Pollock, 1994; Jefferson, 1995.

Conclusions

Social anxiety disorder was recognized as a condition separate from other phobias and panic disorders as recently as the 1960s (Marks and Gelder, 1966). Three decades later, there is still much to learn about the causes and treatment of the disorder.

While social anxiety disorder is common in the general population, it is probably under-reported in general practice because, by its very nature, patients are reluctant to seek treatment. The delay in seeking help for social anxiety disorder may lead to the development of other psychiatric disorders such as major depression, and consequently a greater disability and risk of suicide. In addition, the inability of the patient to function as a normal, productive member of the community places an economic burden on the sufferer and on society. Early diagnosis and treatment of social anxiety disorder may help to alleviate the burdens imposed by the condition.

Optimum treatment strategies for social anxiety disorder have not been clearly defined. However, no one would disagree that treatment should be aggressive in view of the potential disability caused by the disorder. SSRIs show the most promise for the treatment of social anxiety disorder.

POST-TRAUMATIC STRESS DISORDER

There is a sparse but expanding literature on treatment options for PTSD. Most studies in PTSD have been performed in combat-related trauma victims and other serious traumas including rape, sexual molestation and assault. Until recently, the role of pharmacotherapy in PTSD was more of an adjunctive therapy to alleviate depressive and other comorbid symptoms. During the last few years, however, several double-blind studies have been performed which studied the effects on core symptoms of PTSD.

There is a great need for better treatments in this disorder as a recent study has shown that in spite of consistent treatment efforts, more than 30% of patients with episodes of PTSD do not recover even after several years (Kessler *et al.*, 1995). It was recommended by the Expert Consensus Panel for PTSD that the first-line treatment should be psychotherapy, whereas in cases complicated by comorbid psychiatric conditions (which is often the case) a combination of psychotherapy and pharmacotherapy is recommended (The Expert Consensus Panel on PTSD, 1999). PTSD is characterized by different groups of symptoms: intrusive thoughts (recurrent flashbacks and nightmares), avoidance behaviour (active avoiding memories of the traumatic events, numbness) and hyperarousal (insomnia, concentration difficulties). These symptoms are rather specific for PTSD, but it should not be overlooked that many patients also report other symptoms not assessed by DSM-IV criteria such as feelings of shame and guilt and ruminations on existential fear (Wenzel *et al.*, 2000).

Together with the high degree of comorbidity there is the issue of symptom subtypes. If the different symptom-complexes are mediated by different underlying neurobiological systems, it is conceivable that they respond to different psychotropic drugs. Considering the limited number of studies in PTSD this question will be difficult to answer. In addition, we still do not know which scales are the most useful in establishing effective pharmacotherapy in PTSD.

Tricyclics and MAO-Inhibitors in PTSD

Older antidepressants like amitryptiline and desipramine have been shown to be only slightly better than placebo (Davidson *et al.*, 1990; Reist *et al.*, 1989). Only depressive symptoms associated with PTSD responded to treatment in these studies.

The irreversible and nonselective MAOI phenelzine was found to be effective in a number of studies. In comparative studies versus imipramine and placebo in male veterans, phenelzine led to significant improvements in intrusive thought and nightmares. Emotional numbing, however, was not affected (Frank *et al.*, 1988; Kosten *et al.*, 1991). Needless to say, the dietary restrictions posed upon patients during treatment with phenelzine are a disadvantage to this type of drug. The newer generation of MAO-A inhibitors, for which dietary restrictions are not necessary, has been scarcely investigated in PTSD. There are some controlled studies with brofaromine in PTSD with mixed results (e.g. Baker *et al.*, 1995), but since brofaromine has not been registered these studies will not be discussed. Moclobemide, another selective and reversible MAO-A inhibitor which has been marketed for depression was investigated in a 12-week open-label study in 20 patients suffering from PTSD (Neal, Shapland and Fox, 1997). Typical PTSD symptoms subsided and symptoms severity was diminished in most of the patients. Double-blind placebo controlled studies have, however, not been performed with moclobemide.

Benzodiazepines

The use of BDZs in PTSD has been described by several authors, but there are hardly any well designed studies. Clonazepam was found to reduce anxiety and depression in an open-label study (Lowenstein *et al.*, 1988). In a 5-week crossover trial, Braun *et al.* (1990) studied the efficacy of alprazolam in a mixed population ($N = 16$) of therapy-resistant combat-related and civilian PTSD patients. Alprazolam dosages were increased up to 6 mg per day. Although a modest anxiolytic effect (measured with the HAM-A) was described, alprazolam failed to improve typical PTSD symptoms.

SSRIs in the Treatment of PTSD

Fluvoxamine has been studied in three small studies in PTSD: two trials were conducted in war veterans and one in civilians; all studies used an open-label design and included only a small number of patients (Marmar *et al.*, 1996; Davidson *et al.*, 1998; de Boer *et al.*, 1992). The results of these studies suggest that fluvoxamine can diminish the severity of specific PTSD symptoms such as survival guilt, nightmares, insomnia, intrusive recollections and fear. Placebo-controlled studies of fluvoxamine in PTSD are warranted to evaluate the potential role of fluvoxamine in PTSD.

Fluoxetine has been more extensively investigated in PTSD. There are both open-label and double-blind placebo-controlled studies evaluating the effects of fluoxetine in PTSD. Here we will confine the discussion to the placebo-controlled studies. Van der Kolk and co-workers (1994) conducted a 5-week placebo-controlled study and found that fluoxetine was significantly more effective in both combat veterans and civilian casualties. They found the typical PTSD symptoms to respond to treatment with fluoxetine: numbing and arousal. Interestingly, the treatment effect was most robust in the civilian group who suffered from acute traumas, indicating that fluoxetine is perhaps less effective in chronic PTSD. In a more recent study Connor *et al.* (1999a) included 54 civilian PTSD patients (most notably females) and found that fluoxetine up to 60 mg per day had beneficial effects on all typical PTSD symptoms. In a reanalysis of the data it was found that fluoxetine has a broad spectrum effect on the symptomatology of PTSD. The symptoms who were found to be most responsive to treatment with fluoxetine were: being physically upset at reminders of the trauma, avoiding thoughts of the trauma, anhedonia, feeling distant and impaired concentration (Meltzer-Brody *et al.*, 2000). A recent small placebo-controlled study also reported improved quality of

life as a result of fluoxetine treatment as assessed with the Short-Form Health Survey (SF-36; Malik *et al.*, 1999). In a small placebo-controlled study in severe PTSD male combat veterans fluoxetine could not be distinguished from placebo, indicating that severity or gender could influence treatment outcome (Hertzberg *et al.*, 2000).

Two open-label studies evaluated the effects of sertraline in PTSD and most of the core PTSD symptomatology showed a significant decrease (Brady, Sonne and Roberts, 1995; Rothbaum, Ninan and Thomas, 1996). In a 12-week double-blind placebo-controlled multicentre study Brady *et al.* (2000) studied the efficacy of sertraline (50–200 mg per day) in 187 outpatients suffering from non-combat-related PTSD. On three of the main efficacy parameters (Clinician Administered PTSD-scale; Clinical Global Impression Severity and Improvement Scales), sertraline showed a significantly better treatment effect compared to placebo. Around 53% of the patients were considered responders to treatment with sertraline and 32% responded to placebo. Significant efficacy was evident for sertraline on symptoms of avoidance/numbing, increased arousal, but not on re-experiencing traumatic events. Davidson and co-workers (2001) included 208 outpatients suffering from PTSD in a double-blind placebo controlled study. Although their study was not powered enough to evaluate clinical variables such as sex, type of trauma, duration of illness, or presence of comorbidity on treatment response, they reported a 60% response-rate for sertraline and a 38% response rate for placebo.

It has been argued that repeatedly responding to PTSD-related questions can possibly evoke an element of exposure and desensitization (Krakow, Hollifield and Warner, 2000). Thus, since there was a considerable placebo response in this study, this might have been achieved by covert cognitive-behavioural therapy. If this were true, then this reasoning should also apply to other syndromes for which CBT is an effective treatment (e.g. PD, OCD) and since many placebo-controlled studies have been performed yielding significant differences between active treatment and placebo it is almost impossible to disentangle the relative contribution of 'covert' CBT to the treatment. Nevertheless, in PTSD there is a need for studies exploring the interface between pharmacotherapy and psychotherapy.

Paroxetine was found to be effective in a small open-label study in chronic non-combat-related PTSD (Marshall *et al.*, 1998). All typical symptom clusters were improved and there were also beneficial effects of paroxetine on anxiety and depression-scores. Very recently, a number of placebo-controlled studies has been conducted with paroxetine in PTSD. Ruggiero and associates (2001) included 307 patients suffering form PTSD according to DSM-IV criteria in a flexible-dose study. As primary efficacy parameters they used the clinician administered PTSD scale (CAPS-2) and the Clinical Global Impression Scale. Their study showed that paroxetine in a dosage of 20–50 mg was more efficacious than placebo in reducing the symptom clusters of the CAPS-2 and in improving psychosocial functioning. In a 12-week double-blind fixed dose study in 550 outpatients with PTSD, Beebe and co-workers (2000) found paroxetine (20 and 40 mg) to be effective in reducing PTSD symptoms in all symptom domains including re-experiencing, avoidance, intrusion and hyperarousal. A further 12 week double-blind flexible-dose (20–50 mg per day) in 322 outpatients with PTSD showed similar efficacy (unpublished data). Interestingly, when PTSD patients were exposed to individual trauma scripts, another group found autonomic functioning (heart rate and blood-pressure) to be improved after treatment with paroxetine in a 10-week open-label study (Tucker *et al.*, 2000), indicating that a wide range of PTSD symptoms are affected by paroxetine.

In view of the repeatedly reported finding that PTSD patients often suffer from comorbid depression, Stein *et al.* (2001) pooled the data of the three mentioned double-blind placebo-controlled studies described here and found that both the depressed and the non-depressed subgroups showed a statistically significant improvement in the CAPS-total score and the CGI for paroxetine compared to placebo.

Dual Action Antidepressants in PTSD

There is only one small open-label study in which mirtazapine has been studied in PTSD. Six outpatients with chronic PTSD were treated with mirtazapine and three of them were responders (Connor *et al.*, 1999b). Due to the small sample any conclusion would be premature. One case study reported therapeutic effects of venlafaxine in a PTSD patient who did not respond to various serotonergic antidepressants (Hamner and Frueh, 1998).

Serotonin Receptor Antagonists in PTSD

There are a number of open studies in which therapeutic effects of trazodone and nefazodone in PTSD has been investigated. Trazodone was found to be effective in just one open-label study in six combat-related PTSD patients (Hertzberg *et al.*, 1996), but there are no published controlled studies of this compound. The therapeutic potential of nefazodone, an antidepressant which blocks 5-HT_2 receptors and 5-HT reuptake was studied in the beginning of the 1990s in six open-label studies (data pooled and reviewed by Hidalgo *et al.*, 1999). Both civilians and combat veterans were included in these studies, and results indicate beneficial effects on a broad range of typical PTSD symptoms. In chronic therapy-resistant Vietnam veterans, nefazone showed therapeutic effects in depression-scores and typical PTSD symptoms like intrusive recollection, avoidance and hyperarousal in an open-label study (Zisook *et al.*, 2000). Many patients with chronic combat-related PTSD also suffer from comorbid depression. Available evidence indicates that antidepressants affecting 5-HT reuptake are associated with a better outcome compared to compounds predominantly affecting noradrenalin reuptake (Dow and Kline, 1997).

A Role for Atypical Antipsychotics or Antiepileptics in PTSD?

In some cases of severe emotional turmoil in PTSD dopamine blockers can be prescribed and there are case reports indicating beneficial effects of atypical antipsychotics. Leyba and Wampler (1998) report therapeutic effects on nightmares and flashbacks in four cases of PTSD, and another case report describes the use of risperidone in the treatment of intrusive thoughts and subsequent emotional reactivity in combat-related PTSD (Krashin and Oates, 1999). Also in acute stress disorder after physical trauma, preliminary evidence indicates beneficial effects of risperidone on flashbacks (Eidelman, Seedat and Stein, 2000).

In view of the fact that symptoms of PTSD may point to sympathetic hyperarousal and hyperreactivity, it has been suggested that stress-induced limbic kindling could play a role. Consistent with this view is the reported efficacy in PTSD of carbamazepine and valproate. There are only a few open-label studies but all of them support the therapeutic efficacy of carbamazepine and valproate in this condition (Lipper, 1988; Wolf, Alavi and Mosnaim, 1988; Fesler, 1991; Looff *et al.*, 1995). More recently, Clark and co-workers (1999) studied divalproex in 16 combat-related PTSD patients and found reduced intrusion and diminished hyperarousal. Interestingly they also reported significant decreases in depression and anxiety.

Future Directions in the Treatment of PTSD

Based upon the studies described above there is evidence that rarely does a single medication benefit all symptom clusters equally or produce complete remission of the syndrome. Therefore it appears

reasonable to further study the role of specific classes of drugs for particular symptom clusters.

As mentioned before, there is also a need for combination studies using pharmacotherapy and psychotherapy. A number of studies have shown that applying CBT and other psychotherapeutic techniques can reduce core PTSD symptoms in both acute stress disorder and PTSD (Bryant *et al.*, 1998; Devilly and Spence, 1999; Hembree and Foa, 2000). These additional therapeutic strategies probably have additive or synergistic effects on ongoing pharmacotherapy as they focus on the processing of traumatic events, emotional engagement of the traumatic memory, the organization of the trauma narrative and correction of dysfunctional cognitions that often follow traumatic experiences. Therefore, studies combining pharmacotherapy and CBT are clearly needed.

In depression, several studies have suggested potential therapeutic effects of augmentation strategies. The number of augmentation studies in PTSD is limited. One study suggested modest effects of clonidine augmentation to imipramine (Kinzie and Leung, 1989), another open-label study suggested additive effects of buspirone augmentation to SSRIs (and other antidepressants) in combat-related chronic PTSD (Hamner, Ulmer and Horne, 1997). In order to evaluate the therapeutic potential of these augmentation strategies, placebo-controlled studies are warranted.

There is a growing body of literature indicating that there exist dysfunctions in the regulation of the hypothalamic-pituitary axis (HPA) in PTSD (Baker *et al.*, 1999). Dysregulation of CRH is regarded the key component of the human stress response. Based upon a growing body of neurobiological studies it has been shown that other key components of the stress response include changes in the adrenergic system, serotonergic system, neuropeptide Y, opioid system and glutamatergic system. In addition, kindling/sensitization could play a role in developing PTSD. Based upon these findings it has been suggested that in addition to SSRIs future treatment possibilities for PTSD could include CRH antagonists, neuropeptide Y, β-adrenergic blockers, substance P, drugs that influence NMDA receptors and antiepileptics (Friedman, 2000).

If indeed developments in pharmacotherapy of PTSD and other anxiety disorders would be based upon the pathophysiology of the disorder this could lead to a more rational drug design which would greatly influence clinical practice and contribute to the well-being of our patients.

EPILOGUE

Serotonergic drugs have been developed and extensively investigated during the last decade in a range of depressive and anxiety disorders. As a result, a clinical database has been formed which allows us to view, and compare, their efficacy.

Of the serotonergic agents examined in this review, the SSRIs stand out with proven efficacy in the treatment of a wide spectrum of disorders including depression, panic disorder, OCD, PTSD and social anxiety disorder. Initial results with paroxetine have indicated that it may also be a suitable treatment for GAD.

The 5-HT_{1A} agonists have been used extensively in the treatment of GAD and depression, but reports of their efficacy in the treatment of panic disorder, OCD and social anxiety disorder have been equivocal, although they may prove useful in these anxiety disorders as augmenting agents for SSRI treatment-refractory patients.

The 5-HT antagonists are the least well-studied of the agents and have not yet been fully investigated in many of the psychiatric disorders discussed in this article. While they may have activity in the treatment of depression, the initial results in the treatment of panic disorder are disappointing. There are insufficient data concerning the 5-HT antagonists in GAD and social anxiety disorder to draw conclusions on their efficacy in these disorders, but preliminary reports have suggested that the drug class may be of use as adjuvants to the SSRIs in treatment refractory patients with OCD.

Large scale epidemiological studies such as the National Comorbidity Survey have clearly demonstrated that the rate of comorbidity between depression and anxiety disorders is high. Therefore, pharmacotherapy which is effective against a broad range of depressive and anxiety disorders could be of considerable utility to clinicians. The activity profile of the SSRIs in the treatment of depression and anxiety make this class of drug an attractive option for initial monotherapy in these disorders.

It should be stressed, that despite their attractive therapeutic profile, SSRIs have unwanted side effects and that response rates in clinical studies vary between 50% (e.g. OCD) and 70% (e.g. major depression and PD). Future research should focus on much overlooked items, such as pharmacokinetics, sex and ethnic differences and response prediction. Moreover, a sensible combination of clinical and preclinical research into the biological mechanisms is likely to provide information to further enhance (augment) pharmacotherapy of anxiety and depressive disorders.

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XX

Psychobiology of Somatoform Disorders

Winfried Rief and Cornelia Exner

INTRODUCTION: UNEXPLAINED PHYSICAL SYMPTOMS AND THE HEALTH CARE SYSTEM

Unexplained physical symptoms are one of the major problems of the health care system. Depending on the medical setting under investigation, between 15% and 80% of doctor visits are due to persons with physical symptoms which cannot be accounted for by a clear organic cause. Most common symptoms are pains and aches, gastrointestinal complaints, and cardiovascular symptoms. Kroenke and Mangelsdorff (1989) demonstrated that only about 16% of the most common physical symptoms can be explained by a clear organic pathology. In their longitudinal study, patients with multiple unexplained physical symptoms and with an illness duration of more than four months had the worst prognosis. As will be shown below, unfortunately this is the most frequent combination of features in patients with somatoform disorders. Accordingly, this group of patients is one of the most expensive subgroups in the health care system. Fink (1992) analysed a subgroup of high utilizers of the health care system who had had at least ten inpatient treatments during the last eight years. He found that about 20% of these frequent hospitalizations were due to unexplained physical symptoms.

Some experts believe that illness behaviour is the most typical feature of somatization. Typical features of illness behaviour are frequent doctor visits, wandering around from doctor to doctor and from treatment unit to treatment unit, taking unnecessary medication, urging doctors to do unnecessary investigations which may lead to complications, avoidance behaviour and reduction of social activities, a high number of sick-leaves, and reduced social functioning. Health anxiety is a frequent, but not a necessary condition for the development and maintenance of unexplained physical symptoms. It is unclear whether these features are consequences of the disorder or else maintaining factors, or even the cause of additional physical problems.

Patients with somatoform disorders are also characterized by a specific cognitive–perceptual style. Barsky *et al.* (1993) emphasized that patients with hypochondriasis and somatoform symptoms have an over-exclusive concept of being healthy. They conceive health as a state of perfect physical well-being without any physical discomfort. However, physical discomfort is a common sensation even to healthy persons. Therefore persons with somatoform disorders are concerned about normal bodily perceptions; they focus their attention on bodily processes, which leads to an amplified perception of physical changes. This can encourage the interpretation of physical discomfort as illness symptoms.

While Barsky's concept of somatosensory amplification (Barsky and Wyshak, 1990) related primarily to patients with *hypochondriasis*, our own group demonstrated that patients with somatization syndromes without *hypochondriasis* also tend to catastrophize their perception of physical processes (Rief *et al.*, 1998). Patients with somatization syndromes have a bias to interpret minor physical changes (e.g., heart beat acceleration while taking a hot bath) as a possible sign of a severe illness (e.g., cardiomyopathy). This cognitive–perceptual style leads to the behavioural consequences described above. Moreover, the cognitive and behavioural features of somatization interact with biological properties of the disorder and maintain a vicious circle (see Figure XX.1). Affective consequences such as demoralization, negative affectivity, or depression might present a negative feedback loop that helps to maintain the problem.

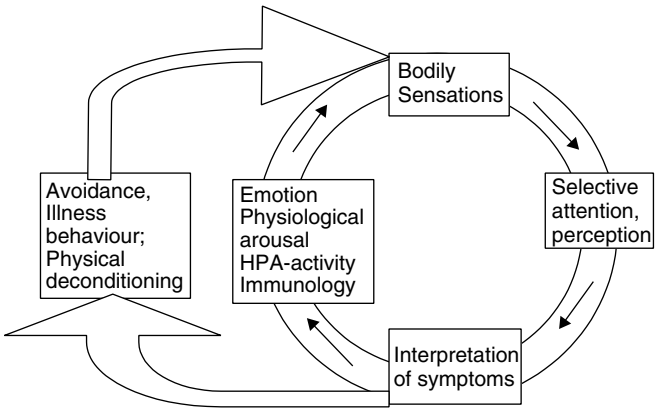

Figure XX.1 Somatization from a cognitive–psychobiological perspective (Rief and Nanke, 1999)

SYNDROMES OF SOMATIZATION AND THEIR CLASSIFICATION

The common feature of the somatoform disorders is the presence of physical symptoms which are not fully explained by a general medical condition, by the direct effect of a substance, or by another mental disorder. The symptoms must cause clinically significant distress or impairment in social functioning. Seeking medical help or para-medical consultation is very frequent. Historically, these syndromes have been labelled 'hysteria', a term which is presently less used because of stigmatizing effects.

Despite the fact that single physical complaints are very common, persons with multiple physical complaints represent the most serious subgroup for the health care system. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (APA, 1994) suggests the diagnosis of *somatization disorder* for polysymptomatic pictures of somatization. The disorder starts typically before age 30 years, extends over a period of years and is characterized by a

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combination of pain, gastrointestinal, sexual, and pseudoneurological symptoms. Eight physical symptoms out of a list of 33 suggested symptoms have to be present or had to be present in medical history. While persons with multiple somatoform symptoms are very frequent, especially in medical settings, they only rarely fulfil the complete criteria of *somatization disorder*. This is one of the most critical points concerning current classification rules of *somatization disorder*. Therefore, following DSM-IV, most patients with polysymptomatic somatoform complaints are diagnosed in the two rest categories *undifferentiated somatoform disorder* or *somatoform disorder not otherwise specified*.

Another subgroup of somatoform disorders refers to clinical pictures with a circumscribed physical symptomatology. The most frequent diagnosis of this subtype is *pain disorder*. To fulfil the criteria for this diagnosis, pain in one or more anatomical sites has to be the predominant focus of the clinical presentation; the pain causes clinically significant distress or impairment, and psychological factors are judged to have an important role in the onset, severity, exacerbation, or maintenance of pain. Especially for the diagnosis of *pain disorder*, it is nearly impossible to differentiate between the influence of psychological factors and a general medical condition. If both psychological factors and a general medical condition are judged to have important roles, the associated medical condition or anatomical site of the pain is coded on axis III. This also applies to pains where an underlying general medical condition is not yet clearly established, for example low back pain, pelvic pain, headache, joint pain, abdominal pain, and urinary pain.

Similar to pain disorder, *conversion disorder* is also characterized by circumscribed physical symptoms without sufficient organic explanation, but the typical symptoms involve voluntary motor or sensory functions. *Conversion disorder* typically suggests a neurological condition and is sometime difficult to diagnose with sufficient certainty.

The following diagnoses are not so much characterized by physical complaints, but by anxiety and cognitive preoccupations. The typical feature of *hypochondriasis* is health anxiety. The unwarranted fear or idea of having a disease persists despite medical reassurance. It can be very difficult to differentiate *hypochondriasis* from somatic delusions that may occur in psychotic disorders.

The essential feature of *body dysmorphic disorder* is the preoccupation with an imagined or exaggerated defect in physical appearance. If a slight physical anomaly is present, the person's concern is markedly excessive. While this disorder can have a dramatic course, and might have high health care relevance (e.g., cosmetic surgery), profound research relating to the condition is still rare.

Table XX.1 presents a brief guideline for the diagnostic process if a somatoform disorder is suspected. Following this guideline, three questions have to be answered.

- Does the patient describe a history of multiple somatoform complaints?
- Does the patient describe focussed, circumscribed symptoms?
- Is health anxiety or concern about the physical appearance a predominant feature?

SOMATIZATION-ASSOCIATED DISORDERS: OVERLAPPING OR DISTINCT FEATURES?

Unexplained physical symptoms are common in all disciplines of medicine. Accordingly, all disciplines have created unique terms for the description of patients with physical complaints which cannot be accounted for by a known medical condition. Examples are cephalgia, dorsalgia, recurrent abdominal pain, vulvodynia, hypomeralgia, chronic fatigue syndrome, fibromyalgia, irritable bowel syndrome, functional disorders, psychosomatic disorder, neurasthenia, multiple chemical sensitivity syndrome, somatized depression. It is

Table XX.1 The three major subgroups of somatoform disorders

Focus on multiple unexplained physical symptoms	Focus on single physical symptoms	Focus on anxiety or concerns
Somatization disorder (DSM-IV; ICD-10)	Pain disorder (DSM-IV; ICD-10)	Hypochondriasis (DSM-IV; ICD-10)
Somatoform autonomic dysfunction (ICD-10)	Conversion disorder (DSM-IV; ICD-10)	Body dysmorphic disorder (DSM-IV)
	(Somatoform autonomic dysfunction; ICD-10)	

Table XX.2 Case definitions for chronic fatigue syndrome

	CDC-1988	CDC-1994	Australia	UK
Minimum duration (month)	6	6	6	6
Functional impairment	50% decrease in activity	Substantial	Substantial	Disabling
Cognitive or neuropsychiatric symptoms	May be present	May be present	Required	Mental fatigue required
Other symptoms	6 or 8 required	4 required	Not specified	Not specified
New onset	Required	Required	Not required	Required
Medical exclusions	Extensive list of known physical causes	Clinically important	Known physical causes	Known physical causes
Psychiatric exclusions	Psychosis, bipolar disorder, substance abuse	Melancholic depression, substance abuse, bipolar disorders, psychosis, eating disorder	Psychosis, bipolar, substance abuse, eating disorder	Psychosis, bipolar, eating disorder, organic brain disease

obvious that there is an enormous overlap between these syndromes as well as with somatoform disorders. Despite the critique of current classification approaches to somatoform disorders, one of the major advantage of the new concept may be the provision of the one term 'somatoform' for a variety of related concepts. One common feature of all these syndromes is that although some symptoms may be the predominant focus of the clinical presentation, most of the patients also report many unspecific physical complaints. Buchwald and Garrity (1994) compared physical complaints of patients with chronic fatigue syndrome, fibromyalgia and multiple chemical sensitivity. Physical complaints that were common in chronic fatigue were also frequent symptoms of fibromyalgia and multiple chemical sensitivity. Wessely (1996) investigated the frequency of somatic symptoms in patients with chronic fatigue and reported that these patients described an enormous amount of unspecific physical complaints. The more chronic the fatigue syndrome was, the more physical complaints patients described.

To summarize, it seems that diagnoses such as fibromyalgia, chronic fatigue syndrome, multiple chemical sensitivity syndrome, etc. cannot be differentiated on the basis of somatic symptoms. The principal difference between these groups are theoretical assumptions on psychophysiological and psychobiological interactions. For some of these syndromes, well-established criteria have been defined which are useful for further research (see Table XX.2 for the example of CFS).

NEW APPROACHES TO THE CLASSIFICATION OF SOMATOFORM DISORDERS

There has been growing concern that current diagnostic concepts of *somatization disorder* fail to cover the large majority of patients presenting with unexplained somatic symptoms (Escobar *et al.*, 1989; Kroenke *et al.*, 1997). Several researchers have therefore suggested alternative diagnostic criteria for *somatization disorder* that require a smaller number of somatic symptoms and also take into consideration the higher symptom report rates of women (see below).

If the number of unexplained physical symptoms presented by the patient is the central diagnostic criterion, the first question is which symptom list should be used. Symptoms that are to be incorporated into a total score should have a sufficient base rate and a sufficient item–total correlation. In an empirical analysis Rief and Hiller (1999) demonstrated that many pseudoneurological symptoms do not fulfil these requirements. Moreover, sexual and menstruation symptoms had an insignificant item–total correlation which was due to unspecifity. These symptoms have comparable base rates in patients with somatization syndromes and in patients with other mental disorders. Our results suggest that for the diagnosis of polysymptomatic somatoform disorder one should focus on pain symptoms, gastrointestinal symptoms, cardiovascular symptoms and a reduced list of pseudoneurological symptoms.

Fink (1996) emphasized that symptom counting should not be the principal part of the diagnostic process, but that further psychological and psychobiological features should be considered, which might present additional aspects for the diagnosis of somatization disorder. In the following sections we will review the evidence concerning the possible psychobiological changes that underlie the chronic tendency to experience bodily complaints.

Abnormal Illness Behaviour

Pilowsky (1993) has outlined the concept of abnormal illness behaviour, which seems to be a predominant feature of somatization. The concept includes an abnormal behavioural reaction to physical discomfort. Doctor visits even for minor reasons, a continuous tendency to search for diagnostic evaluation and interventions, avoidance of physical demands, high number of sick-leave occasions are only some of these aspects.

Sustained Focussed Attention on Bodily Processes

Those patients with somatization syndrome show a tendency to focus attention on bodily processes. This internal attention focusing is often accompanied by a reduction of interests for external events.

Catastrophizing Interpretation of Bodily Sensations

Patients with *hypochondriasis* show a general tendency to misinterpret bodily sensations as evidence for physical illness (Barsky *et al.*, 1993; Rief *et al.*, 1998). While healthy controls can generate many normal attributions for bodily sensations, somatizing patients have a bias towards using only catastrophizing interpretations.

Self-Concept of Being Weak

Many somatizing patients (yet not all) have a self-concept of being weak which is not founded on a physical basis (Rief *et al.*, 1998). A self-concept of being weak is highly associated with disability and abnormal illness behaviour.

Following these empirical analyses, we proposed the diagnosis of *polysymptomatic somatoform disorder*. To fulfil criteria, persons should describe at least seven to eight physical symptoms affecting multiple body sites during the past two years. At least one of the psychological factors which had been mentioned above should be present as a maintaining factor. Further criteria resemble DSM-IV criteria of *somatization disorder* (symptoms not sufficiently explained by any pathological physical condition; symptoms cause clinically significant distress, etc.).

Escobar and colleagues (Escobar *et al.*, 1987, 1989) suggested using the Somatic Symptom Index (SSI) as a classification approach. The criteria for SSI-4/6 are fulfilled if men have at least four physical symptoms and women at least six physical symptoms out of the list of 35 somatoform symptoms suggested for DSM-III-*somatization disorder*. The SSI-4/6 concept of Escobar has been used in a number of studies. However, there are concerns that it may be over-inclusive and the empirical basis of this concept still needs to be improved. Kroenke *et al.* (1997) suggested a new diagnosis of *multisomatoform disorder*. The criteria for this disorder were derived from primary care studies. Patients have to present with at least three current somatoform symptoms out of a 15-symptom checklist along with at least a two-year history of somatoform symptoms. The 15 physical symptoms were chosen because they account for more than 90% of outpatient visits attributable to physical complaints. The major advantage of the Kroenke approach is its feasibility for use in primary care.

COMORBIDITY PATTERNS

Already centuries ago the high overlap between unexplained physical symptoms and depression/melancholia was described (Burton, 1621). Kielholz (1973) has postulated that depression may be the underlying disorder in many patients with bodily complaints, and that functional physical symptoms might be just epiphenomena. Empirical analyses of comorbidity patterns underline the close association of depression and somatization syndrome. In 90–100% of patients seeking help in health care settings and fulfilling the complete criteria of *somatization disorder* a comorbid affective disorder can be found. A history of major depression is reported by 65–80%

Table XX.3 Comorbidity diagnoses of distressed high users of primary care (Katon *et al.*, 1991)

Somatization syndrome	Major depression (lifetime)	Panic disorder (lifetime)
<SSI-4/6	45%	3%
SSI-4/6	75%	20%
SSI-9	71%	19%
Somatization disorder	82%	48%

of patients with *somatization disorder* (Katon *et al.*, 1991; Rief *et al.*, 1996). Despite these findings, the association between somatization and depression should be interpreted with caution. First, high prevalence of comorbid depression (especially in patients in health care settings) is not only common in somatization, but also in most other mental disorders (anxiety disorders, eating disorders and others). Second, the stricter the criteria applied for the diagnosis of *somatization disorder*, the higher is the base rate of comorbid depression (Katon *et al.*, 1991; Hiller *et al.*, 1995). Third, onset and course of depression are quite different from onset and course of somatization with somatoform symptoms having an earlier onset and very frequently a stable and chronic course (Rief *et al.*, 1992). And fourth, there seem to be significant psychobiological differences between depression and somatization, as will be shown in subsequent sections.

Somatization is not only associated with depression, but patients with somatization syndromes are at increased risk also for other disorders. There seems to be a close link to anxiety disorders (Rief *et al.*, 1996). In some studies, patients with *hypochondriasis* showed higher comorbidity rates with anxiety than with affective disorders (Barsky *et al.*, 1992). Therefore *hypochondriasis* was suggested to represent the link between somatoform disorders and anxiety disorders. Other authors emphasize the association of *hypochondriasis* and *body dysmorphic disorder* with obsessive–compulsive disorder (Starcevic, 1990; Simeon *et al.*, 1995; Bienvenu *et al.*, 2000).

Table XX.3 presents comorbidity patterns of somatoform disorders with other mental disorders. Summarizing these results, it seems that somatization, anxiety and depression (as well as other features) are risk factors one for the other.

EPIDEMIOLOGY

Patients who present with multiple physical symptoms that lack an adequate somatic explanation are commonly seen in primary care settings (Rief *et al.*, 2001). This is in contrast to the low prevalence rates of somatoform disorders according to current diagnostic systems (ICD-10 and DSM-IV). Depending on study design, diagnostic criteria (ICD-10 versus DSM-IV) and catchment area *somatization disorder* is estimated to have a lifetime prevalence between 0.03% and 3% (Escobar *et al.*, 1989; Gureje *et al.*, 1997). If less restrictive concepts are used for *somatization disorder* (SSI 4/6 or 3/5), prevalence rates are in the range of 10–20% in the general population (Escobar *et al.*, 1989; Rief *et al.*, 1996, 2001; Gureje *et al.*, 1997).

Reliable information on the prevalence rates of other diagnostic subgroups of somatoform disorders is still lacking. Hypochondrial concerns are present in at least 10–15% of the general population (Kroenke and Spitzer, 1998; Rief *et al.*, 2001). However, only 1–4% meet full diagnostic criteria for *hypochondriasis* (Faravelli *et al.*, 1997; Gureje *et al.*, 1997; Kroenke and Spitzer, 1998).

Pain is probably the most frequent complaint in medical practice. In a representative German sample, for instance, up to 30% of subjects complained about back pain in the last two years (Rief *et al.*, 2001). Pain symptoms often lack an adequate medical explanation. However, little is known about the prevalence of *somatoform pain disorder* according to current diagnostic criteria and reported rates vary from under 1% up to 5% in the general population (Gureje and Obikoya, 1992; Faravelli *et al.*, 1997).

Body dysmorphic disorder (BDD) affects approximately 1% of the general population; however, this is thought to be an underestimate because BDD is frequently underdiagnosed. In clinical populations the frequency might be much higher. Thirteen per cent of psychiatric inpatients were reported to suffer from BDD (Grant *et al.*, 2001). In dermatological patients and those seeking cosmetic surgery the rate of BDD was estimated to be between 7% and 12% (Sarwer *et al.*, 1998; Phillips *et al.*, 2000).

Reported prevalence rates of *conversion disorder* vary widely, ranging from 11/100,000 to 300/100,000 according to DSM-IV. However, in neurological patients the prevalence rates have been reported to be as high as 20% (Mace and Trimble, 1991).

GENDER EFFECTS

Unexplained physical symptoms have consistently been shown to be more frequent in women than in men (Piccinelli and Simon, 1997; Kroenke and Spitzer, 1998; Ladwig *et al.*, 2000; Rief *et al.*, 2001). Kroenke and Spitzer (1998) for instance reported the results of a mental health survey in 1000 primary care patients in the USA. Results showed that physical symptoms in general and somatoform symptoms more specifically were more common in women than in men. Odds ratios adjusted for age, race, education, medical and psychiatric comorbidity were in the range of 1.5 to 2.5. Although anxiety and depression were both strongly associated with symptom reporting the effect of gender on symptom reporting was independent of psychiatric comorbidity. While women in the general population are approximately twice as likely as men to report physical symptoms that lack an adequate somatic explanation the ratio shifts even more to the female side when only patients are considered who fully meet diagnostic criteria for a somatoform disorder. The DSM-IV reports female prevalence rates for *somatization* and *conversion disorder* to be up to 10 times higher in women than in men. However, hypercondriacal concerns in the general population as well as prevalence rates for *hypochondriasis* according to DSM-IV or ICD-10 appear to be equally frequent in women as in men (Gureje *et al.*, 1997; Kroenke and Spitzer, 1998). Men are also as likely as women to suffer from *body dysmorphic disorder*; whilst symptom localization and comorbidity patterns vary between male and female patients (Perugi *et al.*, 1997; Phillips and Diaz, 1997).

The elevated frequency of somatoform symptoms in women has been explained by several authors in terms of a biopsychosocial model of symptom perception (Pennebaker, 1982; Gijsberg van Wijk and Kolk, 1997). Accordingly, symptoms do not just result from the passive registration of bodily changes but are modulated by cognitive processes that guide attention to and the attribution of somatic information. In women there might be simply more somatic information to begin with. The female reproductive process itself produces much physical information that is absent in men. It might further exert an influence on pain perception thresholds that have been shown to be lower in women (Riley *et al.*, 1998) and to vary with the menstrual circle (Riley *et al.*, 1999). Attention towards physical symptoms, attributions applied to them and the willingness to report them to medical professionals might be further influenced by differences between women and men in socialization, sex roles and social position.

NEUROIMAGING STUDIES

Functional Neuroimaging Studies

As somatoform disorders are by their very definition thought to lack an adequate somatic explanation little research has been done to elicit possible neurophysiological abnormalities associated with this type of disorder. Tiihonen *et al.* (1995) reported the case of a 32-year-old woman with a conversive left-sided paralysis and paraesthesiae who had a single photon emission tomography (SPECT) carried out during the electric stimulation of the median nerve of her affected hand. Altered cerebral blood flow was observed with hyperfusion of the right frontal and hypofusion of the right parietal region. After recovery from her conversive symptoms cerebral blood flow measures returned to normal. Results were interpreted as reflecting a simultaneous activation of frontal inhibitory areas and inhibition of somatosensory cortex. A further case study by Marshall *et al.* (1997) reported the case of a 45-year-old woman with left-sided paralysis but without somatosensory loss in whom no organic lesion had been found. Brain activity was studied by positron emission tomography (PET) when the patient prepared to move and tried to move her paralysed (left) leg. Her good (right) leg was used as a control condition. Preparing to move or moving her good leg, and also preparing to move her paralysed leg activated motor and/or premotor areas previously described as participating in movement preparation and execution. However, the attempt to move the paralysed leg failed to activate right primary motor cortex. Instead, the right orbitofrontal and right anterior cingulate cortex were significantly activated. These areas are known from previous studies in both animals and humans as 'negative motor areas' which play a crucial role in the suppression of inappropriate motor responses (Lüders *et al.*, 1995). The interpretation was put forward by Marshall *et al.* (1997) that these two frontal areas inhibited (willed) effects on the right primary motor cortex when the patient tried to move her left leg. Interestingly, the results of their study were paralleled by those of a single case study of brain activity during hypnotic paralysis. Using an identical PET design Halligan *et al.* (2000) showed that hypnotic paralysis activated similar brain areas (orbitofrontal and anterior cingulate areas) as conversive paralysis, supporting the view that hypnosis and *conversion disorder* might share common neurophysiological mechanisms.

Both prefrontal and right parietal regions are thought to be components of a distributed neural network that integrates processes of attention and awareness (Parasuraman, 1998). Thus, the results of the studies cited above are consistent with the notion that altered inhibitory mechanisms at high levels of sensory and motor processing play a role in the formation of conversive symptoms. However, findings to date are far from forming a clear picture: Yazící and Kostakoglu (1998) reported the results of five cases suffering from bilateral conversive symptoms (astasia-abasia) who had SPECT performed at rest. Perfusion decreases were found in left temporal and parietal areas. As symptoms were bilateral in all their cases and no activation paradigm was applied it is difficult to compare the results of Yazící and Kostakoglu (1998) to those of Tiihonen *et al.* (1995) and Marshall *et al.* (1997). Even more care is warranted when trying to generalize neuroimaging findings in *conversion disorder* to other somatoform disorders. Studies in *somatization disorder* patients so far have yielded inconclusive results: James *et al.* (1987) reported regional cerebral blood flow changes in 14 patients with *somatization disorder* who performed a visual matching task known to activate predominantly the right hemisphere. Compared to controls they exhibited a slightly higher right posterior activation. This was interpreted as indicating a hyperactivity of the right hemisphere and the right posterior region in particular and possibly reflecting disturbed processes of selective attention. However, in a recent SPECT study of 11 cases with *somatization disorder* Garcia-Campayo *et al.* (2001) found right hemisphere or bilateral hypoperfusion in different brain areas in the majority of their patients under resting conditions. No control group was studied and no activation paradigm was applied. The seemingly contradictory results highlight the necessity for further research in this field studying larger numbers of carefully diagnosed patients under both resting and activation conditions.

To sum up, the only conclusion that can safely be drawn from functional neuroimaging findings so far is that alternations in regional cerebral blood flow accompany the expression of physical symptoms in *conversion disorder* and *somatization disorder*. These changes might be related to altered processes of attention and awareness in sensory and motor processing.

Structural Neuroimaging Studies

Again, as the definition of somatoform disorder excludes the presence of organic brain disease only few attempts have been made to discover the prevalence of (subtle) structural changes in the brains of patients with somatoform disorders. There is, however, one related condition to which structural brain imaging techniques have been applied. *Chronic fatigue syndrome* (CFS) is a debilitating multisystem condition of unknown origin. Attempts have been made to relate the clinical findings to structural changes in the brain of affected individuals but have so far yielded ambiguous results. Some researchers found an increased frequency of white matter intensities (WMI) in MRI studies of CFS patients compared to normal age-matched controls (Buchwald *et al.*, 1992; Natelson *et al.*, 1993; Schwartz *et al.*, 1994). However, others found no differences in the frequency of WMIs between CFS patients and controls (Cope *et al.*, 1995; Greco *et al.*, 1997). Reported frequencies of white matter lesions in CFS varied greatly from 8% (Cope *et al.*, 1995) to 78% (Buchwald *et al.*, 1992) depending on catchment area, age of subjects and the presence of associated disorders. Even those investigators who found increased frequencies of white matter lesions could not identify a specific pattern of radiological abnormalities and doubt their clinical usefulness (Schwartz *et al.*, 1994). The unspecific and subjective nature of the disease combined with the absence of a known causal agent and pathognomonic abnormalities makes the condition difficult to diagnose. Thus, it seems questionable whether the different investigation carried out even related to the same population of patients.

With relation to *conversion disorder* there is some evidence that lesions of the left hemisphere may present a predisposition for the development of conversive symptoms. Drake (1993) reported that patients who had sustained left hemisphere injuries or infarctions were at greater risk of developing conversion symptoms later in life.

NEUROPSYCHOLOGICAL ASPECTS

Circumstantial evidence for disturbed brain function in somatoform disorders could derive from findings of neuropsychological deficits in affected patients. Indeed, patients with unexplained physical symptoms frequently complain of cognitive deficits such as fatigue, lack of concentration and memory failure. However, little is known about how patients with somatoform disorders perform on standardized neuropsychological tests. Patients suffering from *body dysmorphic disorder* (BDD) have been reported to show performance deficits on verbal and non-verbal learning and memory tests compared to healthy controls (Deckersbach *et al.*, 2000). Learning and memory deficits in BDD patients seemed especially due to their poor organizational strategies, an aspect of memory processing that is attributed to the frontal lobes. These findings were similar to patterns previously observed in obsessive–compulsive

disorder (OCD) and could thus point to the possible relationship between BDD and OCD that has repeatedly been suggested by other investigators (Bienvenu *et al.*, 2000). Further and more direct evidence for this claim comes from the study of Hanes (1998). BDD patients in this study were directly compared to OCD patients and both groups were found to show a similar pattern of neuropsychological performance. Patients with BDD showed normal performance on memory and motor tasks but were significantly impaired relative to normal controls on tests of executive functions, thus again pointing to the frontal lobes as a possible site of disturbances.

Flor-Henry *et al.* (1981) investigated cognitive functioning in 10 subjects with 'hysteria', who according to current diagnostic criteria would have been seen as suffering from *somatization disorder*. Ninety per cent of the test profiles of somatization patients were judged as 'abnormal' according to Reitan's approach (Reitan and Davison, 1974), thus suggestive of cerebral dysfunction. The somatization group was characterized by deficits on tests of sensorimotor, executive and visuospatial function. The test variables that best discriminated somatization patients from controls were Colored Progressive Matrices, Finger Localization (preferred hand) and Verbal Associative Learning. On the basis of these findings the authors concluded that dominant hemisphere dysfunction is related to the symptom of hysteria (today: *somatization disorder*). The high prevalence of cognitive impairment in somatization patients in the study of Flor-Henry *et al.* (1981) has never been replicated and might be partly due to the definition of 'abnormal' test profiles applied in this study. Further investigations of cognitive performance in patients with *somatization disorder* seem mandatory to clarify how prevalent objective cognitive deficits are in somatization patients.

Complaints about cognitive deficits mainly in the area of memory and concentration are also a core feature of *chronic fatigue syndrome* (CFS), a condition often seen as belonging to the 'somatoform spectrum' disorders. Any understanding, however, of the cognitive problems in CFS has to take account of the frequent comorbidity of CFS and depression. Affective disorders themselves are known to cause cognitive deficits (Murphy and Sahakian, 2001). In a recent review Wearden and Appleby (1996) reported that non-depressed CFS patients, although showing mild cognitive impairments, performed within normal limits on various standard neuropsychological tests. In most investigations they did not significantly differ from healthy controls. If impaired at all, CFS patients showed a tendency to perform slower especially on more complex and effortful tasks but there was no evidence for excessive fatigueability. On the basis of the current evidence Wearden and Appleby (1996) concluded that mild cognitive problems in CFS patients are unlikely to relate to grossly altered brain function; instead, psychological variables like motivation, effort, mood and arousal are likely to be involved in the explanation.

LATERALIZATION OF SYMPTOMS

The debate over whether unilateral somatization and conversion symptoms are lateralized dates back to the 1970s and has not yet led to any conclusive result. Psychogenic somatization symptoms in different psychiatric conditions (anxiety, depression, somatoform disorders) have been reported to occur more frequently on the left side of the body (Axelrod *et al.*, 1980; Min and Lee, 1997). Left-sided lateralization of conversion symptoms has been found by Stern (1977) and more recently by Pascuzzi (1994). Galin *et al.* (1977) also found a prevalence of left-sided conversion symptoms but for females only. However, others have found a predominantly right-sided lateralization of symptoms in *conversion disorders* in children (Regan and LaBabera, 1984) and adults (Fallik and Sigal, 1971) or no lateralization of symptoms (Keane, 1989; Roelofs *et al.*, 2000). Apart from the ambiguous results the integration of the existing findings on lateralization of somatoform symptoms is further complicated by differences in sample sizes, patient selection and symptom definition.

Different hypotheses have been put forward to explain the predominantly left-sided lateralization of conversion and somatization symptoms. The *evaluative hypothesis* suggests that the left side of the body is associated with negative connotations and might therefore be the preferred site for the development of somatization (bad) symptoms. This symbolic interpretation has received little empirical support. The *convenience hypothesis* claims that symptoms develop on that side of the body where they cause the least inconvenience. This would predict a left-sided predominance in right-handers and vice versa. However, this claim is contradicted by the finding that left-sided symptoms occur more frequently in both left- and right-handed patients (Galin *et al.*, 1977; Stern, 1977). By the same token, one could also propose the opposite of the convenience hypothesis, namely that symptoms are more frequently on the right side just because they are more incapacitating there and require more attention (Fallik and Sigal, 1971; Regan and LaBabera, 1984). Right-sided lateralization has also been explained as being related to *previous organic lesions* which more often occur on the dominant side of the body (Fallik and Sigal, 1971). Again, this claim is contradicted by the lack of any consistent relation between handedness and lateralization of symptoms. The predominant theory today explains symptom lateralization to the left side in terms of *hemispheric specialization* (Sierra and Berrios, 1999; Roelofs *et al.*, 2000). The right inferior parietal cortex is believed to be involved in higher-order processes of attention and awareness (Parasuraman, 1998). Disturbances of this system might thus compromise sensory and motor processing on the contralateral side of the body. This theory would also allow for the incorporation of current functional imaging findings (Tiihonen *et al.*, 1995; Marshall *et al.*, 1997; Halligan *et al.*, 2000). However, it does not seem necessary to restrict the proposed effects of a dysfunctional right-sided attention and awareness system to the contralateral body side.

To summarize, more investigations of symptom manifestation in somatoform disorder using representative samples are necessary to resolve the question of symptom lateralization. It seems important to stress that most patients suffer from symptoms that are not restricted or not attributable to only one side of the body and any explanation of a possible 'conversion' mechanism has to account for this fact (Roelofs *et al.*, 2000).

GENE–ENVIRONMENT INTERACTIONS

The aetiology and pathogenesis of somatoform disorders are still not well understood. It has been shown that adverse environmental factors such as low socioeconomic background, childhood experiences of death and illness, exposure to traumatic events or high levels of current stress increase the risk for the development of somatoform disorders (Hartvig and Sterner, 1985; Whitehead, 1994; Binzer *et al.*, 1997). There is also evidence that hereditary factors have some influence. Familial aggregation for somatization (Briquet's syndrome) has been reported for women but not for men (Cloninger *et al.*, 1986). Higher concordance rates for somatoform disorders in monozygotic than in dizygotic twins (29% vs. 10%) have been reported in a Norwegian twin study (Torgersen, 1986). However, the differences failed to reach statistical significance and shared environmental influences might have influenced the concordance rates. All the concordant pairs consisted of partners with two different somatoform disorders pointing to an aetiologic and nosologic similarity of the different subtypes of somatoform disorders.

The study further found a high frequency of anxiety disorders, especially generalized anxiety disorders, in the co-twins of somatoform disorder twins.

Familial aggregation and twin studies alone do not provide unequivocal evidence for a genetic aetiology, as shared environmental influences might be responsible for higher frequency of psychiatric disorders in the families of affected patients. More direct evidence can be drawn from adoption studies. A Swedish adoption study using the medical history data of female adoptees found an increased prevalence of alcohol abuse and antisocial behaviour in the biological fathers of somatizing women (Bohman *et al.*, 1984). The results confirmed those of earlier family studies suggesting that somatoform disorders in women and some forms of alcohol abuse and antisocial behaviour in men might share common aetiological factors.

Genetic–epidemiological approaches may also help in clarifying the relationship between somatoform disorders and other comorbid psychiatric diagnoses that are often diagnosed in the same individual. Recent studies suggest that *somatization disorder* and symptoms of anxiety and depression do not share a common genetic background (Battaglia *et al.*, 1995; Gillespie *et al.*, 2000).

The advantages of molecular biology offer new tools for studying those variations in genes and matching gene products that might be responsible for the genetic transmission of psychiatric diseases. So far, little is known about which part of the genome might hold candidate genes related to the aetiology of somatoform disorders. However, a number of the studies point to the involvement of the serotonin transmitter system in *fibromyalgia*, a syndrome of generalized muscular pain which shares clinical features with both *pain disorder* and depression. In patients with fibromyalgia the genotype distributions for both the 5-HT2A receptor gene and the serotonin transporter gene have been found to differ from healthy controls (Bondy *et al.*, 1999; Offenbacher *et al.*, 1999). These results suggest that altered serotonergic neural transmission might present a genetically transmitted vulnerability for the disorder.

NEUROTRANSMITTER SYSTEMS

The serotonergic system has repeatedly been discussed to be involved in the pathophysiology of somatoform disorders and related syndromes. Reduction of central serotonergic transmission has been linked to the pathogenesis of *pain disorder*. This notion is based first on the analgesic effects of tricyclic antidepressants and second on reports of reduced activity of the peripheral and central serotonin system (Van Kempen *et al.*, 1992). Serum levels of serotonin (5-hydroxytryptamine, 5-HT), its precursor tryptophan and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) have also been found to be decreased in patients suffering from *fibromyalgia*. Schwartz *et al.* (1999) found low levels of 5-HIAA and tryptophan to be related to higher pain scores and lower serum concentration of 5-HIAA to be associated with reduced quality of sleep in fibromyalgia patients. There was also a tendency of higher pain scores to be related to higher serum concentrations of the neuropeptide substance P, pointing to the antagonism of substance P and the serotonergic system in nociception. In a recent review Russell (1998) discussed the findings of low 5-HT and high substance P concentrations as signs of a pain amplification syndrome in fibromyalgia. The aetiology of *body dysmorphic disorder*, another somatoform disorder, has also been related to poor regulation and depletion of serotonin (Craven and Rodin, 1987; Barr *et al.*, 1992). However, altered serotonin physiology might be either a consequence or a marker of the disorder.

Current concepts of stress research point to the importance of the monoaminergic neurotransmitters for the response to and adaptation to acute and chronic stressful events. As emotional distress has been shown to be a powerful predictor of somatic complaints across gender, different age groups and different cultures (Piccinelli and Simon, 1997) one would predict that the monoaminergic neurotransmitter system should be involved in the pathophysiology of somatoform disorders. So far, there is only circumstantial evidence to support this claim. Gjerris *et al.* (1987) found CSF adrenaline but not noradrenaline levels to be reduced in 'somatizing' depressive patients. CSF adrenaline concentration was related to ratings of somatic anxiety symptoms and *hypochondriasis* on the Hamilton Depression Scale.

Taken together, most support is there for the involvement of the serotonergic neurotransmitter system in somatoform disorders especially those that are characterized by altered pain perception and obsessive–compulsive features. However, there seem to be few results specific to somatoform disorders as alternations in serotonergic transmission have been shown for other psychiatric conditions such as depression, anxiety, obsessive–compulsive disorder and eating disorders.

ENDOCRINOLOGICAL ASPECTS

Studies on endocrinological aspects of unexplained physical symptoms focus on the activity of the hypothalamic–pituitary–adrenal axis (HPA-axis). Parallels to post-traumatic stress disorder have been drawn as many patients with unexplained physical symptoms report traumatic experiences. Heim *et al.* (1998) examined women with chronic pelvic pain and analysed diurnal salivary cortisol levels and hormonal responses to a corticotropin-releasing factor (CRF) stimulation test as well as a low-dose dexamethasone suppression test. Women with chronic pelvic pain had increased prevalences of abuse experiences and post-traumatic stress disorder. Analysing endocrinological parameters, the authors found normal to low diurnal salivary cortisol levels, normal ACTH, but reduced salivary cortisol levels in the CRF stimulation test. The suppression of cortisol concentrations after dexamethasone was enhanced in patients with chronic pelvic pain.

Taking into account the tendency towards increased cortisol scores in depressive patients, the results for chronically stressed persons with syndromes other than depression are quite surprising. Pruessner *et al.* (1999) demonstrated that the associations between stress and cortisol are not as straightforward as had been thought earlier. In their study, perceived stress correlated with an increase of cortisol levels during the first hour after awakening. However, the overall cortical secretion of persons scoring high on burn-out scales showed lower cortisol concentrations on all sampling days in that study. Therefore the authors postulated differential effect of burn-out and perceived stress on HPA-access regulation.

Other studies have confirmed an association between vital exhaustion and lower basal cortisol levels (e.g., Nicolson and van Diest, 2000). These and other results stimulated Heim *et al.* (1998) to postulate a theory of hypocortisolism in the pathophysiology of stress-related bodily disorders. Chronic or very intense stress may lead to a hypoactivity of the HPA-axis. The authors assume that cortisol may have a protective function; a lack of these protective properties may be of relevance for the development of bodily disorders in chronically stressed individuals.

However, the finding of low cortisol scores in patients with unexplained physical symptoms is not as consistent as might be supposed. For chronic fatigue syndrome patients, Scott and Dinan (1998) confirmed lower urinary free cortisol excretion, while Scott *et al.* (1998) and Young *et al.* (1998) found normal cortisol scores in chronic fatigue patients. Vingerhoets *et al.* (1996) found a tendency towards lower cortisol levels in persons who described many stress-related bodily complaints, whereas Rief and Auer (2000) found normal urinary cortisol, normal salivary cortisol and

normal serum cortisol after dexamethasone suppression tests for persons with somatization syndrome. In another study of Rief *et al.* (1998) we even found increased scores for salivary cortisol in somatizing patients. This effect was still evident after controlling for depression. Results contradictory to the hypocortisolism theory were also reported for persons with burn-out syndrome (Melamed *et al.*, 1999).

Therefore, the theory of hypocortisolism in the pathophysiology of stress-related bodily disorders has less empirical support than proposed by Heim *et al.* (2000). Measurement artefacts, differences in study design, or differences in comorbidity patterns of examined samples might account in part for these discrepancies. If a tendency towards reduced cortisol concentrations in somatoform disorders is confirmed in the future, it is likely to be a rather weak effect.

Neeck and Riedel (1999) presented a sophisticated outline of hormonal perturbations in fibromyalgia syndrome. According to their opinion, fibromyalgia is characterized not only by pain in defined points of the musculoskeletal system, but also by numerous additional somatoform and psychological symptoms. The authors claim that not only the HPA-axis may be deregulated in fibromyalgia, but also other hormonal axes (the thyroid system, regulation of growth hormone, and other parameters). Therefore they conclude that there must be a higher-order central rather than a peripheral origin causing perturbations of the hormonal axes. They favour a model in which hypothalamic CRH neurons play a key role not only in 'resetting' the various endocrine loops, but possibly also in regulating nociceptive and psychological mechanisms as well.

Some other studies have analysed peptides in patients with functional physical symptoms or associated disorders. Jonsson *et al.* (1998) analysed gastrin, cholecystokinin, and somatostatin in blood samples of patients with functional dyspepsia. Mean hormone values did not differ between patients with functional dyspepsia and a matched healthy control sample. Patients with a high degree of dyspeptic symptoms during the week preceding the experiment had a higher mean somatostatin level than patients with a lower degree of dyspeptic symptoms. During a stress interview, cholecystokinin levels increased in patients with functional dyspepsia, but not in controls. Somatostatin increased significantly earlier in patients than in control subjects during the stress interview. Therefore the authors postulated that cholecystokinin and somatostatin may possibly link psychological reactions to the pathophysiology of functional dyspepsia. Maes *et al.* (1998) analysed prolyl endopeptidase and dipepetidyl peptidase IV in patients with fibromyalgia. They found lower serum prolyl endopeptidase in patients with fibromyalgia than in normal volunteers. Moreover, they also described significant negative correlations between serum prolyl endopeptidase and severity of pressure hyperalgesia.

Unfortunately, for patients who were classified according to the somatoform disorders section of DSM-IV, studies on peptides are so far lacking.

IMMUNOLOGICAL ASPECTS

One of the conceptual precursors of somatoform disorders, neurasthenia, was assumed to be triggered by viral infections and other diseases. Hundreds of years later, the terminus *chronic fatigue syndrome* was created and it was postulated that viral infection and subsequent immunological processes may be underlying pathophysiological mechanisms leading to the complaints. While a uniform viral aetiology of chronic fatigue syndrome has been questioned in the following years (for an overview see Wessely *et al.*, 1999), there is convincing evidence to postulate a close link between immunological processes and the perception of physical complaints. The possible trigger function of viral infections continues to be a topic of research. For example, White *et al.* (1998) demonstrated that glandular fever is a risk factor for the development of acute and chronic fatigue syndromes, whereas ordinary upper respiratory tract infections are not (Wessely *et al.*, 1999). White (1997) summarized that specific infections can trigger chronic fatigue syndromes, but these syndromes are not maintained by the infectious agent itself, but by the patient's subsequent maladaptation. This maladaptation may be mediated by changes in sleep patterns, physical deconditioning, and endocrine and immune system changes.

One of the core features of somatization is the patient's belief and self-perception of being sick. This provides a link to the body of immunological research which focuses on 'sickness behaviour'. Sickness behaviour is characterized by reduced activity, a reduction of social interactions and sexual drive and the development of depressed mood. Interestingly, the administration of cytokines in the absence of infection produces the full syndrome of sickness behaviour. For example, all symptoms of sickness were produced by human interleukin-1b administered within rat hippocampus (Linthorst *et al.*, 1994).

Illness behaviour in somatoform disorders is phenomenologically characterized by reduced physical activity, reduced social interactions, and reduced exploration behaviour. As these features are also behavioural correlates of the acute phase-response, a close link between immunological parameters of the acute phase and the symptoms of somatoform disorders can be postulated.

Circumstantial evidence for a relationship between somatization and immunological processes is provided by the stress-dependency of immune processes. Stressors lead to increases in the levels of cytokines circulating in the blood, with IL-6 being the most frequently measured (Maier and Watkins, 1998). Moreover, several reports document the role of IL-1 in the stress response, as well as increased TNF-alpha levels following stress. Thus, both primary as well as secondary cytokines are induced *in vivo* as well as *in vitro* by stressors (Black and Berman, 1999). On the other hand, acute and chronic stressors as well as low stress tolerance are typical concomitants of somatoform syndromes.

Apart from illness behaviour and stress, there is a third line of evidence suggesting a link between somatoform disorders and immune activity. This is the interaction between immunological processes and pain sensitivity. Immune stimulation seems to activate both analgesia and hyperalgesia circuitry (Maier and Watkins, 1998). Stress-induced analgesia is a very rapid response, whereas hyperalgesia follows infectious or inflammatory processes much more slowly. The peripheral administration of LPS or IL-1 results in an increase in pain sensitivity (Watkins *et al.*, 1995). Altered pain sensitivity can lead to the perception of minor physical changes that otherwise would have gone unnoticed. These results could present a framework for research into the relationship between immune system activity and somatization.

Most of the approaches outlined above postulate increased levels of proinflammatory substances in patients with somatization. Moreover, elevated levels of proinflammatory cytokines have been found for the most frequent comorbid psychiatric disorder, major depression (Maes *et al.*, 1997). Neopterin is one of the metabolically stable markers for inflammation. In some (but not all) studies, plasma neopterin levels have been increased in major depression. Bell *et al.* (1998) found that serum neopterin is correlated with variables of somatization. However, this association was only found in a subgroup of persons with chemical intolerance, not in a subgroup of patients with depression or in normal controls.

Substance P, a neuropeptide which is involved in communication between the nervous and the immune system, seems to play a major role in the process of nociception. As outlined above, nociception and alternations of pain sensitivity can be a central process involved in the development of somatoform symptoms. Elevated levels of

Table XX.4 Immunological aspects of somatization (data from Rief *et al.* (2001) and similar studies)

Parameter	Somatization	Depression
IL-6	Reduced	Increased
IL-6R	Reduced	Reduced
IL-1	Unclear	Increased
IL-1RA	Increased	Slightly increased
CD-8 cell count	Reduced	Increased

substance P have been found in cerebrospinal fluid of patients with fibromyalgia. In some studies, there was an association between substance P serum concentrations and pain in fibromyalgia patients (Schwartz *et al.*, 1999). However, a direct investigation of substance P in patients with a DSM-IV diagnosis of somatoform disorders is still lacking.

In a study of Rief *et al.* (2001) we examined serum concentrations of immunological parameters. A total of 150 persons were divided into a subgroup of patients with major depression, patients with somatization, patients with depression and somatization, and healthy controls. Patients with multiple somatoform symptoms were characterized by reduced IL-6 concentrations, increased IL-6R concentrations, increased IL-1RA concentrations and increased concentrations of the anti-cytokine Clara cell protein CC16. Moreover, the T-lymphocyte CD-8 count was reduced for somatizing patients, while patients with major depression had increased scores in comparison to controls. This is one of the few studies comparing multiple clinical groups and providing information about the specificity of the results. To summarize, there is some evidence that immunological processes may be differentially affected in major depression and somatization. In our study we found signs of increased proinflammatory capacity in depression, while some indicators were found for anti-inflammatory capacity in somatization (see Table XX.4).

As the preceding paragraphs highlight, there are reasons for investigating the interaction of immune parameters and somatoform symptoms. To date, only a few studies have been carried out, and we are just at the beginning of understanding these associations.

PSYCHOPHYSIOLOGICAL ASPECTS

Autonomic and Peripheral Physiological Activity

Lehofer *et al.* (1998) suggested a close interrelationship between brainstem centres regulating arousal and pain sensitivity. Higher 'nervousness' has been demonstrated to be associated with increased pain sensitivity. Altered pain sensitivity, on the other hand, can be assumed to be a central feature of somatization. Following this model, somatizing patients may have a reduced threshold for perceiving physiological changes and experiencing multiple unpleasant physical sensations.

However, it is also possible that somatizing patients really have abnormal physiological reactions, and not only changed perception thresholds. There may be different ways in which physiological abnormalities may contribute to an increased risk for the perception of physical symptoms. Abnormal physiological arousal could be of importance not only for the exacerbation, but also for the maintenance of somatoform symptoms. Somatization *per se* is a state of chronic stress which is accompanied by changes of autonomic and muscular activity (Melin and Lundberg, 1997). There are different ways in which an association between arousal abnormalities and perception of symptoms may work.

(a) *Permanent over-arousal*.
Patients with somatoform symptoms can be over-aroused in challenging as well as during relaxing situations. This over-arousal can lead to the subjective feeling of exhaustion which is typical for patients with somatoform disorders.

(b) *Over-activation in response to challenging events; lack of habituation*.
Somatization may be characterized by physiological hyper-reactivity. If the body perceives challenges, physiological over presentation of energy occurs. Moreover, the process of habituation describes the tendency to reduced reactions if stimuli are repeated. If over-activation or a lack of habituation occurs, this may be associated with reduced stress tolerance.

(c) *Lack of recovery and relaxation after challenging events*.
One of the principal human adaptation processes is to relax after physical, emotional, or cognitive challenges. If people are not able to use breaks for relaxation, the activity level which is typical for challenging events remains stable even in periods without demands. Again this may lead to states of exhaustion.

(d) *Increased dishabituation*.
If people are repeating sequences of comparable demands interrupted by shorter breaks, there is a typical increase of activation after the break when people restart activity. However, another process of human adaptation is that the re-increase of arousal after breaks is reduced as the person habituates more and more to the following demands. A process of amplified dishabituation would also lead to insufficient recovery of energy, and feelings of exhaustion and stress.

Some of these pathophysiological hypotheses have been tested in patients with somatization syndrome. In the study of Rief *et al.* (1998) we demonstrated that somatized patients feel more and more tense during and after periods of mental challenges, while healthy controls habituate to the situation. A physiological correlate could be found by assessing heart rate reactions. After breaks, the heart rate of somatizing patients re-accelerated even if the task was well-known and patients were familiar with it. Healthy controls did not show signs of dishabituation in heart rate reactions in this study; obviously, the adaptation process of 'habituation to challenges' is compromised in somatizing patients.

In a subsequent study, this effect was further analysed (Rief and Auer, 2001). As in the first study, a number of muscular and autonomic parameters were assessed (e.g., electrodermal activity, peripheral temperature, muscular activity, peripheral pulse amplitude and others). Again abnormal adaptation processes in heart rate activity were demonstrated in patients with somatoform symptoms. In this study, the most pronounced effects were found for the difference between task period and subsequent breaks: heart rate activity reduced significantly in healthy controls when breaks occurred, demonstrating an adaptation of physiological activity. This process was significantly less pronounced in patients with somatization indicating a reduced ability to benefit from breaks.

Further studies have pointed to the link between cardiovascular activity and somatic complaints, while relationships with other physiological systems seem to be of less importance. Kristal-Boneh *et al.* (1998) reported an association between somatic symptoms and 24-hour ambulatory blood pressure levels. There was a direct association between systolic blood pressure and somatic complaints, while the association with diastolic blood pressure was non-significant. In their study, they also replicated the association between heart rate reactivity and somatic complaints (see Table XX.5).

Yardley *et al.* (1998) confirmed that patients with somatization-associated symptoms react to the provocation of these symptoms with physiological processes which might maintain the problems. They examined persons with vestibular dysfunction with a dizziness provocation technique. After the provocation of dizziness, a

Table XX.5 Heart rate and somatic complaints (data from Kristal-Boneh *et al.*, 1998)

	Low somatic complaints	High somatic complaints	Significance
Diurnal heart rate	76 b.p.m.	81 b.p.m.	$p < 0.001$
Nocturnal heart rate	64 b.p.m.	65 b.p.m.	NS
Work time	76 b.p.m.	83 b.p.m.	$p < 0.01$
Casual	71 b.p.m.	75 b.p.m.	NS

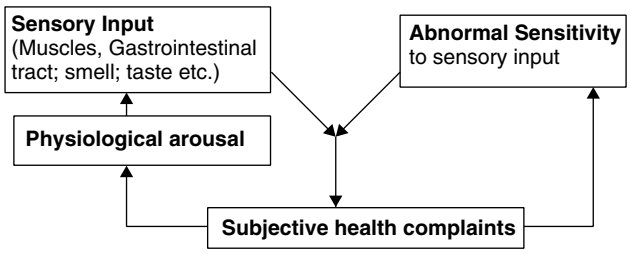

Figure XX.2 Ursin's model of a sensitization feedback loop (Ursin, 1997)

significant greater increase in respiration rate following head movements was found in those patients who complained of more somatic symptoms. These symptom-unspecific physiological effects could be associated with specific physiological changes, such as increased muscle tension or changes of blood circulation in symptom regions. Despite the face validity of the importance of these changes, they need to be better founded with empirical research.

Ursin (1997) presented a model of a psychobiological feedback loop to explain the process of somatization. Psychophysiological over-activation and the experience of complaints may lead to a sensitization of neurons. This might result in an abnormal sensitivity to sensory input from muscles or from the gastrointestinal tract, but also from smell and taste. This positive feedback loop is symbolized in Figure XX.2.

Somatoform Symptoms and the Electrophysiological Activity of the Brain (EEG, Evoked Potentials)

The perception of physical changes is a process of the central nervous system. Therefore it is possible that abnormalities of information processing in the central nervous system might be crucial for the development of somatization symptoms.

Some components of the evoked potentials are direct correlates of perceptual processes. The N1 amplitude, for example, seems to reflect processes of stimulus filtering and selective attention. Only few studies have investigated evoked potentials in somatoform disorders. However, they have yielded promising results: Gordon *et al.* (1986) used the classical 'oddball' paradigm to investigate a tone-discrimination task in *somatization disorders*. Eighty-five per cent of the tones were 1000 Hz in frequency and the remaining 15% were 2000 Hz (target tones). Participants were instructed to ignore the background tones and to count the number of target tones. The results demonstrated a trend for N1 amplitudes to be larger in the somatization group, and this was tentatively interpreted in terms of an impairment in stimulus filtering. For the other components (P1, P2, N2, P3) no significant group differences were found. The authors suggest that a more demanding task of selective attention may reveal more pronounced effects as a result of impaired stimulus processing in *somatization disorder*.

In a second study of the same group abnormalities of event-related potentials were confirmed which are thought to reflect disturbed attention processes in *somatization disorder* (James *et al.*, 1987). In this study the so-called mismatch negativity was assessed. Mismatch negativity is the difference between the potentials seen when subjects have to attend to one specific stimulus (e.g., 1000 Hz tone presented to the left ear) in comparison to potentials raised by the same stimulus when it should not be attended. Mismatch negativity starts prior to the N1 component and lasts for approximately 200 msec. This negative shift represents the difference in attention which is paid to relevant and irrelevant stimuli. In the study of James *et al.* (1987) there was less mismatch negativity in somatizers compared to normal controls at central and parietal midline sites. Somatizing patients seemed to process relevant and irrelevant stimuli more similarly than did normals; they were less capable of dividing their attention efficiently between relevant and irrelevant sensory input. This would help to explain why somatizing patients are more aware of and report more somatic symptoms.

A third study of the same group used auditory stimuli which varied in intensity (James *et al.*, 1990). The slope of P1-N1 amplitude changes as a function of stimulus intensity. This P1-N1 dependency of stimulus intensity was more pronounced in patients with *somatization disorder*, compared with controls, suggesting an enhanced central nervous system response to sensory input.

The results of these three studies are difficult to integrate. While all of them point to alternations in attention processing, the results seem not to be consistent and should be replicated and extended.

An interesting different approach was used by Wittling and colleagues (see Wittley (1997) for review). They did not investigate patients with manifest *somatization disorder*, but persons with high levels of unspecific physical complaints. Participants viewed either emotional stimuli or neutral stimuli, while topographic brain mapping procedures were used. The emotional stimuli evoked a significantly higher negativity in the period between 800 and 1200 msec over the whole posterior region. Group differences were found only for emotional stimuli, but not for the neutral stimulus conditions. Individuals with a low susceptibility to psychosomatic disorders responded to emotional stimuli with a higher negative potential than subjects with a high susceptibility to such disorders. These differences were most pronounced for the right hemisphere. Thus, these results point to abnormalities of information processing not only in simple attention tasks but also in the processing of emotional stimuli. As with other brain imaging techniques, topographic EEG can be one tool to highlight the specificities of the disturbed information processing in somatization. It would be helpful to continue this line of research.

THERAPEUTIC ARMAMENTARIUM

Management of Somatoform Disorders

In most doctor's offices, the time for diagnosis and treatment is quite limited. However, there is evidence that these few minutes can be crucial for whether the patient will be enabled to manage his complaints or whether the complaints might become chronic. Smith *et al.* (1986) and Smith *et al.* (1995) demonstrated that providing information to general practitioners on how to manage patients with somatization complaints was helpful in the avoidance of long-lasting, expensive and useless treatments. Table XX.6 contains the recommendations of Smith *et al.* (1986) as well as some extensions of our group.

Many patients with somatization symptoms are quite suspicious during the first contacts with experts. This means that the experts have to do many empathetic interventions during the first contact to install a constructive therapeutic relationship. One of these

Table XX.6 Guidelines for the management of somatization in primary care

- Confirm the credibility of the complaints
- Anticipate and inform the patient as early as possible that the most probable cause for the complaints is not a severe organic disorder, but a problem in the perception of bodily signals
- Explore the complete history and diversity of physical complaints
- Inform the patient about the next steps of investigations and about possible consequences; anticipate the time point when the procedure of organic investigations will be finished
- Avoid unnecessary investigations and alibi diagnoses
- Arrange regular dates for doctor visits of the patient; avoid spontaneous doctor visits
- Motivate for a healthy way of living and avoid physical deconditioning
- To avoid dysfunctional information processing due to the bias of the patients, use questions and ask the patient for conclusions.

steps should include the confirmation that the symptoms are 'real' for the patient and not 'just in mind'. The introduction of psychophysiological models (such as muscle over-activation, dysfunctional breathing, etc.) can be helpful at an early point in the therapeutic process. Introducing psychophysiological models only after all severe organic causes have been excluded might reduce their credibility.

Physicians may sometimes find it annoying to explore the complete history of complaints all over again. However, patients will not believe in the doctor's treatment recommendations if the doctor does not know all about the complaints. Moreover, some patients describe just those symptoms which fit in the speciality of the doctor, but do not report that they have been in gynaecological, orthopaedic or neurological treatment some weeks earlier. Therefore the diagnostician has to explore actively and ask for complaints in all parts of the body.

Some patients find it difficult to accept the doctor's opinion that no more medical investigations are necessary despite the fact that no organic reason has been found. It is easier for the patients to accept the decision to stop the diagnostic process if they have been informed in advance when this step will be taken. Unnecessary investigations increase the risk of iatrogenic harm, and unnecessary 'alibi diagnoses' reduce the probability of using successful self-help strategies.

If patients are in need of frequent doctor visits, it may be helpful to install a regular scheme for visits to avoid spontaneous, symptom-contingent procedures. The installation of regular doctor visits may be one of the first and most important steps to encourage self-help strategies for the patient: the patient knows that the doctor is still involved in the treatment process, but the patient tries to use self-help strategies in between appointments.

Some patients think that the avoidance of physical activity may be helpful to overcome the complaints. However, these patients should be informed that the avoidance of physical activity may be a helpful short-term strategy (e.g., in acute pain), but considering a long-term perspective it might increase disability. Therefore the patients should be encouraged to continue and even to expand regular physical activities.

Most patients with somatoform complaints have an information processing bias. The information provided by the doctor is transformed until it fits into the patient's reasoning. A doctor's statement of "Cancer is really improbable in your case" could be transformed into "It is probable that you have cancer" by the patient. To prevent this information transformation, the doctor can use questions and ask the patient for summaries.

As mentioned above, these simple behaviour strategies can be done even during very short-term contacts, and have a proven efficacy in the management of patients with unexplained physical symptoms. Most patients with somatoform symptoms are treated by general practitioners. Therefore it is important to transform the knowledge of experts into intervention strategies for GPs. If patients need more sophisticated psychological treatment, there are also

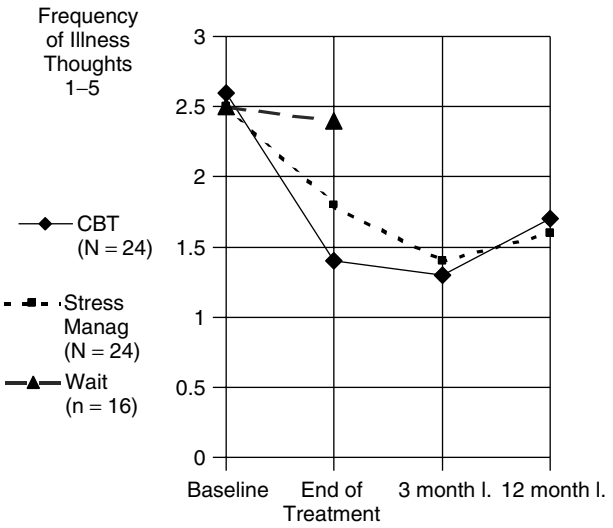

Figure XX.3 Treatment of hypochondriasis (Clark *et al.*, 1998)

some treatment studies demonstrating the efficacy of psychotherapeutic approaches. Kroenke and Swindle (2000) presented a critical review of controlled clinical trials for cognitive–behavioural therapy (CBT) in somatization. They summarized that CBT can be an effective treatment for patients with somatization or somatoform syndromes. Benefits can occur whether or not psychological distress is ameliorated. However, compared to some outstanding studies proving the efficacy of CBT in *hypochondriasis* (Clark *et al.*, 1998), the number of studies reporting psychotherapeutic approaches in other somatoform disorders is still small (see Figure XX.3).

Pharmacological Treatment

Although somatoform disorders are highly prevalent and present a rising problem to the health services there is still no standard pharmacological treatment. Only few placebo-controlled clinical trials have been performed. This might be partly due to the very concept of somatoform disorders that excludes somatic causes and therefore seems to call for other than pharmacological treatment. Further difficulties for the establishment of a standard pharmacological management of somatoform disorders arise from the heterogeneous characteristics of sub-syndromes summarized under the heading of somatoform disorders, inconsistencies in diagnostic concepts and outcome measures and generally elevated placebo response rates in somatoform disorder patients (Volz *et al.*, 1994). From the limited data base to date there is reason to believe that antidepressants are beneficial in the treatment of somatoform disorders (Volz *et al.*,

1994; Escobar, 1996). Open clinical trials suggest that selective serotonin reuptake inhibitors (SSRIs) like fluvoxamine might lead to improvement in about 60% of patients (Noyes *et al.*, 1998). In one of the few placebo-controlled clinical trials Volz *et al.* (2000) reported that the tricyclic antidepressant opipramol proved to be more effective than placebo in reducing somatic symptoms in patients with certain somatoform disorders (*somatization disorder*, *undifferentiated somatoform disorder*, *somatoform autonomic dysfunctions*). Additional improvement on other psychopathological outcome measures and the good tolerability profile further recommended the compound, especially for use in a patient group characterized by high psychiatric comorbidity and a focus on even minor body sensations.

Body dysmorphic disorder (BDD) has been considered an obsessive–compulsive spectrum disorder, owning to the features it shares with obsessive–compulsive disorder (OCD). As in OCD open trials suggest a good therapeutic response to serotonin reuptake inhibitors, such as clomipramine, fluoxetine and fluvoxamine; other pharmacological agents, such as neuroleptics, trazodone, lithium, benzodiazepines, tricyclics and anticonvulsants, have been less beneficial or ineffective in BDD (Hollander *et al.*, 1989; Phillips *et al.*, 2001; Phillips *et al.*, 2001). In a randomized, double-blind, crossover-design study of 29 BDD patients clomipramine, a potent serotonin reuptake inhibitor, was superior to the active control desipramine, a potent norepinephrine reuptake inhibitor in the treatment of BDD symptoms according to all outcome measures (Hollander *et al.*, 1999). As was later confirmed by other researchers delusional patients (which required the diagnosis of delusional disorder, somatic type) responded as well as non-delusional patients to SRI treatment (Hollander *et al.*, 1999; Phillips *et al.*, 2001). Thus, delusional patients may not require neuroleptic treatment and might be spared possible adverse side effects.

Another subtype of somatoform disorder, *hypochondriasis*, has also been related to obsessive–compulsive disorder. Although the comorbidity profile, clinical course and family history data do not support this hypothesis there is evidence that hypochondriac concerns respond to the same class of drugs that is effective in OCD: serotonin reuptake inhibitors. Open trial data suggest that SSRIs such as fluvoxamine might be useful therapy for hypochondriac patients (Fallon *et al.*, 1993; Perkins, 1999). A preliminary report of placebo-controlled trial in a small group of patients partly confirmed these results. However, due to high placebo response rates differences between fluvoxamine (66% responders) and placebo (50% responders) felt short of statistical significance (Fallon *et al.*, 1996). Tricyclic antidepressants seem not to be effective in *hypochondriasis* (Fallon *et al.*, 2000). As is the case in BDD there seems to be a spectrum of insight in *hypochondriasis* ranging from good to absent. Patients at the latter end of this continuum would require the diagnosis of delusional disorder, somatic type, formerly called monosymptomatic hypochondriacal psychosis. For those patients single case studies and small clinical series suggest that low doses of neuroleptics such as pimozide or olanzapine might be the treatment of choice (Opler and Feinberg, 1991; Weintraub and Robinson, 2000). However, as SSRIs have proved to be effective in treating delusionality in delusional BDD patients there might be reason to expect beneficial effects also on delusional *hypochondriasis* (Perkins, 1999).

The possible analgesic effects of antidepressants for the treatment of chronic pain have been evaluated in a number of double-blind studies. In a recent meta-analysis Fishbain *et al.* (1998) reviewed 11 randomized, placebo-controlled treatment studies on *pain disorder*. The results showed that antidepressants decreased pain intensity more than placebo in patients with psychogenic pain or *somatoform pain disorder* (mean overall effect size = 0.48). The authors argued that low dosage and short duration of treatment in most studies made it unlikely that the medication could have exerted an appreciable antidepressant effect. Thus, the analgesic effect of the treatment was considered not to be just a function of the antidepressant properties of the drugs.

The analgesic properties of both tricyclic antidepressants and new antidepressant compounds have also proved to be therapeutically useful in related syndromes such as the somatoform spectrum disorder *fibromyalgia* (Goldenberg *et al.*, 1986; Dwight *et al.*, 1998).

Taken together, there is evidence that antidepressant drugs have a beneficial effect in somatoform disorders, with traditional tricyclic antidepressants being especially useful in those conditions that are characterized by pain and multiple somatic complaints (*pain disorder*, *somatization disorder*) and modern SSRI antidepressants being especially effective in conditions being related to anxiety disorders and obsessive–compulsive disorder (*body dysmorphic disorder*, *hypochondriasis*). However, there is still a need for more controlled studies using larger patient samples. Issues of differential indication and long-term maintenance of treatment effects need considerably greater attention.

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XXI

The Emerging Psychobiology of Trauma-Related Dissociation and Dissociative Disorders

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INTRODUCTION

Mental dissociation is an intriguing and complex phenomenon that has been studied clinically and theoretically for more than 150 years. Major empirical studies have been conducted during the last two decades. For example, these studies have established that the prevalence rates of the DSM-IV dissociative disorders (APA, 1994) are considerable among psychiatric inpatients, with an overall prevalence among nine studies of 18.9% for dissociative disorders in general, and 4.4% for dissociative identity disorder (DID; Friedl *et al.*, 2000). Only in recent years has dissociation begun to receive attention from a combined psychological and biological perspective.

The study of dissociation has been impeded by several factors. First, conceptual confusion and controversy exist regarding the nature of this phenomenon. For example, dissociation is described as a process, as various symptoms, as mental structure, as psychological defense, and as a deficit in integrative capacity. In addition, it is confused with retraction of the field of consciousness (that which is within awareness at a given time) such as absorption, daydreaming, and states of inattention. Although negative dissociative symptoms such as amnesia are acknowledged, positive ones, such as intrusive re-experiences of trauma, are often not understood as dissociative in nature.

Second, diagnostic and aetiological issues are far from resolved in psychiatric disorders in which dissociation is an essential feature. For example, it is debatable that the DSM-IV category of dissociative disorders encompasses the actual range of dissociative disorders. As discussed below, although post-traumatic stress disorder (PTSD) is classified as an anxiety disorder, there are arguments to regard it as a dissociative disorder (e.g., Nijenhuis *et al.*, 2002b). And the ICD-10, for instance, includes dissociative disorders of movement and sensations that are defined in the DSM-IV as conversion disorders. It has been theoretically argued (Kihlstrom, 1994; Nemiah, 1991; Nijenhuis and Van der Hart, 1999a) and empirically documented (Nijenhuis, 1999) that conversion symptoms are dissociative in nature. Conversion is better conceptualized as somatoform dissociation in that the symptoms of concern are at least equally characteristic of DSM-IV dissociative disorders as dissociative symptoms that manifest in psychological variables, i.e., so-called psychoform dissociation (Van der Hart *et al.*, 2000). Moreover, somatoform dissociation correlates very strongly with psychoform dissociation (Nijenhuis, 1999; Nijenhuis *et al.*, 1996, 1997, 1999b; Sar *et al.*, 2000; Waller *et al.*, 2000), and evidence for the hypothesis that psychological conflicts can be converted into physical symptoms, and thus reduce mental strain, is lacking.

Although the DSM-IV dissociative disorders have received wide acceptance, there exists a vocal minority of clinicians and researchers who express their scepticism about the validity of the most complex dissociative disorder, DID (Spanos and Burgess, 1994). As we will discuss in more detail below, patients with DID have severe somatoform and psychoform dissociative symptoms that relate to the existence of two or more self-aware dissociative systems, i.e., dissociative systems of ideas and functions that involve a sense of self. However, sceptics doubt that dissociative personalities are genuine phenomena. Simulated or factitious cases of DID certainly exist (Draijer and Boon, 1999) along with genuine cases, and the psychobiological differences between genuine and false positive cases of DID are a fruitful area of research. Some authors have also questioned whether chronic childhood traumatization is an aetiological base for DID and other dissociative disorders, but research has converged to indicate traumatic events are a key element in the psychopathology of dissociation and dissociative disorders. Retrospective (e.g., Boon and Draijer, 1993; Coons, 1994; Kluft, 1995; Lewis *et al.*, 1997; Nijenhuis *et al.*, 1998b) and prospective studies (Ogawa *et al.*, 1997) — some of which have provided external corroboration for reported trauma — have confirmed the clinical observation that dissociative symptoms and disorders are associated with reported and factual traumatization. Studies have also demonstrated discrete and long-lasting alterations in neurobiological systems in relation to trauma and dissociation, which will be discussed below.

The current chapter focuses on dissociation related to traumatization. The first aim of the chapter is to reduce confusion about the concept of dissociation. Next, possible neuroendocrine parameters of dissociation and key brain structures that seem to be involved in the phenomenon are discussed. Several neurobiological models of dissociation will be reviewed, including Putnam's discrete behavioural states model (Putnam, 1997). Finally, the theory of structural dissociation will be presented (Nijenhuis and Van der Hart, 1999b; Van der Hart *et al.*, 1998; Nijenhuis *et al.*, 2002b; Steele *et al.*, 2001), along with supporting evidence. The theory attempts to explain trauma-related dissociation as it manifests in disorders ranging from simple PTSD to DID, and serves an important heuristic function with regard to the emerging psychobiological study of trauma-related dissociation.

THE CONCEPT OF DISSOCIATION

Dissociative symptoms are often misunderstood as indicators of other disorders or problems. Thus, it is quite common for dissociative disorder patients to hear internal voices (easily confused with psychosis); to experience disorientation to time, place and person during intrusive re-experiences (confused with intoxication, delirium, delusions, hallucinations, and psychosis); to have

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rapidly alternating shifts in mood (confused with bipolar disorders); to experience cognitive and attentional difficulties (confused with attention deficit disorder and cognitive processing deficits); to have intractable dissociative pain and somatoform disturbances (confused with malingering, factitious disorder; hypochondriasis, drug-seeking behaviour, and somatization disorder); to have unusual sleep disturbances due to night-time dissociation (confused with disorders of sleep); and to experience lapses in awareness (confused with malingering, manipulation, organic memory dysfunction, and iatrogenic suggestibility). Therefore, efforts to define dissociation (e.g., Braude, 1995; Cardeña, 1994; Janet, 1907; Nijenhuis *et al.*, 2002b; Putnam, 1997; Van der Hart, 2000) are important, both for clinical and research purposes.

Janet (1907, p. 332) postulated that dissociation involves a lack of integration of 'systems of ideas and functions that constitute personality' due to 'a form of mental depression'. In the context of traumatic experience, dissociation ensues when the integrative capacity of the individual is not sufficient to integrate experiences and functions. As a result, (components of) experiences can be encoded and stored as discrete systems involving sensations, body movements, affects, perceptions of the environment, and factual knowledge. Because conscious experience implies a subject, dissociative systems also involve their own sense of self, however rudimentary. While integrative failure that results in fragmentation of the personality denotes the essence of dissociation, Janet did not specify the underlying psychobiological nature of the different dissociative 'systems of ideas and functions' that traumatic experience tends to evoke.

Psychoform and Somatoform, and Negative and Positive, Dissociative Symptoms

Dissociation manifests in psychoform and somatoform symptoms (Janet, 1889; Kihlstrom, 1994; Nijenhuis and Van der Hart, 1999a; Nijenhuis *et al.*, 2002b; Van der Hart *et al.*, 2000). Both categories include negative and positive symptoms. Negative symptoms manifest when the individual fails to integrate dissociative systems of ideas and functions that, at least in principle, are available. Positive symptoms occur when these systems are retrieved (e.g., flashbacks), but without integration. Negative psychoform symptoms include losses such as amnesia, depersonalization, and derealization. Positive psychoform symptoms include re-experiencing the mental contents of traumatic memories such as thoughts, images, and feelings, as well as hearing voices that are emitted by dissociative systems (e.g., as in dissociative fugue; APA, 1994). Negative somatoform dissociative symptoms involve anaesthesia of one or more sensory modalities and motor inhibitions, such as paralysis, visual disturbances like tunnelling and haziness, deafness, or inability to feel parts of the body. Examples of positive ones are pain, sexual sensations, and freeze, flight, fight and submissive states pertaining to traumatic re-experiences.

Retraction of the Field of Consciousness and Low Levels of Conscious Awareness

Phenomena such as absorption, imaginative involvement and trance-like behaviour are often referred to as normal or non-pathological dissociation (e.g., Putnam, 1997) or as dissociative detachment (Allen *et al.*, 1999). However, these experiences result from automatic and unconscious reduction of the number of phenomena in current conscious awareness, called retraction of the field of consciousness. They do not involve encoding and storage of experience, knowledge, and skills. For example, selective attention for threat cues does not necessarily imply encoding and storage of 'peripheral' cues. While retraction of the field of consciousness may accompany dissociation (Janet, 1907; Nijenhuis *et al.*, 1996), the concepts are different.

Retraction of the field of consciousness can involve different levels of conscious awareness. The level of awareness can be high when dissociative patients focus on threat cues, but low when they enter trance states during which they are only minimally aware of themselves and their surroundings, and thus fail to encode their experience at the time. Extremely high and low levels of awareness may set the stage for dissociative processes, but are not dissociative in themselves.

The conceptual confusion with regard to dissociation continues to plague the field. For example, many instruments that evaluate the severity of dissociation suffer from under-inclusion of negative somatoform dissociative symptoms, as well as positive psychoform and somatoform dissociative symptoms, and from over-inclusion of symptoms that evaluate retraction of the field of consciousness and lowering of the level of consciousness.

PTSD, DISSOCIATION, AND STRESS RESPONSES

Defining dissociation as a lack of integration among self-aware 'systems of ideas and functions' implies that dissociation also characterizes PTSD in that this psychobiological disorder (Kardiner, 1941; Van der Kolk, 1994; cf. Vermetten and Bremner, 2001a, 2001b) involves two dissociative parts of the personality that can take control over consciousness and behaviour (Nijenhuis and Van der Hart, 1999b). One is numb and avoidant of traumatic memories and trauma-related reminders, but more or less functional in daily life, and another is enmeshed in traumatic memories. The enmeshed dissociative system intermittently intrudes or replaces the numb dissociative system. As we will discuss below, this split represents a form of structural dissociation of the personality that also constitutes the basis of more complex dissociative conditions.

To the extent that this conceptualization is correct, pre-clinical and clinical studies of severe aversive stress and PTSD are also relevant for the psychobiology of trauma-related dissociation more generally. Although a review of these studies is beyond the scope of the present chapter (for reviews, see Coupland, 2000; Van der Kolk, 1994; Vermetten and Bremner, 2001a, 2001b), the main findings are briefly noted. Animal studies have contributed to the understanding of human reactions to overwhelming events. For example, they have allowed for insights into delay conditioning of fear and defensive reactions patterns in which the conditioned stimulus immediately follows the unconditioned stimulus, as well as trace conditioning, in which the conditioned stimulus and unconditioned stimulus are separated by an interval. Delay conditioning depends on long-term potentiation within the basolateral amygdala, whereas trace conditioning requires involvement of the hippocampus. These forms of classical conditioning are relevant for dissociation, since dissociation as a process (i.e., the presumed psychobiological process that yields splits among self-aware dissociative systems) can become a conditioned response to previously neutral cues. Yet, the issue is complex: clinical observations and experimental research (Nijenhuis *et al.*, 1999a; Reinders *et al.*, in preparation; Van Honk *et al.*, 1999, 2001), to be discussed below, suggest that different dissociative systems can display different psychobiological responses to identical stimuli. Somehow the associations between unconditioned aversive stimuli (UCS) and conditioned stimuli (CS), or the responses to these associations, may depend on the particular dissociative system that dominates consciousness and behaviour.

Pre-clinical studies have also been valuable in delineating other learning processes that are relevant for trauma-related dissociative disorders. These processes include contextual conditioning (i.e., learning to respond to contextual cues such as the place where a foot shock was received), sensitization (i.e., response increment with repeated exposure to aversive cues), kindling, and extinction. It was once thought that the disappearance of conditioned responses

indicated loss of the conditioned association between the CS and the UCS. However, the evidence suggests that once encoded, CS-UCS associations cannot be extinguished (Bouton, 1994), but rather become inhibited. The hippocampus and medial prefrontal and orbitofrontal cortex seem to have major roles in this process.

Animal studies provide a model for understanding a range of neurobiological alterations in PTSD, and probably are relevant for (other) dissociative disorders as well. The pathophysiology of stress reflects enduring changes in biological stress response systems with implications for learning and memory. The affected biological systems include systems mediating corticotropin-releasing factor (CRF), adrenocorticotropin hormone (ACTH), glucocorticoids (HPA-axis), neuropeptide Y (NPY), and norepinephrine (locus coeruleus/autonomic nervous system). Stressors also tend to affect serotonergic, dopaminergic, neuropeptide and central amino acid systems. Combat-related PTSD was marked by decreased benzodiazepine receptor binding in the prefrontal cortex (Bremner *et al.*, 2000b). These systems interact with brain structures involved in learning and memory, including the hippocampus, amygdala, and prefrontal cortex. Pre-clinical studies have demonstrated that the intensity and duration of the stressor, as well as the timing of the stressor in life, amplify the neurobiological effects of stressors. For example, maternal separation, lack of handling, and repetitive pain affect the developing brain of animals.

There is evidence for a number of parallels between animal responses to aversive stress and similar mechanisms in PTSD (Coupland, 2000), as well as other trauma-related disorders in humans. Thus, clinical studies of PTSD have found that brain areas such as the hippocampus, amygdala, insula, cingulate and prefrontal cortex are critically involved in responding to severe stressors, as well as in the learning from and memorization of these events (e.g., Rauch *et al.*, 1996; Van der Kolk, 1994; Vermetten and Bremner, 2001b). Some findings (to be discussed below) suggest that these brain structures are also of major importance in other dissociative conditions. Neural circuits affected in PTSD include the HPA-axis, catecholaminergic and serotonergic systems, neuropeptide systems, the hypothalamic-pituitary-thyroid axis, and neuro-immunological alterations. In both animals and humans, the HPA-axis and the autonomic nervous system are important stress response systems that, within limits, serve to maintain homeostasis during exposure to stress. Consistent with the animal studies, converging evidence has emphasized the role of early life trauma and attachment in the development of PTSD and other trauma-related disorders (Schore, 2001). Yet, there are also differences between neurobiological correlates of animal and human traumatization. An example is the finding of low cortisol levels in several studies of PTSD that contrasts with stress-induced increases of cortisol in animals (for a review see Yehuda, 2000).

POSSIBLE INVOLVEMENT OF NEUROCHEMICALS IN DISSOCIATION

Dissociation involves a failure to integrate systems of encoded and stored experiences and functions. From a biological point of view, integrative functions can be hampered by the release of neurochemicals provoked by severe threat (Ludwig, 1972; Krystal *et al.*, 1991; see also Siegel, 1999) and that are concentrated in brain regions implicated in integrative mental acts, such as the hippocampus and the prefrontal cortex.

Enhancement and Impairment of the Memory Function

The extent to which substances such as glutamate (Krystal *et al.*, 1998), norepinephrine, epinephrine, glucocorticoids, endogenous opiates, and several others (McGaugh, 1990, McGaugh *et al.*, 2000) interfere with integration is dose-dependent. For example, retention of recently learned material is enhanced when moderate doses of epinephrine are administered after training, but impaired at high doses. Memory impairment also depends on experimental conditions such as degree of arousal produced by the learning situation and the strength of the memory involved. For example, when the β-adrenergic antagonist propanolol was administered after training, memory was strongly impaired in rats that exhibited good learning during the acquisition session, but not in rats that exhibited poor learning (Cahill *et al.*, 2001, discussed in Cahill, 2000). While long-lasting exposure to threat cues such as predators may produce failure to execute previously memorized tasks (Diamond and Park, 2000; Woodson *et al.*, 2001), memories of emotional events can also be excellent, requiring little or no cognitive rehearsal (Guy and Cahill, 1999). This apparent paradox may be resolved by considering that patients with PTSD and several other dissociative conditions may alternate between hyperamnesia and memory loss. Hence, memory retrieval may depend on the dissociative part of the personality that is dominant.

Substance-Induced Dissociative-Like Symptoms in Healthy Individuals

Several substances may induce phenomena in healthy individuals that mimic dissociative symptoms, i.e., antagonists of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors, cannabinoids, and serotonergic hallucinogens (Krystal *et al.*, 1994a, 1994b, 1998). Because glutamate has a central role in corticocortical, thalamocortical, amygdalacortical, and hippocampocortical connectivity, higher cognitive functions could basically involve glutamatergic systems. Pre-clinical data have indeed suggested that (traumatic) stress stimulates the corticolimbic release of glutamate, and clinical studies have documented that antagonists of the NMDA receptor may transiently stimulate glutamate release, inducing symptoms that resemble dissociative symptoms in humans (Chambers *et al.*, 1999).

Ketamine blocks the NMDA receptor-mediated component of glutamatergic connections. This substance produces dose-dependent impairment of perceptual, sensory, and proprioceptive experiences that could reflect retraction of the field of consciousness, lowering of the level of consciousness, and negative dissociative symptoms. Ketamine does not seem to evoke positive dissociative symptoms, such as manifestations of dissociative parts of the personality or intrusive recollection of experiences involving negative emotions. Ketamine-induced learning and memory impairments have also been observed, as were psychotic-like symptoms such as delusions, thought disorder and negative symptoms of schizophrenia (e.g., Breier *et al.*, 1997). Hence, it seems doubtful that glutamatergic dysregulation is a *specific* inductor of dissociation, i.e., an inductor of dissociative fragmentation of the personality.

The hippocampus is implicated in the synthesis of experiences and memory encoding, and the NMDA receptor is highly concentrated in this brain structure. Since blocking of the Schaffer collateral-CA1 NMDA receptors in the hippocampus can inhibit long-term potentiation (Tsien *et al.*, 1996), ketamine could affect memory. Substances that enhance NMDA receptor function may perhaps reduce trauma-related dissociation (see Krystal *et al.*, 1998). Consistent with this hypothesis, a drug that reduces glutamate release attenuated the effects of ketamine (Chambers *et al.*, 1999). Modulatory GABAergic neurons regulate pyramidal neurons that use glutamate as their primary neurotransmitter (Krystal *et al.*, 1998). Thus, future research should assess whether substances that reduce trauma-related glutamate release or enhance gamma-aminobutyric acid (GABA) functions promote the integration of traumatic experience. Benzodiazepines are prototypical

GABA enhancers, and were useful in the treatment of some PTSD symptoms of patients with dissociative disorders (Loewenstein *et al.*, 1988). Yet, benzodiazepines can evoke positive dissociative symptoms, discussed below, and at high doses they may promote amnesia. Krystal *et al.* (1998) have proposed to evaluate whether drugs that facilitate NMDA receptor function have antidissociative effects in PTSD patients.

Depersonalization, derealization, perceptual alterations, and time distortions have also been provoked by high doses of cannabinoids (Dittrich *et al.*, 1973; Melges *et al.*, 1970), and were correlated with increased cerebral blood flow (Mathew *et al.*, 1993). Moreover, cannabinoids also produce amnestic effects due to interference with hippocampal memory functions (Hampson and Deadwyler, 1999). Some cannabinoid effects may be mediated by stimulation of glucocorticoid receptors (Eldrigde and Landfield, 1990) and blockade of NMDA receptors (Feigenbaum *et al.*, 1989). Serotonergic hallucinogens produce visual hallucinations, illusions, depersonalization, derealization, as well as body image distortion and, occasionally, emotional detachment (e.g., Freeman, 1986; Strassmann *et al.*, 1994). However, ketamine, its cousin phencyclidine, cannabinoids, and serotonergic hallucinogens do not produce dissociative disorders or positive dissociative symptoms such as hyperamnesia and flashbacks with respect to stressful and aversive experiences. As applies to ketamine, there is no evidence that the symptoms that cannabinoids and serotonergic hallucinogens produce relate to structural splits among dissociative parts of the personality.

Substance-Induced Dissociative Symptoms and States in PTSD Patients

A range of substances, including barbiturates and benzodiazepines, can reactivate traumatic memories and flashbacks in PTSD (for a review, see Krystal *et al.*, 1998). The effects of these drugs seem to be indirect, as they may reduce anxiety, and thus suppress mechanisms that inhibit access to traumatic memories (e.g., Kopelman *et al.*, 1994).

Autonomic activation, flashbacks and recall of traumatic memories can be provoked in a subset of patients with chronic PTSD using intravenous administration of sodium lactate (Rainey *et al.*, 1987), yohimbine (Southwick *et al.*, 1993), and metachlorophenylpiperazine (m-CPP; Southwick *et al.*, 1991). In some cases, yohimbine-induced flashbacks and traumatic memories were accompanied by depersonalization and derealization. Yohimbine blocks $\alpha 2$ receptors located on noradrenergic neurons that have a role in the regulation of fear and arousal. The $\alpha 2$ receptors partly mediate feedback inhibition of noradrenergic neurons (Starke *et al.*, 1975). Hence, yohimbine activates central noradrenergic neurons, producing increased release of norepinephrine in the brain. Clinical observations have suggested that autonomic arousal can reactivate traumatic memories, possibly because the autonomic arousal that was part of the traumatic experience has become a conditioned stimulus. (Thus, some PTSD patients and traumatized patients with other dissociative disorders tend to avoid aroused states, such as physical exertion, as well as negative and positive emotions.) However, Southwick (see Krystal *et al.*, 1998) reported that in some cases autonomic arousal followed yohimbine-provoked traumatic memories. This observation led Krystal and colleagues to conclude that 'noradrenergic systems might be involved in the elicitation of dissociative symptoms [i.e., traumatic memories, and accompanying features] as a direct consequence of central pharmacological actions of yohimbine on neural circuitry contributing to dissociation and memory retrieval' (p. 326). On the other hand, even small increments of arousal may perhaps trigger traumatic memories when the individual has developed a phobia for bodily arousal that originally accompanied trauma.

Norepinephrine/Locus Coeruleus

The autonomous nervous system is regulated by the locus coeruleus and its projection areas. The activity of the HPA-axis is partly mediated by the release of CRF by the paraventricular nucleus of the hypothalamus. Both systems are key players in stress responsivity and are functionally interrelated. They both have differentiated anatomical and functional responses to different kinds of stressors. For example, the hypothalamic paraventricular nucleus neuron appears to be affected by multiple sources, including brainstem aminergic and peptidergic afferents; blood-borne information; indirect input from limbic system and associated regions, including the prefrontal cortex, hippocampus, and amygdala; and local-circuit interactions (Herman *et al.*, 1996).

The locus coeruleus houses the majority of noradrenergic neurons. Exposure to chronic stress may yield long-term alterations in locus coeruleus firing and in norepinephrine release in target brain regions of the locus coeruleus (Bremner *et al.*, 1996). Norepinephrine also has a role in fear conditioning and sensitization, and there is strong evidence that noradrenergic activation of the amygdala is critical for neuromodulatory influences on memory storage (McGaugh *et al.*, 2000).

Both animal and human studies have shown that high levels of norepinephrine release are associated with a decrease in metabolism in the cerebral cortex. Following administration of yohimbine, PTSD patients had a tendency towards decreased brain metabolism in hippocampal, orbitofrontal, temporal, parietal, and prefrontal cortex areas; healthy controls had a tendency toward increased metabolism in these regions (Bremner *et al.*, 1997a).

Endorphins

Alcohol and opiate withdrawal activate central noradrenergic systems and can increase flashbacks and other PTSD symptoms (e.g., Kosten and Krystal, 1988). Many dissociative patients report that self-mutilation and substance abuse may be instrumental in temporarily reducing autonomic arousal, intrusions of traumatic memories, and internal imperative voices. These effects could be mediated by the release of β-endorphins that dampen norepinephrine firing in the locus coeruleus. Endogenous opiates contribute to stress-induced analgesia, dissociative symptoms, and possibly the high rate of opiate abuse in patients with PTSD: this disorder is associated with decreased baseline levels, but increased post-stimulation effects of β-endorphins (Newport and Nemeroff, 2000). The opioid antagonist naltrexone decreased the frequency of flashbacks and other dissociative phenomena in borderline personality disorder (Bohus *et al.*, 1999).

HPA-Axis

Several studies have suggested that PTSD involves low baseline levels of cortisol (Yehuda, 2000), and lower cortisol responses to CRF and ACTH challenge (Vermetten and Bremner, 2001b). While there is evidence for excessive CRF release from the hypothalamus in PTSD, and excessive ACTH from the pituitary, baseline cortisol is low. According to Yehuda (2000), low cortisol in PTSD is due to negative feedback inhibition in the HPA-axis. Yet, since the number of glucocorticoid receptors are high in PTSD, the HPA-axis would be very sensitive to stressors. In some studies, however, PTSD was associated with increased cortisol levels (e.g., De Bellis *et al.*, 1999; Lemieux and Coe, 1995). Bremner (1999) suggested that perhaps low cortisol characterizes chronic PTSD, and high cortisol levels the acute stages of the disorder. For example, women with a history of prior rape or assault had relatively lower cortisol levels immediately after rape than raped women without such a history (Resnick *et al.*, 1995). On the other hand, in individuals who had developed PTSD

in response to motor vehicle accidents, the cortisol response in the immediate aftermath of the accident was significantly lower than in individuals who had developed major depression. This group effect remained after controlling for covariates such as minutes post accident, time of day, severity of trauma, and past PTSD (Yehuda *et al.*, 1998).

The neuropeptide CRF and ACTH are involved in the modulation of memory functions and the stress response. Bremner *et al.* (1997b) found an increase of CRF in PTSD patients, which is consistent with the impaired memory function in PTSD (Bremner *et al.*, 1998b) and the DSM-IV dissociative disorders. Heim *et al.* (2000) documented strongly increased ACTH levels and heart rate increases in response to mild stressors, but normal cortisol levels in women who had been sexually and physically abused early in life. This increase is presumably due to CRF hypersecretion.

Interactions Among Neuropeptide Y (NPY), Corticotropin-Releasing Factor (CRF), Cortisol, and Norepinephrine

Anxiety and stress vulnerability may relate to dysregulation of interrelated neurotransmitter systems that augment and attenuate threat responses. Whereas CRF promotes anxiety, pre-clinical studies suggest that NPY has anxiolytic properties (Helig *et al.*, 1989, 1993). Chronic stress exposure may result in the development of low baseline levels of NPY, as well as a blunted NPY response to subsequent stress (Corder *et al.*, 1992). It thus seems that deficits in NPY may promote anxiety and distress, whereas augmentations in NPY can buffer these effects.

The interactions between CRF and NPY are relevant to human stress responses in that abnormalities of both CRF and NPY have been noted in individuals suffering from post-traumatic stress, anxiety, and symptoms of dissociation (Bremner *et al.*, 1997b; Rasmusson *et al.*, 2000). Compared with healthy subjects, PTSD patients reported more anxiety, had low baseline levels of NPY, and had a blunted NPY response to yohimbine (Rasmusson *et al.*, 2000). Rasmusson and colleagues also found a negative correlation between the degree of combat exposure and plasma NPY and a negative correlation between baseline NPY levels and yohimbine-induced increases in plasma 3-methoxy-4-hydroxyphenylglycol and in systolic blood pressure. Thus sympathetic dysregulation in PTSD may be related to deficits in the NPY response to stress.

Twenty-four hours after cessation of uncontrollable stress during military survival school training, NPY had returned to baseline in special forces soldiers (Morgan *et al.*, 2000). However, other soldiers exhibited a significant depletion of NPY. The stress-induced release of NPY was negatively correlated to negative dissociative symptoms. Studying healthy subjects enrolled in US Army survival school, Morgan *et al.* (2001) found that release of salivary cortisol, NPY, and norepinephrine induced by stressful military interrogation during training were positively correlated. Release of cortisol, but not of NPY, was also correlated with dissociation and military performance during stress in this study. The dissociation measure (Clinician Administered Dissociative States Scale, CADSS, Bremner *et al.*, 1998a) administered in this study is problematic, however. The CADSS includes items that evaluate retraction of the field of consciousness, and lowering of the level of consciousness, disregards positive psychoform dissociative symptoms, and includes only a few items that assess somatoform dissociation. These flaws are found in many dissociation scales.

Urinary Catecholamines and CSF Norepinephrine

De Bellis *et al.* (1999) examined the relationship between trauma, psychiatric symptoms and urinary-free cortisol and epinephrine, norepinephrine, and dopamine excretion in abused pre-pubertal children with PTSD. These subjects were compared to a group of non-traumatized children with overanxious disorder, and with healthy controls. The children with PTSD excreted significantly greater concentrations of urinary dopamine and norepinephrine over 24 hours than the other subjects, had greater concentrations of 24 hour urinary-free cortisol than control subjects, and excreted greater concentrations of urinary epinephrine than subjects with overanxious disorder. They also had more dissociative symptoms and other psychiatric symptoms than control groups. Urinary catecholamine and urinary-free cortisol concentrations showed positive correlations with the duration and severity of PTSD symptoms. However, urinary catecholamine levels bear little relation to brain activity. A recent study found presence of greater central nervous system noradrenergic activity under baseline conditions in patients with chronic PTSD than in healthy individuals (Geracioti *et al.*, 2001). While plasma norepinephrine concentrations showed no significant relationship with the severity of PTSD symptoms, CSF norepinephrine levels strongly and positively correlated with PTSD symptoms.

In conclusion, progress in the study psychobiology of dissociation depends on more sophisticated conceptualization and measurement of dissociation. Excessive glutamate could be associated with negative dissociative symptoms, whereas excessive norepinephrine may provoke positive dissociative symptoms in individuals who have been traumatized. Traumatized individuals also display ACTH hypersecretion in response to even mild stressors — probably induced by hypersecretions of CRF. Assuming that the subjects studied by Heim *et al.* (2000) had increased numbers of glucocorticoid receptors, the hippocampus would be exposed to levels of cortisol that exceed normal values. The role of cortisol in dissociation requires further study. Some studies have found low-to-normal cortisol levels in PTSD, perhaps indicating negative feedback inhibition. However, abused children with PTSD and dissociative symptoms may have increased levels of cortisol. The inconsistent findings with respect to cortisol perhaps indicate that the functioning of patients with dissociative conditions depends on the dissociative system that dominates at a given time. Consistent with this hypothesis, Mason *et al.* (2001) found an inverse relationship between urinary cortisol levels and a symptom complex composed of two closely related clinical subgroupings, i.e., 'disengagement' (emotional numbing; relatively lower cortisol levels) and 'engagement' in trauma memories (relatively higher cortisol levels). Anisman *et al.* (2001) found that one month after an ice storm, salivary cortisol levels were elevated among moderately affected victims. However, they were diminished among those with the highest Impact of Event Scale scores. It seems possible that the more seriously affected victims remained in a numbed state when tested. The hypothesis that the psychobiological functioning of patients with dissociative conditions depends on dissociative systems will be discussed in detail below.

KEY BRAIN STRUCTURES IN INTEGRATIVE FUNCTIONS

The integrative failure that is characteristic of traumatized individuals may relate to structural and functional brain changes. For example, some data suggest temporal lobe abnormalities in dissociation. EEG recordings of dissociative patients usually involved temporal and frontal slow wave activity (Putnam, 1997). Studies of psychiatric patients reporting childhood abuse (Teicher *et al.*, 1993), as well as traumatized children (Ito *et al.*, 1993) have also documented EEG abnormalities. Using SPECT tomography, Yazici and Kostakoglu (1998) found regional cerebral blood flow changes in patients with somatoform dissociative disorder involving astasia–abasia, i.e., bizarre gait and the inability to stand unaided. Four of the five patients had left temporal, and one patient had left parietal perfusion decreases.

A range of temporal lobe structures have been implicated in dissociation, including the amygdala, hippocampus, and thalamus. Cortical regions involve the orbitofrontal and medial prefrontal cortex, and the sensory association areas.

Amygdala

The amygdala plays a critical role in responding to unconditioned threat stimuli, in the acquisition and expression of classical conditioning effects (Davies, 2000; Dolan, 2000), and in encoding and retrieval of the evaluative tone of episodic memories (Dolan, 2000). For example, LeDoux (2000) stressed that inactivation of the amygdala during learning prevents classical fear conditioning from taking place, and inactivation immediately after training precludes effects on memory. These data suggest that the amygdala is essential for fear learning.

There is evidence for involvement of the amygdala in PTSD. Electrical stimulation of the amygdala and hippocampus — as components of distributed neural memory networks — may automatically retrieve memories in a rather inflexible way, including sensorimotor re-experiencing of frightening experiences (Halgren *et al.*, 1978; Penfield and Perot, 1963). Relative inflexibility and involuntary retrieval are prominent features of traumatic memories (Van der Kolk and Van der Hart, 1991). The responsiveness of the amygdala to threat cues does not depend on conscious awareness of threat: Morris *et al.* (1999) found that the amygdala is responsive to masked angry faces, and Rauch *et al.* (2000) reported functional MRI-evidence for exaggerated amygdala response to masked facial stimuli in PTSD.

Hippocampus

The hippocampus supports the integration and modulation of modality-specific information (e.g., of the sensory, motor, and visual cortices). This processing is required for the development of coherent experiences and memories (Squire and Knowlton, 1995). The hippocampus also serves to evaluate context, which is important in the context-dependent modulation of acquired associations between unconditioned and conditioned (threat) stimuli. Thus, the amygdala stores (LeDoux, 1996) and modulates (Cahill, 2000; McGaugh *et al.*, 2000) the mostly permanent CS-UCS associations, but information processing involving the hippocampus is required to evaluate whether the CS-UCS association applies in the current context. If the CS does not signal the probable occurrence of the UCS in the present circumstances, the hippocampus should inhibit (with the medial prefrontal and orbitofrontal cortex) amygdala-based responses to CS.

Stress hormones interfere with functioning of the hippocampus, and may even damage it: animal studies have shown that direct glucocorticoid exposure results in a loss of pyramidal neurons and dendritic branching in the hippocampus (Woolley *et al.*, 1990). This suggests that the organic structure of the hippocampus is affected by cortisol, probably resulting in a decrease of neuronal synapses that would facilitate integration. Glucocorticoids might have similar effects on the human hippocampus. Compared with healthy controls, patients with combat-related PTSD (Bremner *et al.*, 1995), adults reporting childhood physical and sexual abuse with PTSD (Bremner *et al.*, 1997c), as well as a patient with DID (Tsai *et al.*, 1999) exhibited smaller hippocampal volume.

Whereas trauma-reporting female patients with borderline personality disorder had smaller hippocampal volumes than control groups, levels of neuropsychological functioning were associated with severity of depression but not with hippocampal volume (Driessen *et al.*, 2000). Stein *et al.* (1997) found smaller left-sided hippocampal volume for women reporting childhood sexual abuse compared to controls, with volume correlating strongly ($r = -0.73$) with dissociative symptom severity, but not with indices of explicit memory functioning. It thus seems that the relationship between hippocampal volume and memory functioning may be mediated by dissociation. Whether smaller hippocampi constitute a premorbid risk factor for PTSD and possibly for DSM-IV and ICD-10 dissociative disorders, or whether they are caused by chronic stress exposure, is a question for further study. Recently, Bonne *et al.* (2001) reported that smaller hippocampal volume was not a necessary risk factor for developing PTSD and did not occur within 6 months of expressing the disorder. A controlled study of hippocampal volume in patients with DID is underway (Ehling *et al.*, 2001).

Medial Prefrontal Cortex

Stress hormones also interfere with the activation of the medial prefrontal cortex. For example, elevated levels of norepinephrine were associated with dysfunction of the prefrontal cortex (Arnsten, 1999). This interference presents a major problem of affect regulation in that hippocampal (McCormick and Thompson, 1982) and medial prefrontal (Armony and LeDoux, 1997) information processing are crucially involved in inhibiting the amygdala. As discussed above, yohimbine-provoked traumatic memories and reduced hippocampal and medial prefrontal blood flow in PTSD.

Bremner *et al.* (2000b) found decreased benzodiazepine receptor binding in the medial prefrontal cortex and Bremner *et al.* (1999) documented medial prefrontal and anterior cingulate dysfunction in women with and without PTSD who reported childhood sexual abuse (CSA). The participants were exposed to neutral personal memories and to descriptions of personalized CSA events. CSA scripts were associated with greater increases in regional cerebral blood flow in portions of the prefrontal cortex, posterior cingulate, and motor cortex in women with PTSD than in those women without PTSD. These scripts also induced alterations in brain metabolism in the medial prefrontal cortex, i.e., decreased blood flow in subcallosal gyrus and the anterior cingulate. Compared with women who had not developed PTSD, those with PTSD also had decreased blood flow in the right hippocampus, fusiform/inferior temporal gyrus, supramarginal gyrus, and visual association cortex.

Using SPECT, Sar *et al.* (2001) assessed regional cerebral blood flow in 15 patients with DID without structural lesions and epilepsy, and in eight healthy controls. The blood flow ratio was decreased in orbitofrontal region bilaterally and increased in the left lateral temporal region among patients with DID when compared to the control group. Comorbid diagnoses or ongoing drug treatment did not have any significant effect on perfusion in these regions, and there was no statistically meaningful difference in regional cerebral blood flow ratios among dissociative parts of the personality, also described as dissociative personalities. Sar *et al.* (2001) concluded that the persistency of the findings over different dissociative personalities suggests a trait measure for DID. As we will discuss below, until specific *types* of dissociative personalities are compared, this conclusion might be premature.

In conclusion, dysfunction in the hippocampus and the prefrontal cortex may be related to memory deficits, affect dysregulation, impaired inhibition of conditioned responses, and dissociative symptoms in PTSD and possibly in more complex dissociative disorders as well.

Thalamus

Krystal *et al.* (1998) argued that dissociative-like phenomena in healthy individuals tend to ensue when they are exposed to extremely low or high levels of sensory stimulation. This led them to propose that the thalamus might contribute to dissociation-like alterations in consciousness in that it serves as a sensory gate or filter that directly and indirectly modulates the access of

sensory information to the cortex, amygdala and hippocampus. In relay mode, the thalamus facilitates accurate transmission of sensory information to the cortex, amygdala, and hippocampus. When slow oscillatory firing patterns predominate, the flow of sensory information to amygdala, cingulate and frontal cortex is impeded, leaving the individual focused on internally generated thought processes and affective and sensory experiences. While Krystal *et al.* (1998) hold that this mode might be associated with dissociation, they have not detailed in what modes the thalamus would operate during detachment from and overengagement in trauma memories.

Cingulate Gyrus

The cingulate gyrus is responsive to shifts in thalamic sensory processing functions and is an important point of convergence for a network involving amygdala, prefrontal cortex, and mediodorsal thalamic nucleus (cf., Krystal *et al.*, 1998). The cingulate may be involved in dissociation. For example, Shin *et al.* (2000) reported that Vietnam veterans with PTSD failed to activate the affective division of the anterior cingulate during the Emotional Counting Stroop, while non-PTSD veterans did not (Whalen *et al.*, 1998). Maltreated children with PTSD also manifested metabolic abnormalities in the anterior cingulate (De Bellis *et al.*, 2000). Women with histories of childhood sexual abuse had increased anterior cingulate activity in response to trauma scripts, but those with PTSD had less regional cerebral blood flow increases in this region than those without PTSD (Shin *et al.*, 1999).

Motor Cortex

Marshall *et al.* (1997) reported that attempts of a patient with dissociative paralysis (somatoform dissociation) to move her affected leg failed to activate right primary motor cortex. Instead, the right orbitofrontal and the right anterior cingulate cortex were significantly activated. The authors suggested that these areas may inhibit willed effects on the right primary motor cortex. Preparing to move the paralysed leg produced normal brain activation. Findings by Spence *et al.* (2000) also suggest that somatoform dissociative motor symptoms involve intact ability to generate motor plans, but inhibition of actual motor action. All patients had deactivation of the left dorsolateral prefrontal cortex, regardless of the side of the symptoms. This frontal region is specifically activated by willed action. Feigning controls did not exhibit this deactivation but had hypofunction of the right anterior prefrontal cortex compared with controls. However, studying stress-related dissociative unilateral sensorimotor loss in seven patients using SPECT, Vuilleumier *et al.* (2001) found that these symptoms may represent lack of motor readiness. Compared with a resting condition, passive vibration of both hands-known to activate sensory and motor brain regions — generated bilateral and symmetrical regional cerebral blood flow increases in parietosomatosensory, frontal premotor, and anterior prefrontal cortical areas. Blood flow was reduced in the thalamus, putamen, and caudate contralateral to the side of the symptoms These hemispheric asymmetries disappeared in the four scanned patients who recovered. Vuilleumier *et al.* (2001) suggested that the decreased blood flow in circuits involving the basal ganglia and thalamus may reflect impaired motor readiness through inhibitory input from the amygdala and orbitofrontal cortex in response to emotional stressors.

Somatosensory Association Areas:

Depersonalization is related to several negative somatoform dissociative symptoms, e.g., experiencing the body as a foreign object. Comparing eight patients with depersonalization disorder and 24 healthy controls, Simeon *et al.* (2000) reported that the disorder was associated with functional abnormalities along sequential hierarchical areas, secondary and cross-modal areas of the sensory cortex (visual, auditory, and somatosensory), as well as areas responsible for an integrated body schema. More specifically, they found less metabolism in right temporal cortex (auditory association area), and more metabolism in parietal somatosensory association area and multimodal association area. Dissociation and depersonalization scores among the patients with depersonalization disorder were strongly correlated with metabolic activity in the posterior parietal association area. It thus seems that integrative failure with respect to bodily cues — which may be at the heart of basic forms of consciousness (Damasio, 1999) — is related to dysfunctioning of the temporal, parietal, and occipital association areas. Indeed, '[t]here is a hierarchy of sensory processing in the brain, from primary sensory areas to unimodal and then polymodal association areas and finally to the prefrontal cortex' (Simeon *et al.*, 2000, p. 1786). Depersonalization and negative somatoform dissociative symptoms may thus relate to dysfunction of the posterior association areas, that affects the input into the prefrontal cortex.

Insula:

Reiman *et al.* (2000, p. 399) observed that 'a region in the vicinity of anterior insular cortex [. . .] appears to be preferentially involved in the emotional response to potentially distressing cognitive stimuli, interoceptive sensory stimuli, and body sensations'. For example, regional cerebral blood flow increases have been observed in the anterior insular region during recall-generated sadness (Lane *et al.*, 1997), normal anticipatory anxiety, (Reiman, 1996), lactate-induced panic (Reiman *et al.*, 1998), and the perception of temperature and pain (Bushnell *et al.*, 1995). Based on these and other studies, Reiman *et al.* (2000) suggested that the insula participates in the evaluation of potentially distressing thoughts and body sensations with negative emotional significance, and may serve as an internal alarm centre, 'alerting the individual about potential dangers inside the body' (p. 399). The hypothesized alarm function could perhaps be executed in conjunction with the amygdala in that the insular cortex has afferent and efferent connections with the amygdala (Aggleton and Saunders, 2000).

Because traumatic memories essentially involve unintegrated emotional and somatosensory reactivity, re-experiences of trauma could be associated with altered metabolism in the insula. Indeed, exposing PTSD patients to recorded trauma memory scripts was associated with increased metabolism in the right insula (Rauch *et al.*, 1996). Other areas involved in processing these trauma scripts included a range of other right-sided limbic and paralimbic structures.

NEUROBIOLOGICAL MODELS OF DISSOCIATIVE DISORDERS

DID and Epilepsy

It has been suggested that DID actually represents epilepsy (e.g., Mesulam, 1981; Schenk and Bear, 1981). However, video EEG monitoring of DID patients previously diagnosed as cases of epilepsy documented EEG abnormalities, but not epileptiform EEG activity (Devinsky *et al.*, 1989). In addition, compared to patients with epilepsy, DID patients and patients with pseudo-epileptic seizures had more symptoms of psychoform and somatoform dissociation (Bowman and Coons, 2000; Devinsky *et al.*, 1989; Kuyk *et al.*, 1999; Loewenstein and Putnam, 1988). In some cases, epilepsy and DID may be concurrent disorders. In other cases, apparent epilepsy may represent dissociative convulsions

(WHO, 1992) reflecting a more complex dissociative disorder (e.g., Bowman, 1993; Kuyk *et al.*, 1996, 1999). Moreover, DID responds to psychotherapy, but not to the pharmacological treatment for epilepsy. The evidence thus reveals that DID does not represent epilepsy.

Dissociative Disorders and Cerebral Hemispheric Laterality

The hypothesis that dissociative personalities involve left- or right-sided hemispheric dominance (Tiihonen *et al.*, 1995) is difficult to reconcile with the fact that most DID patients encompass more than two dissociative personalities, and that these personalities display bilateral perfusion defects (Sar *et al.*, 2001). It has been suggested that dissociative disorders of movement and sensation involve symptom lateralization, and perhaps hemispheric lateralization (e.g., Marshall *et al.*, 1997; Tiihonen *et al.*, 1995). However, the results from different studies were contradictory, and a recent study of 114 cases found no evidence for lateralization (Roelofs *et al.*, 2000).

Dissociative Personalities as Discrete Behavioural States

While particular neuroendocrine substances and brain structures may be involved in trauma-related integrative failure, they do not explain why dissociation of the personality is not a random phenomenon, but tends to be orderly within limits. To account for this, Putnam (1997) has proposed that complex dissociative disorders involve discrete behavioural states. He argued that the first years of life are important in laying the groundwork of personality organization that is rather cohesive across contexts, such as place, time, and state. Infants tend to automatically move from one discrete state to another, initially lacking much integration. These states are referred to as discrete behavioural states because emotional states in young children are closely tied to behaviour, producing a highly state-dependent sense of self (Wolf, 1990; Wolff, 1987).

The relatively low integrative capacity of young children can be related to the immaturity of the brain regions that have major integrative functions, notably the prefrontal cortices and the hippocampus. Hippocampal maturity may not occur for up to three years (Seress, 1998), and full maturation of the orbitofrontal and prefrontal cortex requires many years (Benes, 1998). Furthermore, myelination in the hippocampus increases from childhood until adolescence, after which the pattern remains unchanged (Arnold and Trojanowski, 1996). As a result, emotional states involving the amygdala operate in relatively uncoordinated and unintegrated ways in early childhood, even under normal conditions.

The integrative capacity of children is also limited because of a relative absence of experience-derived templates that are helpful as 'attractors' (Siegel, 1999) to integrate new and/or emotionally charged experiences. Thus (young) children seem to be dependent on their social environment for regulation of instinctual emotional systems. In support of this hypothesis, Ogawa *et al.* (1997) found that dissociation in early childhood was a common response to disruption and stress, whereas persistent dissociation in adolescence and young adulthood was indicative of psychopathology.

Although little is known about the development of integration among emotional states, it is in interaction with caretakers that young children begin to acquire skills to sustain, modulate, and integrate states (Schore, 2001; Siegel, 1999). Social sharing of parallel or complementary states between the child and caretaker is associated with synchronizations of physiological processes between the child and the adult that assist the child in regulating states, and lack of synchronization has disruptive effects (Field, 1985). While adequate caretaking activities critically enhance integration of behavioural states in the child, inadequate or inconsistent caretaking will result in the child having difficulty integrating emotional states and constructing a stable sense of self.

Trauma may interfere with this developmental integrative process (Putnam, 1997) by compromising the integrative functions of the developing brain (Gurvits *et al.*, 2000; Teicher *et al.*, 1997; for reviews, see Bremner, 1999; Glaser, 2000; Schore, 2001; Siegel, 1999). Thus, repeated activation of trauma-related states can promote state-dependent functioning and concomitant neurobiological 'hard-wiring' of the brain (Perry, 1999; Perry *et al.*, 1995), depending on the child's life experiences, particularly before age six. While prospective studies should assess to what extent particular brain features are a risk factor for, or sequelae of traumatization and dissociation, the evidence to date suggests that maltreatment in childhood is associated with adverse influences on brain development (De Bellis *et al.*, 1999), and increased sensitivity to the effects of stress in later life (Graham *et al.*, 1999). Thus, adverse social experiences during early critical periods result in permanent alterations in CRF, opiate, corticosteroid, dopamine, noradrenaline, and serotonin receptors (cf. Schore, 2001).

Several studies suggest a potential causal relationship between severe traumatization in early childhood and compromised integrative functions. For example, younger children had more PTSD symptoms than adolescents (Anthony *et al.*, 1999), and the severity of psychoform and somatoform dissociation among DID patients and psychiatric controls were best predicted by reported trauma during the first six years of life (Draijer and Boon, 1993; Nijenhuis *et al.*, 1998b). Also, the age of onset, chronicity, and severity of childhood trauma were associated with psychoform dissociative symptoms up to 19 years later (Ogawa *et al.*, 1997). Recently, Nijenhuis *et al.* (2001a) found that adult psychiatric patients who reported more than five different types of traumatization during their lives had higher levels of PTSD symptoms, and more psychoform and somatoform dissociation than did psychiatric patients who reported fewer than five different types of trauma, only emotional abuse and neglect, or no traumatization at all. In most cases, the reported severe traumatization had commenced in (early) childhood, and severity of somatoform dissociation was associated with composite bodily threat scores, including age at onset, duration, and subjectively rated impact of the traumatization (Nijenhuis *et al.*, 2001a).

Dissociative Personalities in DID

Consistent with Putnam's theory (Putnam, 1997), dissociative systems in DID tend to display psychophysiological differences that are not reproduced by DID-simulating controls. Differences have been reported in electrodermal activity (skin conductance; Ludwig *et al.*, 1972; Larmore *et al.*, 1977), EEG — in particular in the beta 2 band (Coons *et al.*, 1982; Hughes *et al.*, 1990; Ludwig *et al.*, 1972; Putnam *et al.*, 1993), visual evoked potentials (Putnam *et al.*, 1992), regional cerebral blood flow (Mathew *et al.*, 1985; Saxe *et al.*, 1992), autonomic nervous system variables (Putnam *et al.*, 1990), optical variables (Birnbaum and Thomann, 1996; Miller, 1989; Miller and Triggiano, 1991; Miller *et al.*, 1991), and arousal (Putnam *et al.*, 1990).

While these studies are valuable, it is difficult to say what the data actually tell beyond suggesting that these physiological data sets 'are most parsimoniously explained by regarding the alter personalities as discrete states of consciousness' (Putnam, 1997, p. 138). Advances in the field critically depend on theoretical predictions with respect to the *kind* of differences that exist among different *types* of dissociative personalities. The theory of structural dissociation (Nijenhuis *et al.*, 2002) offers such predictions.

THE THEORY OF STRUCTURAL DISSOCIATION

Many individuals with PTSD or more complex dissociative disorders alternate between being fixated on the trauma and being

detached from the experience (Nijenhuis and Van der Hart, 1999a, 1999b). Using metaphors that Myers (1940) proposed, the 'emotional' personality (EP) has encoded and stored trauma, and is dedicated to defense from major threat, in particular, threat to the integrity of the body. The 'apparently normal' personality (ANP), on the other hand, is detached from trauma, experiences some degree of amnesia or is depersonalized from some or many components of the experience, and is dedicated to fulfilling functions in daily life, some of which serve the survival of the species. According to the theory of structural dissociation (Nijenhuis *et al.*, 2002b), this alternating pattern reflects failed integration between the ANP and EP.

Structural dissociation of the personality may involve constellations of one ANP and one EP, as in PTSD; one ANP and more than one EP, as in complex PTSD (Herman, 1992), also known as disorders of extreme stress (Pelcovitz *et al.*, 1997), and more than one ANP and more than one EP, as seen only in DID. According to the theory, the extent to which the personality becomes fragmented depends on the severity of the traumatization in terms of developmental age at trauma onset, chronicity and intensity of the traumatization, and factors such as the relationship to the perpetrator and lack of support and social recognition of the trauma. A limitation of the theory of structural dissociation is that it does not address structural dissociation of the personality unrelated to trauma (for a remarkable case of latent abilities, see Braude, 2000).

ANP and EP are two different psychobiological systems, each endowed with its own sense of self. According to Damasio (1999, p. 26), consciousness and sense of self are essentially grounded in 'a feeling that accompanies the making of any kind of image — visual, auditory, tactile, visceral — within our living systems' and may involve several integrative levels: (1) the *proto-self* that emerges from the activity of brain devices that continuously and nonconsciously maintain the body state within the narrow range and relative stability required for survival; (2) the *core self* that relates to core consciousness defined as conscious awareness of the here and now based on the mental representation of how the organism's own state is affected by the organism's processing of an object; and (3) the *autobiographical self* that involves extended consciousness, i.e., conscious awareness of one's personal existence across subjective time.

The existence of the EP can be limited to re-experiencing traumatic memories, i.e., sensorimotor experiences that hardly involve narrative components, if at all (Nijenhuis *et al.*, 2001b; Van der Kolk and Fisler, 1995; Van der Kolk and Van der Hart, 1991). In this case, the EP may involve little more than core consciousness and core self. Yet clinical observations suggest that with recurrent reactivations of traumatic memories and chronic traumatization (and treatment), the EP may develop a degree of extended consciousness and autobiographical self. Even in these cases, however, extended consciousness and the sense of autobiographical self tend to remain quite limited. EPs are typically fixed in past trauma with absent or only partial awareness of the present or the passage of time. ANPs typically have developed a more substantial degree of extended consciousness, yet lack personification of the traumatic past and the associated EPs. EPs and ANPs have a narrowed field of consciousness focused on issues relevant to the functions they exert. Although Nijenhuis *et al.* (2002b) use the metaphor of dissociative personalities, it should be noted that such systems range from quite simple to highly complex: some dissociative personalities encompass just one psychobiological state, others are complex assemblies of such states. Dissociative personalities involve senses of self that are different from the pretraumatic self. However, because young children encompass psychobiological systems that are still relatively unintegrated, their pretraumatic sense of self is rather inconsistent and changeable, thus sense of self may not be stable even prior to early traumatization.

A major question is what psychobiological systems drive the EP and ANP, including their sense of self? These systems should meet a range of shared criteria: (1) they must be self-organizing and self-stabilizing within windows of homeostasis, time and context to control and integrate all the rather coherent complexes of psychobiological phenomena exhibited by ANP and EP; (2) they should be functional systems that have been developed in the course of evolution, and should be rather analogous to animal biological systems. Clinical observations suggest that the ANP typically engages in tasks of daily life such as reproduction, attachment, care taking, and socialization, and avoidance of traumatic memories that supports focus on daily life issues. In contrast, the EP primarily displays evolutionary defensive and emotional reactions to the (perceived) threat on which they seem to be fixated; (3) they should be very susceptible to classical conditioning, because, as we discuss below, the EP and ANP strongly respond to unconditioned and conditioned threat cues; (4) they should involve stable characteristics, but also allow for case-dependent variation as well, as ANP and EP exhibit both invariant and idiosyncratic variations; and (5) they should be available early in life, since dissociative disorders can manifest from a very early age.

Emotional Operating Systems

The theory of structural dissociation holds that the psychobiological systems that drive EP and ANP are *emotional operating systems*, or briefly, emotional systems, described by Panksepp (1998). Defense, attachment of offspring to parents, parental attachment to and care for offspring, procreation, sociability, energy management, exploration, and play constitute the major emotional systems, and may encompass a range of subsystems. Panksepp argued that basic emotional processes arise from distinct psychobiological systems that reflect coherent integrative processes of the nervous system (cf. Ciompi, 1991). In his view, the affective essence of emotionality is organized on subcortical and precognitive levels, and each of the emotional systems involves specific patterns of activation of neural networks and associated neurochemical activity in the brain.

Emotional systems are psychobiological in nature and closely meet the five criteria of dissociative personalities described above: they are organizational, evolutionary derived, functional, flexible within limits, and inborn but epigenetic. Emotional systems are functional in that they activate various types of affective feelings which help identify events in the world that are either biologically useful or harmful, and generate adaptive responses to many life-challenging circumstances. The basic behavioural patterns involved in emotional systems are approach and avoidance. Although the resulting behaviour is unconditionally summoned by the appropriate cues, approach and avoidance are adaptable to prevailing environmental conditions within limits, rather than being mere inflexible responses. For example, flight involves not just running away from threat, but running that is adapted to the current situation in form, direction, and duration. Thus, threat as an unconditional stimulus does not evoke a single 'unconditional' response, but integrated series of psychobiological responses.

Emotional systems are epigenetic, i.e., the result of influences by nature and nurture. Experiences, especially early ones, can change the fine details of the brain forever. These experiences include learning associations between events. Emotional systems are susceptible to classical conditioning: i.e., learning that some previously neutral events predict or refer to unconditioned stimuli. These conditioned stimuli tend to reactivate a representation of the unconditioned stimuli, and thus the once unconditionally summoned emotional system. Subsystems of defense are of particular interest in dissociation because of exposure to threat. Each defensive

subsystem controls a pattern of psychobiological reactions that is adapted to meet a particular degree of threat imminence (Fanselow and Lester, 1988). This degree of imminence can be expressed in terms of the time and space that separate the subject from the threat (i.e., the distance between predator and prey), as well as in terms of an evaluation of the defensive abilities of the subject (e.g., the subject's psychosocial influence and physical force).

Pre-encounter defense involves an apprehensive state with increased arousal, potentiated startle response, interruption of 'normal life' behaviours, and nearly exclusive attentional focus on the potential threat. *Post-encounter defense* includes several subsystems: flight, freeze, with associated analgesia, and fight. *Post-strike defense* involves total submission and bodily as well as emotional anaesthesia. Upon survival, a recuperative subsystem is activated that allows for a return of affective awareness and body sensations (e.g., pain, fatigue), and that drives wound care and rest through social isolation, as well as sleep. Upon recovery, there will be a reactivation of (sub)systems that control daily life interests such as consumption of food, reproduction and taking care of offspring.

The EP is Dedicated to Survival Under Threat

According to the theory of structural dissociation, EPs are primarily manifestations of the emotional system that controls defense in the face of threat — particularly threat to the integrity of the body by a person — and potentially also of the emotional system that controls separation panic in relation to caretakers. Both systems serve survival interests and strongly influence the mental and physical experiences and actions of the EP. While EPs essentially rely on evolutionary derived mechanisms, their manifest form will be shaped by environmental conditions, especially traumatic experiences that evoke threat, in particular those that occurred in early childhood, and subsequent external and internal conditions. These conditions include the degree and quality of social support in the aftermath of trauma, repetition of trauma, and the degree of dissociation between EP and ANP.

In cases of *primary structural dissociation*, which would characterize acute stress disorder, simple PTSD, and simple cases of somatoform dissociative disorders (i.e., the ICD-10 dissociative disorders of sensation and movement), a single EP can include all defensive subsystems. *Secondary structural dissociation* is a manifestation of a range of defensive subsystems that have not, or not sufficiently, been integrated among each other. Thus the EP may become fragmented into several EPs that serve different defensive functions. In secondary structural dissociation, some EPs typically display freezing and are analgesic, others are inclined to physically resist threat and experience anger, or totally submit to threat while being severely anaesthetic. This threat often consists of re-experiencing (traumatic) memories of severe and chronic childhood abuse and neglect, or in responding to cues that are salient reminders of these events. Insecure attachment to caretakers can also become associated with one or more EPs in secondary structural dissociation. This level of integrative failure is mediated by traumatization that is more severe than that associated with simple PTSD. Secondary dissociation is characteristic of complex acute stress disorder, complex PTSD, complex cases of somatoform dissociative disorders, many cases of dissociative disorder not otherwise specified (APA, 1994), and perhaps borderline personality disorder (APA, 1994) as well.

We repeat that the literature generally refers only to states of freezing and submission as dissociative (and often these states are not regarded as different from each other), whereas sympathetic hyperarousal states are not (Perry *et al.*, 1995). However, defining dissociation as a lack of integration among emotional systems that may include single or clusters of states implies that hyperarousal states can also be dissociative. But these, as well as states involving analgesia and motor inhibition (freezing), bodily and emotional anaesthesia, disengagement from environmental cues, and submission (total submission, regulated by the parasympathetic nervous system (Porgess *et al.*, 1994; Schore, 1994)) may all be manifestations of unintegrated dissociative subsystems of defense.

The ANP Involves Systems that Manage Daily Life and Promote Survival of the Species

Clinical observations suggest that emotional systems of the ANP primarily function to direct performance of daily tasks necessary to living (work, social interaction, energy control), and of tasks related to survival of the species (reproduction; caretaking of children). Some ANPs may execute daily life emotional systems with passion, while others do so in more or less depersonalized and automatic ways (e.g., caretaking). Depersonalized functioning in caretaking and attachment may interfere with synchronizations of physiological processes between adult and child that assist the child in regulating states (Field, 1985), potentially leading to dissociation in the offspring of dissociative parents (Schore, 2001).

When trauma by caretakers begins early in the life of the child, a particular style of attachment often develops in the child, termed *disorganized/disoriented* (Liotti, 1999; Main and Morgan, 1996). In normal, middle class families about 15% of the infants develop this attachment style, but in cases of maltreatment its prevalence may be up to three times higher (Van IJzendoorn *et al.*, 1999). Thus frightened or frightening parental behaviour predicted infant disorganized attachment (Schuengel *et al.*, 1999). Prospective longitudinal research has demonstrated that disorganized and avoidant attachment in early childhood, along with age of onset, chronicity, and severity of abuse, predicted dissociation in various developmental stages, up to late adolescence (Ogawa *et al.*, 1997). Both ANP and EP may be insecurely attached to original abusive caretakers or to (positive or negative) substitute caretakers.

Disorganized attachment may neither be disorganized nor disoriented. Instead, it involves concurrent or rapid successive activation of the attachment system and the defense system when primary attachment figures are both the source of protection from threat and the threat itself for the traumatized child. Separation from attachment figures activates the innate attachment system, which evokes mental and behavioural approach to the caregiver. However, approach yields an increasing degree of imminence of threat, and therefore evokes a succession of defensive subsystems (flight, freeze, fight, submission). This approach and avoidance conflict cannot be resolved by the child and promotes a structural dissociation of the attachment and the defensive system.

In addition to secondary structural dissociation (fragmentation of the defensive system, thus of the EP), fragmentation of the ANP may also occur. Thus, this *tertiary structural dissociation* (Nijenhuis and Van der Hart, 1999b; Nijenhuis *et al.*, 2002; Van der Hart *et al.*, 1998), characteristic only of DID, involves fragmentation among two or more emotional operating systems that serve functions in daily life and in survival of the species. For example, one ANP regarded herself as the mother of her children, and another ANP engaged in a job. Remaining as the mother, the patient did not appreciate or understand the interests that she had as a worker, and vice versa. Tertiary structural dissociation does not occur during trauma, but rather emerges when certain inescapable aspects of daily life become associated with past trauma, such that systems of daily life become dissociated. Apart from extreme generalization of stimuli that reactivate traumatic memories, tertiary dissociation can also result from traumatization that started before the individual had been able to create a cohesive personality. Early and chronic traumatization may lead to some

unclear mix of ANP/EP, where neither can be clearly distinguished. Such complexes are clinically observed in more dysfunctional DID patients.

SIMILARITIES BETWEEN THE HUMAN AND ANIMAL DEFENSIVE SYSTEM

At a general level, Nijenhuis *et al.* (1998c) drew a parallel between animal defensive systems/recuperative systems and characteristic somatoform dissociative responses of trauma-reporting patients with dissociative disorders. Their review suggested that there are similarities between animal and human disturbances of normal eating patterns and other normal behavioural patterns in the face of diffuse threat; freezing and stilling when serious threat materializes; analgesia and anaesthesia when strike is about to occur; and acute pain when threat has subsided and recuperation is at stake.

Nijenhuis *et al.* (1998a) performed a first empirical test of the hypothesized similarity between animal defensive reactions and certain somatoform dissociative symptoms of dissociative disorder patients who reported trauma. All 12 somatoform dissociative symptom clusters tested were found to discriminate between patients with dissociative disorders and patients with other psychiatric diagnoses. Those clusters expressive of the hypothesized similarity between animal and human models — freezing, anaesthesia–analgesia, and disturbed eating — belonged to the five most characteristic symptom clusters of dissociative disorder patients. Anaesthesia–analgesia, urogenital pain and freezing symptom clusters independently contributed to predicted caseness of dissociative disorder. Using an independent sample, it appeared that anaesthesia–analgesia best predicted caseness after controlling for symptom severity. The indicated symptom clusters correctly classified 94% of cases that constituted the original sample, and 96% of an independent second sample. These results were largely consistent with the hypothesized similarity to animal defense systems.

Among Dutch and Flemish dissociative disorders patients, the severity of somatoform dissociation — as measured by the Somatoform Dissociation Questionnaire (SDQ-20; Nijenhuis *et al.*, 1996, 1999b) — was best predicted by threat to the integrity of the body in the form of childhood physical abuse and childhood sexual trauma (Nijenhuis *et al.*, 1998b). The particularly strong association between the SDQ-20 — which includes many items that assess anaesthesia, analgesia, and motor inhibitions — and physical abuse has also been found in a range of other populations: nonclinical subjects (Waller *et al.*, 2000), gynaecology patients with chronic pelvic pain (Nijenhuis *et al.*, 1999c), women reporting childhood sexual abuse (Nijenhuis *et al.*, 2001b), psychiatric outpatients (Nijenhuis *et al.*, 2001a), as well as North American (Dell, 1997) and Ugandan patients with dissociative disorders (Van Duyl and Nijenhuis, in preparation).

According to the theory of structural dissociation, EPs involve defensive (sub)systems. We will discuss below recent experimental research suggesting that (1) animal defense-like reactions particularly characterize the EP, and that (2) EPs and ANPs have different psychophysiological stress responses to threat-related stimuli, even if these stimuli are presented preconsciously. Future research will need to decipher whether various EP-subtypes have the hypothesized features of animal defensive subsystems.

PSYCHOBIOLOGICAL INTERFERENCE WITH INTEGRATION OF ANP AND EP

Peritraumatic Integrative Failure

Evocation of the defense system or any other psychobiological system is not dissociative in itself, rather the lack of integration between various systems and subsystems is what constitutes dissociation. As discussed above, extremely high levels of arousal may interfere with the execution of normal integrative processes (Ludwig, 1972; Krystal *et al.*, 1991; see also Siegel, 1999), and integrative functions may be compromised by long-lasting neuroendocrine instability induced by severe stress in early childhood. It is likely that some emotional systems can be integrated more readily than others. As Panksepp (1998) argued, multiple feedbacks within and across emotional systems promote synthesis of components of a system (e.g., perceptions, behaviours, sense of self) and integration across emotional systems. However, integration across emotional systems that involve quite different and sometimes conflicting functions may be far more demanding than synthesizing components of a particular emotional system or integrating functionally related systems. If this is correct, the integration of systems dedicated to daily life and survival of the species (ANP), and systems dedicated to survival of the individual in the face of that threat (EP) will fail more readily than integration across subsystems of these two complex systems. Structural dissociation between the ANP and the EP will thus be the basic type of integrative failure, i.e., primary structural dissociation, when overwhelming trauma occurs. When stress levels rise, integration of subsystems of defense may be compromised as well, yielding secondary dissociation, i.e., fragmentation of the EP.

Post-traumatic Integrative Failure

Since living organisms have a natural tendency toward integration (Siegel, 1999), what maintains dissociation when trauma has ceased and stress-induced monoaminergic reactivity has returned to baseline? According to the theory of structural dissociation, apart from integrative deficiency that relates to enduring neuroendocrine changes induced by stress in early life, integrative failure in the aftermath of trauma also involves fear conditioning.

Trauma and Classical Conditioning

Trauma-related classical conditioning involves association of stimuli that saliently signalled or accompanied the overwhelming event. As a result, these previously neutral cues will thereafter reactivate a representation of the traumatic experience. Thus the essence of classical conditioning is the development of an anticipatory (CS signals UCS) or referential response (CS refers to UCS). For example, the specific mood (e.g., anger) of the caretaker when abusive, as well as the stimuli that apparently tended to elicit this mood, will tend to become conditioned stimuli.

Phobias of Traumatic Memories and Dissociative Personalities

Classical trauma conditioning can also generate effects that support continued structural dissociation (Nijenhuis *et al.*, 2002). First of all, structural dissociation is less than perfect. When the EP's traumatic memories are reactivated by potent external (e.g., certain smells, sounds, sights) or internal (e.g., feelings or body sensations) CS, they can intrude into the experiential domain of the ANP. Since traumatic memories represent the trauma, they are formally CS. But the sensorimotor and highly affectively charged properties of these unintegrated experiences are inherently aversive for the ANP and *will therefore act as UCS.* Indeed, when traumatized patients re-experience their traumas, it is as if the trauma happens 'here and now'. When the integrative capacity of the ANP does not suffice for integration of the intruding traumatic memory, the ANP will respond to intrusions (UCS) with typical behavioural and mental defensive reactions (UCR; Nijenhuis and Van der Hart, 1999b). The ANP cannot escape from the highly stressful intrusions by behavioural means, but mental escape can be effective, as applies to factual (inescapable) trauma. Thus, typical reactions of the ANP

include retracting the field of consciousness, lowering the level of consciousness (with pseudo-epileptic loss of consciousness as an extreme), and (re)dissociating the EP and the traumatic memories. At the same time, the ANP learns to fear and avoid internal and external CS that signal or refer to the EP. As time progresses and the dissociative condition continues, there is an ever widening range of CS that the ANP will avoid due to stimulus generalization.

Evaluative Conditioning

In addition to classical fear conditioning, evaluative conditioning (Baeyens *et al.*, 1993) of external and internal stimuli may occur. This type of associative learning produces robust effects and involves the presentation of two conjointly stimuli: a neutral stimulus and a stimulus that the individual evaluates in a negative (or positive) manner. As a result of this simple procedure, the previously neutral stimulus adopts a similar negative (or positive) tone. The ANP and EP evaluate traumatic memories differently (Nijenhuis *et al.*, 1999a), and clinical observations strongly suggest that evaluative conditioning applies to trauma-related dissociation. For example, when the trauma involved a shameful event, the ANP may learn to be ashamed of the EP, and to despise it.

In cases of secondary and tertiary dissociation, EPs and ANPs may learn to fear, reject, and avoid each other along similar pathways of evaluative and classical conditioning. In tertiary structural dissociation, avoidance of different ANPs may be based on similar trauma-related issues and conflicts. In summary, many dissociative personalities become phobic of each other. These conditioned effects interfere with normal integrative tendencies. Hence, *structural dissociation involves a strong tendency toward chronicity*.

In some individuals, alternations between the ANP and EP manifests from the acute phase onward, but other individuals function apparently well for extended periods of time before displaying post-traumatic stress symptoms. However, upon close scrutiny it often appears that the latency period was marked by avoidance of the trauma and associated internal and external cues, yielding a condition of chronic depersonalization. In cases of trauma-related dissociative amnesia as a disorder (APA, 1994), access to the memory of the trauma and to other parts of one's previous non-traumatic life seem to be inhibited (Markowitsch *et al.*, 2000; Van der Hart and Brom, 2000; Van der Hart and Nijenhuis, 2001; Van der Hart *et al.*, 1999).

Relational Factors that Maintain Structural Dissociation

When significant others deny trauma instead of assisting in the integration of the painful experience, or prohibit talking about it, dissociative tendencies are enhanced. These adverse social influences prevail in intrafamilial childhood sexual abuse (Freyd, 1996), and seem to promote dissociative amnesia (Vanderlinden *et al.*, 1993). PTSD has been associated with lack of support in the aftermath of trauma (King *et al.*, 1998), and in another study, patients with complex dissociative disorders reported total absence of support and consolation when abused (Nijenhuis *et al.*, 1998b). As the structural dissociation theory predicts, social support can buffer negative effects of trauma exposure (Elklit, 1997; Runtz and Schallow, 1997). It might be that social support provides safety cues, assists the individual in modulating the affective state and biological stress levels, and thus promotes the integration of the EP and ANP.

PSYCHOBIOLOGICAL RESEARCH OF ANP AND EP

To summarize the essence of the structural dissociation theory, it proposes that (1) traumatic experiences — especially trauma that occurs early in life and involves severe threat to the integrity to bodily integrity — activate evolutionary derived defensive systems, (2) these emotional operating systems may remain unintegrated to varying degrees due to extreme stress levels, classical and evaluative conditioning to traumatic memories, approach–avoidance conflicts with regard to defense and attachment, and lack of social support. ANPs involve emotional operating systems dedicated to survival of the species and normal life, and EPs systems dedicated to survival of the threatened individual. From this theory, a range of hypotheses can be derived, some of which have been tested in pioneering studies.

Differences Between ANP and EP on Subliminal Threat Exposure

Preconscious information processing plays a key role in responding to unconditioned and conditioned threat cues and in fear-related learning (Davies, 2000; Dolan, 2000; LeDoux, 1996; Morris *et al.*, 1998). The theory of structural dissociation considers that ANPs aim to avoid this threat, and that EPs will selectively attend to these cues. Thus in an original study, Van Honk *et al.* (1999, 2001) hypothesized that dissociative personality-dependent reactivity to (un)conditioned threat will be evident following exposure to cues that are presented very briefly in order to preclude consciously aware perception. More specifically, the effects of exposing the ANP and EP in DID patients to masked neutral, fearful, and angry facial expressions were tested.

Whereas ANPs named the colour of the mask that immediately followed the experimental stimuli more quickly when this stimulus involved angry facial expressions compared to exposure to neutral facial expressions, EPs did not show differential responses to these cues. DID-simulating controls showed the reverse pattern: a tendency toward longer response latencies after exposure to angry faces when enacting ANPs, and a tendency toward shorter reaction times after exposure to angry faces when enacting EPs. The interaction 'group (genuine DID vs DID-simulators) $\times$ condition (angry vs neutral faces)' was statistically significant. Because this effect was absent when comparing fearful and neutral faces, it was specific for cues that signal an increased possibility of attack. The reaction time effects were associated with a decrease of the pre-ejection period (PEP; the interval between ventricular depolarization and ventricular ejection onset) for the EP when exposed to angry faces, indicating increased sympathetic nervous system activity in this condition.

The results are consistent with the hypothesis that ANPs avoid subliminal threat cues by means of gaze aversion, and that EPs particularly attend to bodily threat from a person, with increased sympathetic tone. The response of the EP is pathological in that angry faces represent social threat that can be reduced by gaze aversion: a social cue that signals submission to a dominant individual. On the other hand, the results of the study are at odds with the theory that DID involves effects of suggestion and role-playing.

If ANPs can preconsciously avoid externally presented (un)conditioned threat cues, it is reasonable to assume that they can also preconsciously avoid *internal* (un)conditioned threatening stimuli. Hence, it seems possible that the ANP preconsciously avoids the EP and its memories, as the theory of structural dissociation holds. Some neurobiological data are consistent with the interpretation that dissociative amnesia involves inhibited access to episodic memory. Markowitsch and his colleagues have demonstrated that trauma-related dissociative amnesia as a disorder (APA, 1994) can be associated with reduced blood flow in parts of the brain that are normally activated during retrieval of autobiographical memories (Markowitsch, 1999; Markowitsch *et al.*, 1997a, 1997b, 1998, 2000). Moreover, partial regaining of these memories was correlated with a return to normal brain metabolism in

these areas (Markowitsch *et al.*, 2000). The mechanisms of this presumed inhibitory process could be studied using functional MRI analysis.

Symptom Provocation by Exposure to Neutral and Trauma Memory Scripts in DID

In several studies, imagery of personal trauma, audiotaped descriptions of traumatic experiences, and videotaped traumatic scenes have been used to provoke post-traumatic stress reactions in PTSD patients. Compared to controls, many PTSD patients have increased psychophysiological responses (heart rate, blood pressure, skin conductance, EMG) to these types of trauma-related cues (Keane *et al.*, 1998; Orr *et al.*, 1993, 1998; Pitman *et al.*, 1999), and more severe pathology was associated with a higher magnitude of psychophysiological reactions to trauma cues (Orr *et al.*, 1998). However, using psychophysiological responses to trauma-related cues as a classificatory criterium of PTSD yielded a substantial number of false positives and false negatives (Keane *et al.*, 1998), i.e., the positive and negative predictive values of these factors were limited. It seems possible that the 'physiological nonresponders' to trauma cues remained as ANP, whereas 'physiological responders' failed to avoid cued intrusion by the EP, or switched into the EP. In this regard, it is interesting that physiological nonresponders with PTSD manifested less re-experiencing symptoms (Keane *et al.*, 1998), perhaps indicating ANP dominance.

In the first study of its kind, Nijenhuis *et al.* (1999a) assessed several sensorimotor, affective, and psychophysiological reactions in 11 women with DID, assessed by the SCID-D (Steinberg, 1994). Regional cerebral blood flow was evaluated with PET (Reinders *et al.*, in preparation). ANPs and EPs were subjected to audiotaped descriptions of neutral personal memories and traumatic personal memories. In support of the hypotheses, it was found that in response to the trauma scripts, EPs, but not ANPs displayed decreases of heart rate variability as measured by time domain variability. Stress induces reductions in heart rate variability. In response to the trauma scripts, EPs but not ANPs, also showed statistically significant increases of heart rate frequency and systolic blood pressure. Neither personality types had differential psychophysiological responses to the neutral memories. Whereas EPs experienced a wide range of affective and sensorimotor reactions to the trauma scripts, ANPs did not have these reactions, or only to a minor degree. The ANP's lack of psychophysiological reactivity to supraliminal threat (trauma scripts) could reveal inhibition of responding to threat cues by means of mental avoidance.

As hypothesized, there were statistically significant differences in regional cerebral blood flow between EPs and ANPs when they were exposed to trauma memory scripts, but not when they were exposed to neutral memory scripts (Reinders *et al.*, in preparation). There were also differences in brain metabolism for exposure of EPs to trauma memory scripts and neutral memory scripts. The observed neurobiological differences could not be explained as a result of the variability in subjective and psychophysiological reactivity, and thus reflected effects of the experimental conditions, i.e., types of memory scripts and types of dissociative personality. The PET scan results — described in more detail in Reinders *et al.* (in preparation) — converged with the subjective and psychophysiological data. Jointly, the findings were supportive of the hypothesis that EPs and ANPs have different patterns of psychobiological reactivity to traumatic memories, and that the psychobiological reactions of EPs to trauma memory scripts differ from their reactions to neutral memory scripts: EPs responded to trauma memory scripts in somatosensory and emotionally charged ways, whereas ANPs responded to these cues with a psychobiological pattern that reflects depersonalization and numbing.

POSITIVE FEEDFORWARD EXCITATION (EP) AND NEGATIVE FEEDFORWARD INHIBITION (ANP)?

As detailed above, the ANP tended to avoid masked angry faces, whereas the subliminal perception of angry faces interfered with performance for the EP (Van Honk *et al.*, 1999, 2001). This may imply that the ANP and EP involve different degrees or kinds of amygdala activity when exposed to external threat cues. One EP inadvertently recognized the angry faces, became very anxious, fixed her gaze on the screen, froze, and could not name the colour of the mask for several minutes. She finally mentioned the wrong colour. Remaining as ANP, the patient did not recognize the angry faces, however. (Note: the scores of this patient were excluded from the calculations.) The EP's fixation on threat cues is also suggested by the increased heart rate frequency and blood pressure, as well as the reduced heart rate variability of EPs in response to trauma memory scripts (Nijenhuis *et al.*, 1999a). Moreover, EPs but not ANPs, reported analgesia and strong negative evaluative reactions in response to the trauma scripts. A major riddle to solve regarding dissociation is how DID patients can display the EP-dependent and ANP-dependent psychobiological activity in response to identical threat stimuli (i.e., angry faces and trauma scripts).

Before presenting some thoughts on the issue, we remark that our experimental DID research was limited to women, which may have affected the results. Pre-clinical trials have suggested large gender differences with respect to responsivity to identical stressors, e.g., c-fos expression in the prefrontal cortex and other brain regions (Trentani *et al.*, 2002). Hence, the degree of activation of various brain structures may be dependent on gender. Next, there is evidence for differential responding of the left and right amygdala to stressors (Cahill *et al.*, 2001). For the time being, we disregarded this complexity, but remark that future research should control for gender.

EP-Dependent Responsivity to Threat

Exposure to major external threat causes rapid activation of the defensive system by the amygdala and related structures. This is mandatory for survival, as is learning and memorizing by means of classical conditioning that which signals or refers to threat. The range of threat-related responses orchestrated by the amygdala includes activation of the sympathetic nervous system and the HPA-axis, defensive behaviour (through the central grey), startle response, and stress-induced hypoalgesia (Aggleton, 2000). The lateral amygdala receives sensory inputs directly from the sensory thalamus and indirectly from the sensory cortex. The lateral amygdala projects to the central nucleus of the amygdala, which projects to structures controlling defensive behaviour (flight, freeze, fight, submission), autonomic arousal, hypoalgesia, stress hormones, and potentiated startle. Thus, the amygdala will be hyperactivated in the face of threat (UCS), will encode and store CS-UCS propositions (e.g., CS signals UCS), and will modulate trauma memories more generally. When CS (re)appear, the amygdala and other aspects of the emotional brain and the defensive systems tend to become reactivated.

Hyperactivation of the defensive system during traumatic stress may produce hyperamnesia through mediation of the basolateral nucleus of the amygdala. The basolateral amygdala has a major role in stress-mediated neuromodulatory influences on memory storage (Cahill, 2000; McGaugh *et al.*, 2000). Post-event memory consolidation for emotional experiences involves not only the basolateral amygdala, but the stria terminalis as well. This is a major afferent/efferent amygdala pathway, which interacts with peripheral stress hormone feedback locally in the amygdala, and with emotional memory storage elsewhere in the brain (Cahill, 2000).

Reactivated traumatic memories represent internal threat in that these memories are not narratives but somatosensory and

emotionally charged experiences. Findings of Reiman and his colleagues, discussed above, suggest that internal threat cues (including body signals) are associated with activity of the insula. Because the insula have afferent/efferent connections with the amygdala, internal threat cues may activate the amygdala through this path.

Hyperactivation of the amygdala in the EPs exposed to external and internal threat cues (angry faces) may be related to failed inhibition of the amygdala and insula by the hippocampus and the medial prefrontal cortex due to excessive release of stress hormones: uninhibited positive feedforward loops would seem to stabilize the defensive system, and impede the integration of EP and ANP. In this context, reactivation of traumatic memories and the defensive system (the EP) by CS implies sensitization rather than modulation of CS-UCS propositions. Finally, hyperactivation of the defensive system/limbic structures and potent somatosensory activity combined with relatively low levels of prefrontal cortical activity could perhaps explain why in most cases EPs have only developed a quite limited degree of extended consciousness.

Thus it seems that exposure of the EP to (perceived) threat cues (re)activates defensive responses with concomitant lack of contextual information processing, uninhibited conditioned emotional responding within limits of homeostasis, and hampered integration of traumatic memories. Hyperamnesia, sensitization, and maintenance of structural dissociation between ANP and EP can co-occur.

ANP-Dependent Responsivity to Threat

Now consider the ANP. Because ANPs displayed reactivity to masked angry faces (Van Honk *et al.*, 1999, 2001), it seems unlikely that the amygdala and related brain structures were not activated at all when ANPs were exposed to these threat cues. However, in the EP, the emotional brain was strongly activated (Nijenhuis *et al.*, 1999a; Reinders *et al.*, in preparation; Van Honk *et al.*, 1999, 2001). While the EP selectively attends to threat cues, the ANP averts its gaze from threat and selectively attends to cues that matter to daily life functioning. It seems possible that the lateral amygdala is activated for a short time by means of input from the sensory thalamus when the ANP is exposed to threat. However, due to ANP's mental avoidance of threat cues and retraction of the field of consciousness to matters of daily life, the lateral amygdala could become readily subject to a form of negative feedback inhibition. When emotional systems that regulate daily life are in executive control, the amygdala — which has a role in selective attention (Gallagher, 2000) — and related structures may operate in a mode that is different from the mode associated with the defensive system.

Because ANPs are often depersonalized, studies of DID could help understand ANP reactivity to threat in various trauma-related disorders. Depersonalization is related to several negative somatoform dissociative symptoms, e.g., experiencing the body as a foreign object, and involves functional abnormalities along sequential hierarchical areas, secondary and cross-modal areas of the sensory cortex (visual, auditory, and somatosensory), as well as areas responsible for an integrated body schema (Simeon *et al.*, 2000). Perhaps the ANP's lack of sensory perception, including bodily and peripheral stress hormone feedback, could be instrumental in inhibiting the defensive system, hence, the insula and amygdala, and the responsivity it orchestrates. One way to study the presumed negative feedback and positive feedforward loops would be to apply functional MRI while ANPs and EPs are exposed to external and internal threat cues.

PSYCHOBIOLOGICAL DIFFERENCES AMONG DISSOCIATIVE PARTS OF THE PERSONALITY IN PTSD

Recent studies of PTSD have begun to appreciate that the condition in which the patient remains may affect psychobiological functioning. For example, in a recent functional MRI study using script driven imagery of traumatic situations, Lanius *et al.* (2000) found that a subgroup of PTSD patients developed a depersonalization response, while other PTSD patients relived their trauma. Both groups showed less activation of the thalami and the dorsolateral prefrontal cortices compared with healthy controls, but the depersonalization group also displayed activation of the right anterior cingulate. The depersonalization group may perhaps have represented the ANP, and the group that relived trauma, the EP. In any case, the Lanius study confirms that the activity in different brain regions depends on the dissociative part of the personality that is activated during testing. The same interpretation may apply to the finding that some PTSD patients produced (contrary to the expectation of the investigators) slower vocal responses to trauma cues in the emotional Stroop task, whereas others exhibited faster vocal responses to trauma-related than to neutral cues (Stewart *et al.*, 2000). Furthermore, Liberzon and Taylor (2000) reported that a PTSD patient who experienced a flashback in a symptom provocation study had very different regional cerebral blood flow from other PTSD patients and controls with greater uptake in subcortical regions than in cortical regions, in particular the thalamus. We conclude that future psychobiological studies of PTSD should control for the dissociative part of the personality that dominates during testing. However, controlled activation of ANPs and EPs of PTSD patients may be difficult. As a prototype that can be more readily self-controlled in ANP–EP switches, DID deserves far more study from a psychobiological angle than has been performed to date.

CONCLUSION

The integrative functions of the human mind can be hampered by overwhelming events, especially when these events begin early in life, are recurrent, involve threat to the body and to life itself, and are accompanied by compromized attachment, and lack of social recognition and support. While substances such as glutamate, norepinephrine, and CRF may have a role in dissociative processes, these substances in themselves do not define what dissociative structures ensue. Trauma-related dissociation does not split the personality in accidental ways. Clinical, empirical, and experimental evidence rather suggests that structural dissociation of the personality reflects a lack of integration among specific psychobiological systems, described in this chapter as emotional operating systems. The primary form of this structural dissociation involves failed integration between systems dedicated to daily life and survival of the species, and systems dedicated to the survival of the individual in the face of severe threat.

Key brain structures involved in responding to (perceived) threat include the amygdala, insula, locus coeruleus, HPA-axis, hippocampus, cingulate, medial prefrontal cortex, and orbitofrontal cortex, as well as the somatosensory association areas situated in the temporal, parietal and occipital cortex. The evidence to date suggests that in PTSD and the dissociative disorders, (re)activation of the defensive system — metaphorically addressed as the 'emotional' personality — by trauma-related cues implies increased activation of the amygdala, insula, and related structures, and decreased activation of the hippocampus, cingulate gyrus, medial prefrontal cortex, and perhaps other prefrontal areas as well. The amygdala orchestrates a range of unconditioned and conditioned reactions to threat, including sympathetic and parasympathetic nervous system activity, analgesia, defensive motor reaction patterns, subjective emotional feelings such as fear, and retraction of the field of consciousness to threat cues in the immediate, subjective present. These reactions seem to lack modulation by the hippocampus and prefrontal cortex. However, when the psychobiological systems that involve daily

life functioning — i.e., the 'apparently normal' personality — are dominant, threat cues are avoided (gaze aversion, mental inhibition), and attention is directed to cues that have a bearing on daily life. The depersonalization and negative somatoform dissociative symptoms that characterize the ANP may be related to disturbed metabolism in the somatosensory association areas. While structural dissociation may be adaptive when the integrative level is not sufficient to integrate both systems, continued structural dissociation is maladaptive when integration of traumatic experiences would be feasible.

To date, research of PTSD and most research of dissociative disorders has largely overlooked that findings may depend on the type of dissociative psychobiological system that dominates the functioning of the patient at the time of measurement. (It must be noted that in parallel dissociation, two or more dissociative personalities may be activated simultaneously, and conflicts among them may occur.) In this regard, at a minimum, the theory of structural dissociation can serve as a heuristic for future research of trauma-related dissociation. To date, we have studied global differences between ANP and EP, but future studies should also address emotional operating subsystems. As the theory predicts, EPs engaged in flight, freeze, fight, or total submission would have different psychobiological reactivity to threat cues and, for example, ANPs engaged in work or reproduction and caretaking would have different responses to attachment cues.

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XXII

The Psychobiology of Sexual and Gender Identity Disorders

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INTRODUCTION

Interest in human sexual function has increased in the past decade, in large part as a result of increased recognition of the sexual side effects of various medications, the high incidence of sexual dysfunction among men and women, and the highly publicized success of some treatments for sexual dysfunction (e.g., Viagra for erectile dysfunction). This paper will describe the present knowledge of the endocrine, neurotransmitter, and central and peripheral nervous system mechanisms governing sexual function and dysfunction. The primary focus will be the underlying physiological processes although it should be noted at the outset that it would be misleading to assume that sexual dysfunction is best conceptualized in this manner.

Psychological problems, such as depression or anxiety, and relationship issues, such as marital discord or stress, can have a profound effect on sexual functioning. Although such cognitive and emotional factors are often integral to a sexual problem, these aspects will be reviewed only briefly here.

SEXUAL DESIRE DISORDERS

Hypoactive Sexual Desire Disorder

Sexual desire is commonly defined as the broad interest in sexual objects or experiences. One of the difficulties in diagnosing inhibited desire is determining exactly what constitutes low desire. Sexual desire cannot be measured exclusively by frequency of sexual activity — a person may desire sexual activity a great deal more or less often than their actual level of activity. It is problematic to measure sexual desire based on a discrepancy between partners; a man who desires sexual activity once a day may be frustrated by a partner who desires sexual activity twice a week, yet both partners have a level of sexual desire that falls within the normal range. Because there is no objective physiological criterion for desire, it is generally inferred by self-reported frequency of sexual thoughts, fantasies, dreams, wishes, and interest in initiating and/or engaging in sexual experiences. However, it is also problematic to diagnose hypoactive sexual desire based on a simple comparison with typical levels of desire. A couple who both prefer sexual activity only once a month would be exhibiting levels of desire below normal, yet it is unlikely that they would be unsatisfied with their degree of activity (LoPiccolo and Friedman, 1988). In order to meet the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), criteria for hypoactive sexual desire disorder, the person must not only experience a persistent or recurrent deficiency or absence of sexual fantasies and desire for sexual activity, but the situation must create marked distress or interpersonal difficulty — indeed, it should be noted at the outset that in order to be diagnosed with any of the sexual disorders a person must be experiencing significant distress or interpersonal difficulty (American Psychiatric Association, 1994).

Hypoactive sexual desire disorder is much more common in women than in men. Thirty-two percent of women between the ages of 18 and 29 years old reported a lack of sexual interest compared to 14% of men in the same age group. Women did not demonstrate a change in rates of inhibited desire according to age while men were significantly more likely to report lack of sexual interest as they aged, particularly after age 50 years old. Women did not differ in rates of inhibited desire based on marital status whereas married men were significantly less likely to report inhibited desire compared to divorced or never married men. Women who had less than a high school level of education reported significantly higher rates of inhibited desire compared to women with more education. Perhaps more educated women are more open to improving sexual communication and sexual knowledge. Exploring what is sexually pleasurable, and communicating sexual needs are techniques used for enhancing sexual desire. Unlike women, men showed no significant differences in desire according to education. African-American women reported significantly higher rates of inhibited desire compared to Caucasian or Hispanic women whereas men demonstrated no ethnic differences in sexual desire (Laumann *et al.*, 1999).

Physiological Factors

Cases of low desire in men are often related to medical conditions or treatments that affect hormone levels. Hypogonadal men (i.e., men with deficient secretion of gonadal hormones) receiving testosterone replacement therapy demonstrated a significant drop in sexual interest following removal of the hormone treatment, and a return in sexual interest when the hormone treatment was resumed. This indicates that very low testosterone levels may impair sexual desire in men. Once testosterone levels reach a certain threshold, additional testosterone does not affect sexual desire — thus, testosterone administration to a male with normal testosterone levels will not increase sexual desire. In adolescent males, higher testosterone levels are associated with increased frequency of sexual fantasies and sexual activity but this relationship does not hold true in adult men. Perhaps during and around puberty internal factors (e.g., hormones) trigger sexual appetite while in adulthood external cues (e.g., relationship factors) play more of a central role. Some evidence suggests that oestrogen and progesterone administration reduces sexual desire in men with excessive or inappropriate desire,

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although few studies have been published on this topic (Meston and Frohlich, 2000).

Unusually low testosterone levels that result from removal of the adrenal glands (adrenalectomy), removal of the ovaries (oophorectomy), or as a consequence of menopause, may impair sexual desire in women. Testosterone is effective in restoring sexual desire in these women with abnormally low testosterone levels. It should be noted that most women with hypoactive sexual desire disorder do not have abnormally low testosterone levels and administering exogenous testosterone to women with normal testosterone levels does not enhance sexual desire and can lead to a number of adverse side effects (e.g., acne, facial hair). Oestrogen levels do not significantly affect sexual desire in women and evidence is mixed regarding the affects of progesterone administration on sexual desire in women. Some evidence suggests that increases in prolactin levels that occur with breast-feeding may diminish sexual desire in women but myriad other psychological factors (e.g., post-partum depression) could account for these changes (Meston and Frohlich, 2000).

A variety of psychoactive medications affect sexual drive. Selective serotonin reuptake inhibitors (SSRIs, used most commonly for treating depression), which acutely increase the amount of serotonin in the synapse, produce a variety of sexual side effects including diminished libido in both men and women. Sexual dysfunction secondary to SSRI use is believed to result from the activation of the $serotonin_2$ receptor, although it is unclear whether activation of this specific receptor type is responsible for SSRI-induced loss of sexual drive *per se*.

Drugs that facilitate dopamine activity, such as the antiparkinsonian medication levodopa, tend to increase sexual desire in men. Dopamine activity also increases sexual drive in animals; selective dopamine agonists and apomorphine increased mounting behaviour in male rats (for review, see Meston and Frohlich, 2000). The role of dopamine activity in female sexual desire is not known although one report described a case of a middle-aged woman who exhibited increased sexual behaviour while receiving antiparkinsonian medication, and a return to normal sexual behaviour when the dosage was decreased (Uitti *et al.*, 1989). Long-term opioid (e.g., heroin) use diminishes libido in men and women, perhaps due to the testosterone and luteinizing hormone reducing properties of these drugs (Meston and Frohlich, 2000).

Although it is generally believed that sexual drive is controlled by the central nervous system, the specific brain regions and mechanisms controlling drive are not well understood. In male rats, lesion to the medial preoptic area impairs copulatory behaviour by disrupting the animal's ability to identify potential partners. Some evidence suggests that the medial amygdala plays an important role in sexual motivation in males. Large temporal lobe lesions have been reported to produce hypersexuality, although it is believed that damage to inhibitory neurons in the pyriform cortex may be responsible for these results (McKenna, 1999). It is feasible that activity in one or more of these regions may be abnormal in cases of hypoactive sexual desire.

Psychological Factors

Daily hassles (e.g., worrying about childcare, bills, etc.) and high stress jobs are probably the worst offenders for suppressing sexual desire, as are a multitude of relationship or partner-related issues. If the couple is experiencing conflict, one or both partners may experience a drop in desire. One member of a couple may experience a drop in desire because he or she no longer feels attracted to his or her partner. This typically occurs when the partner undergoes a dramatic change in appearance (e.g., significant weight gain). A person may experience a drop in sexual desire if their partner is unwilling to experiment sexually or lacks sexual skill, making the sexual experience frustrating, unpleasant, or in other ways unappealing. Differences in the desired amount of emotional intimacy or time spent together can impair sexual desire; one person may feel frustrated by the lack of closeness while the other may feel suffocated. Some people fear intimacy, perhaps as a result of being hurt in the past, and thus may have little desire for intimacy in the form of sexual activity. If one partner has more power in the relationship, the other partner may feel bullied or harassed, and may experience a drop in desire (LoPiccolo and Friedman, 1988). Religious concerns and certain psychological difficulties, such as depression or obsessive–compulsive disorder, may also be associated with inhibited desire.

Treatment

Treatment for inhibited sexual desire may be difficult because the person with low desire may be seeking treatment at the urging of their partner, and thus they may not be internally motivated for therapy. Provided physiological causes have been ruled out (e.g., low testosterone levels) it is essential that treatment begin by structuring the impaired desire as a couple problem, rather than as an individual problem (Pridal and LoPiccolo, 2000). Cognitive therapy is often used to restructure thoughts and beliefs about sexuality that may be inhibiting desire (e.g., good women should not desire sex) to reflect ideas more conducive to sexual enjoyment, and to address negative underlying relationship issues. Behavioural interventions are often used to treat physical expressions of affection and intimacy and to increase sexual communication. In some cases, couples have ceased not only sexual activity, but all physical affection — the partner with low desire may have stopped showing any affection for fear that it be interpreted as interest in sexual activity. In such cases, the couple is encouraged to begin expressing affection through non-sexual means such as holding hands, cuddling, hugging, and brief kissing, and then to gradually reintegrate sexual intercourse into their relationship.

Sexual Aversion Disorder

In the DSM-IV, sexual aversion disorder is defined as the recurrent or persistent extreme avoidance of or aversion to all, or nearly all, genital sexual contact with a sexual partner. The association between sexual aversion disorder and anxiety has led some to argue that sexual aversion disorder may be best conceptualized as a type of anxiety disorder, like a snake phobia or a fear of heights. Sexual aversions in some people may be related to a history of sexual trauma or unwanted sexual activity. Men may also develop sexual aversion as a result of fear of erectile failure and/or a desire to avoid unpleasant sensations associated with anxiety (Gold and Gold, 1993).

Sexual aversion disorders may be treated by addressing the underlying anxiety. Anxiolytic medications may be used to reduce the anxiety, sometimes in conjunction with sex therapy. If the sexual aversion appears to result from a history of sexual abuse, counselling directed at coming to terms with this history may be most effective. For men, treatment may involve sex education regarding realistic expectations about performance and female response (Gold and Gold, 1993).

SEXUAL AROUSAL DISORDERS

Closely connected with sexual desire, sexual arousal is defined in both psychological (e.g., 'feeling sexually excited') and physiological terms (e.g., genital blood flow). Physiological sexual arousal in males involves signals from central (brain and spinal cord) and peripheral nervous systems, and on a complex interplay between

neurotransmitters, vasoactive agents and endocrine factors. Within the penis is a central artery and veins that exit and drain the erectile bodies. The muscles that line the sinusoidal spaces and the central artery are contracted during the non-aroused state. Erection begins with muscle relaxation that is controlled by autonomic nerves and by the release of nitric oxide (described below). Smooth muscle relaxation reduces vascular resistance and the erectile bodies fill with blood. Once the erectile bodies become engorged with blood, the veins are compressed under the penis's tough fibroelastic covering and blood is trapped in the penis. Normally, detumescence (i.e., loss of erection) occurs with the release of catecholamines during orgasm and ejaculation.

Physiological sexual arousal in women begins with vasocongestion of the vagina, vulva, clitoris, uterus, and possibly the urethra, and can occur within only a few seconds of sexual stimulation. Vaginal lubrication occurs when the blood vessels of the vaginal epithelium (vaginal wall) become engorged with blood, causing fluid to pass between the cells of the vaginal epithelium and emerge on the vaginal wall as sweat-like droplets. These droplets can quickly build up to form a lubricating film that facilitates penetration of the penis. Nitric oxide has also been implicated in female sexual arousal (see below).

Female Sexual Arousal Disorder

According to the DSM-IV, a woman may have female sexual arousal disorder if she experiences repeated and persistent difficulty attaining or maintaining, until sexual activity is completed, a sufficient lubrication-swelling response of sexual excitement. As with many sexual disorders, female sexual arousal disorder can be subdivided into lifelong versus acquired types, generalized versus situational types, and due to psychological versus organic, or combined factors (American Psychiatric Association, 1994). Women of all ages may experience difficulty lubricating, although it tends to be more of a problem in later life, typically after menopause. Approximately 20% of women aged 18–49 years old reported problems lubricating compared to 27% of women aged 50–59 years old. Difficulty lubricating is not associated with marital status or level of education (Laumann *et al.*, 1999).

Physiological Factors

Oestrogen levels can have a profound effect on sexual arousal. This is most apparent through the menopause transition. Oestrogen levels decline during menopause, which results in atrophy of the vaginal epithelium (vaginal walls), and a decline in blood flow into the capillaries of the vaginal wall. A loss of oestrogen following menopause can also indirectly impair sexual arousal by impairing mood. Oestrogen replacement therapy can help remedy some of the vasomotor symptoms and vaginal atrophy in postmenopausal women (Sherwin, 1991). Several researchers have examined whether hormonal fluctuations across the menstrual cycle affect sexual arousal but failed to find any consistent relationship (Meuwissen and Over, 1992).

As noted earlier, some studies have shown that activation of the sympathetic nervous system can facilitate female sexual arousal. When sexually functional women and women with low sexual desire engaged in vigorous exercise (stationary cycling, which stimulates the sympathetic nervous system), they demonstrated significantly higher levels of physiological sexual arousal to an erotic film than without exercise (Meston and Gorzalka, 1995a, 1996). Physiological sexual arousal was measured using a vaginal photoplethysmograph, a tampon-like device that the woman inserts into her vagina, which measures blood flow in the vaginal capillaries. It is important to note that in these studies exercise alone did not facilitate sexual arousal — it was only when the women viewed an erotic film that vaginal blood flow was increased. This suggests that exercise somehow prepares the women's body for sexual arousal so that when she enters a situation she views as sexually appealing, her level of physiological sexual responding is intensified. Meston and Gorzalka (1995b) found a facilitatory effect of exercise at 15 and 30 minutes following exercise. Whether the effect remains past 30 minutes has not been examined. When the sympathetic nervous system is activated using a medication to increase sympathetic nervous system activity, such as ephedrine, physiological sexual arousal to an erotic film is also facilitated (Meston and Heiman, 1998). The reverse has also been shown; medications such as clonidine that block sympathetic nervous system activity impair sexual arousal (Meston *et al.*, 1997).

Animal studies and studies examining the physiology and side effects of medications suggest that neurotransmitters in the central nervous system and neuropeptides in the periphery of the body impact sexual arousal. Nitric oxide activity in the penile tissue triggers a cascade of events leading to increased blood flow into the penile capillaries. The medication Viagra, which is an orally administered treatment for erectile dysfunction, acts by initiating these events (the mechanism of action of Viagra will be described in more detail below under the erectile dysfunction section). Recent studies show that nitric oxide is also produced in clitoral tissue.

Convergent evidence suggests that serotonin activity in the periphery of the body may be involved in female sexual function and dysfunction. Serotonin is a powerful vasoactive substance that, depending on the site and tissue type, may produce vasodilation or vasoconstriction. Normal vaginal lubrication is dependent upon adequate vasocongestion of the genital tissue and it is feasible that serotonin may be involved in this process. If so, abnormalities in serotonin mechanisms may impair vaginal vasocongestion (e.g., with SSRI use). Serotonin is also active in several other peripheral mechanisms that are likely to affect sexual functioning such as nonvascular smooth muscle contraction, endocrine functions, and the spinal cord and peripheral nerves (Frohlich and Meston, 2000).

Mild abnormalities in cutaneous sensation, the sense of touch, have been associated with difficulty becoming lubricated. Cutaneous sensation was measured using Von Frey monofilaments, hair-like fibres that when pressed against the skin reliably apply a specific amount of force — the amount of force applied depends upon the diameter and length of the hair. College aged women with sexual arousal disorder required a higher degree of stimulation (more force) to their skin before they perceived the stimulation as compared to women with normal sexual functioning. This suggests that women with sexual arousal disorder may have mild abnormalities in peripheral nervous system functioning (Frohlich and Meston, 1999).

Very little research has been published regarding brain areas involved in female sexual arousal although several studies have implicated the paraventricular nucleus of the hypothalamus. Transneural viral labelling indicates that the clitoris and uterus are connected to the paraventricular nucleus and the findings from one study in rats suggests that the paraventricular nucleus is active during copulation. During sexual arousal, oxytocin produced in the paraventricular nucleus is secreted into the blood stream by the posterior pituitary (McKenna, 1999). It is feasible that paraventricular nucleus activity is abnormal in women with sexual arousal disorder.

Psychological Factors

In addition to affecting sexual desire, factors such as performance demand, anxiety, and expectancies can also affect physiological sexual arousal (i.e., vaginal vasocongestion and lubrication). Laan *et al.* (1993) found that sexually functional women became more sexually aroused after being asked to become as sexually aroused

as possible, versus being told their level of sexual arousal was not important to the study. This suggests that for women without sexual difficulties, performance demand can facilitate sexual arousal. Expectancies may interact with autonomic arousal to influence sexual arousal as well. Palace (1995) found that sexually dysfunctional women who were falsely informed that they displayed strong physiological sexual arousal to an erotic film, displayed greater physiological and self-reported sexual arousal to a subsequent erotic film. Moreover, this response was greater if the erotic stimulus was preceded by an anxiety/fear provoking film versus a neutral film. This suggests that sympathetic nervous system arousal paired with the expectation of sexual arousal may be effective in increasing physiological sexual arousal in women.

Because of the close link between sexual desire and sexual arousal in women, many of the same psychological factors that impair sexual desire also inhibit vaginal lubrication. Briefly, these include individual, relationship, and cultural factors. An estimated 49% of women who were sexually abused as children report impaired sexual arousal. Inadequate or inappropriate sexual stimulation may interfere with sexual arousal, as would negative emotions such as fear of rejection, anger, or relationship conflict (Morokoff, 1993).

Male Erectile Disorder

According to the DSM-IV, male erectile disorder is characterized by a persistent or recurrent inability to attain or maintain an adequate erection until completion of the sexual act. Erectile dysfunction may result when a medical problem or condition, such as diabetes mellitus, surgical injury, aging, or pharmaceutical intervention, affects vascular blood flow to the penis or neural innervations to and from the penis. Patients are diagnosed with erectile dysfunction when their erectile difficulties are exclusively psychogenic in nature, or are caused by a combination of psychological and medical factors (American Psychiatric Association, 1994).

Men of all ages occasionally have difficulty obtaining or maintaining an erection, but true erectile disorder is more common after age 50 years. Approximately 7% of men aged 18–29 years have erectile troubles compared to 18% of men aged 50–59 years. Level of education and ethnicity are not associated with erectile difficulties, but married men are less likely to report erectile problems compared to never married or divorced men (Laumann *et al.*, 1999).

Physiological Factors

Testosterone does not affect erectile functioning unless it falls below a critical level. Hypogonadal men, or men with unusually low testosterone levels, often experience erectile problems that are successfully treated with testosterone replacement therapy. Testosterone administration does not improve erectile response in men with normal testosterone levels. Prolactin levels also affect erectile functioning although the process is complex; men with abnormally high prolactin levels and men with abnormally low prolactin levels may experience erectile dysfunction (Besser and Thorner, 1975; Deutsch and Sherman, 1979). In normally functioning men, prolactin and oxytocin levels increase significantly during sexual arousal (Meston and Frohlich, 2000).

Acetylcholine, vasoactive intestinal peptide, and nitric oxide have also been implicated in penile tumescence (i.e., erection). Activation of cholinergic receptors produces relaxation of the penile smooth muscles, allowing blood flow into the penis, thus producing an erection (Saenz de Tejada *et al.*, 1988). Sexual stimulation leads to the production of nitric oxide in penile tissue. Nitric oxide stimulates guanylate cyclase release, which triggers the conversion of guanosine triphosphate to cGMP. cGMP activity relaxes the smooth muscles of the penile tissue allowing vasocongestion and erection (Burnett, 1995). The pharmaceutical company, Pfizer, capitalized on this process by developing the medication Viagra, which is designed to treat erectile dysfunction. Viagra potentiates the activity of cGMP by inhibiting phosphodiesterase type 5, the endogenous substance responsible for cGMP deactivation. This increases and prolongs cGMP activity, which increases and prolongs vasocongestion, and enables erection. Interestingly, Viagra was discovered by accident when researchers for Pfizer noticed that men taking an experimental drug for heart disease, which worked on nitric oxide systems, had erections as a side effect.

A variety of psychoactive medications produce erectile dysfunction. Antiparkinsonian medications are dopamine agonists and are reported to facilitate erection (Bowers *et al.*, 1971) while antipsychotic medications are dopamine antagonists and facilitate erection at low doses and impair erection at high doses (Aizenberg *et al.*, 1995; Marder and Meibach, 1994). Cocaine is a dopamine agonist and high doses can disrupt erectile capacity (Miller and Gold, 1988), perhaps due to its vasoconstrictive properties. Opioid abuse (e.g., heroin) can lead to erectile dysfunction.

Erection is dependent upon spinal reflexes and is controlled by descending inhibitory and excitatory input from the brainstem. This is most apparent when studying the effects of spinal cord injury. Transection of the spinal cord often facilitates erectile response (depending upon the region of the spinal cord injured) — animal and human studies suggest that following spinal cord injury, less stimulation is required to obtain erection and erection occurs more frequently (McKenna, 1999).

Several brain regions have been implicated in male erection. Animal studies indicate that oxytocin released from the paraventricular nucleus of the hypothalamus can produce erection as can electrical stimulation of the paraventricular nucleus of the hypothalamus and hippocampus. Electroencephalographic studies indicate that the right temporal lobe is activated when right-handed men are presented with visual sexual stimulation. Perhaps the most definitive study to date used positron emission tomography (PET scan) to examine brain activity in healthy men presented with visual sexual stimulation. The areas of the brain activated included visual, sensory, and neuroendocrine and autonomic areas. Specifically, visually presented information produced bilateral activation of the inferior temporal cortex, a visual association area, activation of the right inferior frontal cortex and right insula, paralimbic areas that relate motivational states with highly processed sensory information, and activation of the left anterior cingulated cortex, a paralimbic area that controls neuroendocrine and autonomic functions (McKenna, 1999).

Psychological Factors

It is common for men to have occasional episodes of erectile failure without it developing into a full blown erectile disorder. Barlow (1986) argued that men with erectile dysfunction respond differently in sexual situations compared to normally functioning males. When placed in a sexual situation, men with erectile dysfunction focus on non-sexual cues such as fears about inadequate performance, and worries about inability to control performance. These thoughts lead to increased anxiety, increased focus on non-erotic cues and fears of erectile failure. This process inhibits erection and thereby confirms the men's fears. Since the men's fears were confirmed, they are likely to repeat the process in subsequent sexual situations and a negative feedback loop develops. In contrast, when placed in a sexual situation, men with normal erectile responding focus on erotic cues and subsequently become aroused and are able to obtain and sustain an erection. They experience the sexual situation as pleasurable and look forward to future sexual situations creating a positive feedback loop.

Treatment

A variety of tools can be used to assess whether the erectile dysfunction is of psychological or physiological origin. If the male exhibits nocturnal erections, or obtains an erection when a vasoactive substance is injected into the corpora, the erectile problem is likely to be psychological in nature. Treatments for erectile dysfunction include vacuum devices and constriction rings, intracavernosal injections, intraurethral pharmacotherapy, topical pharmacotherapy, oral pharmacotherapy, and penile implants. The vacuum device consists of a tube that is placed over the penis, and a vacuum pump that draws blood into the penile arteries. A constriction ring is placed at the base of the penis to prevent venous outflow so that the erection is maintained until completion of the sexual act. Several medications are available that can be injected into the corpus cavernosum of the penis to induce erection including papaverine, phentolamine, and prostaglandin E_1. These all act to dilate penile capillaries, allowing blood to flow into the penis. Although intracavernosal injections are fairly effective (between 70–90%), between 50% and 80% of patients discontinue treatment, citing problems such as inconvenience, cost, and invasiveness of treatment. An intraurethral-administered medication, MUSE (Medicated Urethral System for Erection — active ingredient prostaglandin E_1), has recently been introduced. This medication is administered in suppository form and is absorbed through the urethral mucosa. It is most effective when used in conjunction with a constriction ring. Side effects of the medication include urogenital pain and urethral bleeding. Topical medications, such as Minoxidil, are also available, although these types of treatments are not commonly used, in part because their effects on vaginal mucosa are not well understood. Viagra, an orally administered treatment, was introduced to the market in 1998, and since then, many of these rather cumbersome and involved treatments have become less popular. Viagra is well tolerated by a variety of patients and is an effective treatment for both organic and psychogenic impotence (Montorsi *et al.*, 1999). Because sildenafil produces vasodilation and a minor drop in blood pressure, it may be contraindicated for patients diagnosed with or receiving treatment for cardiovascular disease. Data are not presently available regarding sildenafil-use in patients with certain cardiac conditions such as unstable angina, stroke, or recent myocardial infarction (heart attack) and/or arrhythmias. Nonetheless, sexual activity increases the likelihood of ischaemia or infarction and thus patients with cardiac risk factors are often referred for an exercise stress test prior to receiving sildenafil. Sildenafil can safely be administered in conjunction with some cardiovascular medications, such as most antihypertensives, but if it is taken in conjunction with organic nitrates, it can produce a major and life-threatening drop in blood pressure (Klonger, 2000).

Penile implants are considered a last resort and are used when tissue damage or deterioration is severe, and when other treatments have failed. Penile implants typically consist of a cylinder (implanted in the penis) that can be mechanically inflated and deflated (Rowland and Burnett, 2000).

Studies are currently underway to determine whether Viagra and other drugs that act as vasodilators on genital tissue will be effective for treating Female Sexual Arousal Disorder. The first Federal Drug Administration (FDA) approved treatment for female sexual arousal disorder was recently introduced to the market. The treatment is a hand-held battery-operated device, called the EROS-CTD, which contains a soft plastic cup and a suction device. When the cup is placed over the clitoral tissue and the device is activated it draws blood into the genital tissue.

ORGASM DISORDERS

The normal ejaculatory response typically occurs following sensory stimulation to the penis. The stimulation initiates a nerve signal that travels along the pudendal nerve (i.e., genital sensory nerve) and synapses at the sacral level of the spinal cord. The spinal cord input stimulates an autonomic and a somatic response. The autonomic response involves adrenergic neurons (within the sympathetic nervous system), which stimulate contraction of the smooth muscles of the vas deferens, seminal vesicles, and prostate. This leads to closure of the bladder neck and movement of seminal fluid into the urethral duct. In both men and women, orgasm is characterized by a peak in sexual pleasure that is accompanied by rhythmic contractions of the genital and reproductive organs, cardiovascular and respiratory changes, and a release of sexual tension. In men, during the emission stage of orgasm, seminal fluid is propelled into the bulbar urethra via the release of norepinephrine that acts on alpha-adrenergic receptors, the smooth muscles of the vas deferens, prostate, and seminal vessels. During the ejaculatory phase, which is mediated by a sacral spinal reflex, semen is released through the urethra via contractions of muscles that surround the bulbar urethra. The extent to which central neurophysiologic events are related to the intensity or experience of orgasm is not known. While orgasm is generally the result of both genital and psychological stimulation, evidence suggests central stimulation alone may trigger orgasm.

Female Orgasmic Disorder

Female orgasmic disorder is diagnosed when the woman experiences persistent or recurrent delay in, or absence of, orgasm following a normal sexual excitement phase. In order to meet DSM-IV diagnostic criteria, the woman's orgasmic capacity is less than would be reasonable for her age, sexual experience, and adequacy of sexual stimulation she receives (American Psychiatric Association, 1994). Between 22–28% of women ages 18 to 59 years report that they are unable to attain orgasm. Married women and women who have some college education are significantly less likely to report being unable to attain orgasm as compared to never married and divorced women, and women who have not attended college (Laumann *et al.*, 1999).

Physiological Factors

Among normally orgasmic women oxytocin levels were positively correlated with subjective intensity of orgasm, and prolactin levels were elevated for up to 60 minutes following orgasm (Meston and Frohlich, 2000). It is feasible that oxytocin and prolactin regulation is abnormal in women with orgasmic disorders, although a study examining oxytocin and prolactin levels in orgasmic disordered women has not yet been published.

The SSRIs frequently affect orgasmic functioning, leading to delayed orgasm or anorgasmia. The antidepressant, nefazodone, produces fewer sexual side effects in women (Feiger *et al.*, 1996), possibly because it increases serotonin activity in general while simultaneously inhibiting serotonin activity at the $serotonin_2$ receptor — the receptor implicated in SSRI-induced sexual dysfunction (Eison *et al.*, 1990). Cyproheptadine is also a $serotonin_2$ receptor antagonist and has been an effective antidote to SSRI-induced orgasmic dysfunction (although it is not an ideal antidote as it can disrupt the effectiveness of the antidepressant medication). Drugs that affect dopamine mechanisms, such as antipsychotics (which inhibit dopamine) or cocaine (which facilitate dopamine), delay or inhibit orgasm in women. Female heroin addicts also report delayed or inhibited orgasm. Taken together, these findings suggest that serotonin, dopamine, and opioid mechanisms may be functioning abnormally in orgasmic disordered women.

Studies examining blood plasma levels of neuromodulators before, during, and after orgasm suggest that epinephrine and norepinephrine levels peak during orgasm in normally functioning women (Exton *et al.*, 1999; Wiedeking *et al.*, 1979). This process

may be impaired in anorgasmic women. One study showed that when vigorous exercise (which stimulates the sympathetic nervous system) is followed by an erotic stimulus, sexual arousal is facilitated in normally functioning women, but inhibited in anorgasmic women (Meston and Gorzalka, 1996).

Brain and spinal cord mechanisms are integral to the orgasm response. The neuronal pathway connecting the genitals to the brain was identified via transneuronal labelling. Virus injected into the clitoris revealed that sexual afferent neurons synapse on neurons in the lumbosacral spinal cord and connect to the nucleus paragigantocellularis in the brainstem. Lesions to the nucleus paragigantocellularis and spinal cord transection can suppress tonic inhibition of the orgasm-like response, suggesting that this pathway exerts inhibitory control over orgasm. Neurons in this region stain positively for serotonin suggesting that it may be implicated in SSRI-induced anorgasmia in women. Studies in humans suggest that the paraventricular nucleus of the hypothalamus is also involved in the orgasmic response. The paraventricular nucleus produces oxytocin that is released from the posterior pituitary during arousal and orgasm (McKenna, 1999).

The neurological disease, multiple sclerosis, often results in orgasmic disorders. In multiple sclerosis patients, orgasmic problems are related to neurological problems such as changes in genital sensation, muscle weakness in the pelvis (Lundberg and Hulter, 1996), and brain abnormalities (in the pyramidal and brain-stem regions) (Barak *et al.*, 1996). It is feasible that similar anatomical structures are involved in orgasmic disorders, albeit non-disease related.

Psychological Factors

The degree to which a woman is distracted during sexual situations may affect orgasm ability. Orgasmic women tend to focus on their own arousal and their partners' arousal throughout the sexual situation, while anorgasmic women tend to focus on trying to attain orgasm, focus on non-sexual thoughts, and are more easily distracted by non-sexual thoughts. Anorgasmic women are also more likely to report discomfort with sex compared to orgasmic women; they are more likely to be uncomfortable discussing direct clitoral stimulation, and are more likely to have guilt about sex, to endorse sex myths, and to report negative attitudes about masturbation. Anorgasmic women are less likely to be aware of their physiological sexual arousal as compared to consistently orgasmic women (Stock, 1993).

Treatment

Treatment for anorgasmia involves education about female anatomy and physiology, self-exploration, and directed masturbation. The woman is encouraged to explore her body and identify regions and types of stimulation that produce sexual arousal, to experiment with different fantasies, and to use various tools (such as a vibrator) that may enhance arousal. Once she has successfully learned to attain orgasm during masturbation, she is encouraged to teach her partner which parts of her body and which types of stimulation are likely to bring her to orgasm. Directed masturbation is a highly effective treatment for anorgasmia, with outcome studies showing up to a 90% success rate (Heiman and Meston, 1998).

Male Orgasmic Disorder

DSM-IV criteria for male orgasmic disorder include recurrent or persistent difficulty obtaining orgasm, or inability to obtain orgasm, even after sufficient sexual stimulation. The clinician takes a variety of factors into account before making the diagnosis, such as age and amount of stimulation. In most cases of inhibited male orgasm the patient is able to attain orgasm but only through manual or oral stimulation, and when orgasm occurs through intercourse it is only possible after prolonged manual or oral stimulation (American Psychiatric Association, 1994).

For the patient and his partner (male or female), sexual activity is experienced as 'hard work' (Dekker, 1993). Very few studies of male orgasmic disorder have been published, likely due to the fact that it is a rare disorder. The prevalence in the general population is estimated at 1.5 in 1000, and among those presenting for sex therapy, 0–13 in 100 (Dekker, 1993).

What is presently known about the mechanisms underlying orgasm is drawn from animal studies and from studies examining side effects of recreational and pharmaceutical drugs. One of the more common side effects of SSRI medications is delayed or inhibited orgasm, suggesting that serotonin activity may play a role in normal orgasm functioning. Animal studies indicate that serotonin$_{1A}$ receptor activation facilitates orgasm (Bitran and Hull, 1987) — it is feasible that men with inhibited orgasm have abnormal serotonin$_{1A}$ receptor activity. Dopamine may also be implicated in orgasm functioning. Dopamine agonists, such as apomorphine and cocaine, have been reported to inhibit orgasm, although apomorphine has also been reported to facilitate orgasm. The fact that dopamine can both inhibit and facilitate orgasm may be a function of dose; in rats, a low dose of a dopamine agonist increased ejaculation latency while a high dose decreased ejaculation latency (Clark *et al.*, 1983). In normally functioning men, blood plasma levels of norepinephrine significantly increased at orgasm (Kruger *et al.*, 1998) and oxytocin levels were significantly positively correlated with subjective orgasm intensity (Carmichael *et al.*, 1994). Some evidence suggests that inhibited orgasm may result from spinal reflex abnormalities. Studies examining men with spinal cord injuries and normally functioning men suggest that the dorsal penile nerve and the perineal nerve are involved in the ejaculatory response. Stimulation to these nerves in normally functioning men resulted in contraction of the bulbocavernosus muscle, the muscle involved in ejaculation (Yang and Bradley, 1999).

The brain and spinal cord are regions implicated in orgasm. The neuronal pathway from the genitals to the brain was transneuronally labelled by injecting a virus into the penile tissue. Sexual afferents enter the lumbosacral region of the spinal cord and via interneurons and neurons connect to the nucleus paragigantocellularis in the brainstem. Lesions to the nucleus paragigantocellularis impair normal tonic inhibition of the orgasmic response. Neurons in this region stain positively for serotonin suggesting this region may be implicated in SSRI-induced anorgasmia in men. During arousal and orgasm in men, oxytocin produced in paraventricular nucleus of the hypothalamus is released from the posterior pituitary (McKenna, 1999). Single photon emission computed tomography was used to examine brain physiology during orgasm in eight healthy right-handed men. During orgasm, blood flow significantly increased in the right prefrontal cortex and decreased in all other cortical regions (Tiihonen *et al.*, 1994). Although these processes have been implicated in normal orgasmic functioning in men, no evidence is currently available on men with orgasmic disorders. Nonetheless, it is feasible that these regions and mechanisms function abnormally in men with orgasmic disorders.

Premature Ejaculation

When seminal fluid enters the urethral duct, it triggers a somatic response — known as ejaculatory inevitability. The bulbocavernosus and ischiocavernosus muscles contract and semen is ejaculated through the urethral opening. This event is typically associated with the subjective pleasure of orgasm (Rowland and Burnett, 2000). When ejaculation occurs with minimal sexual stimulation before, on, or shortly after penetration, it is referred to as premature ejaculation. The clinician determines whether a diagnosis should be

made after taking a variety of factors likely to affect ejaculation latency into account, such as age and novelty of partner. A diagnosis is only made if it is a recurrent or persistent problem (American Psychiatric Association, 1994).

Premature ejaculation is a fairly common problem. Approximately 30% of men ages 18 to 59 report that they orgasm too early. Marital status and ethnicity are not significantly associated with premature ejaculation, but men who have attended college or graduated from college have lower rates of premature ejaculation than men with less education (Laumann *et al.*, 1999).

Physiological Factors

As described above, several medications inhibit or delay orgasm suggesting that neurotransmitter activity affected by these medications may be involved in normal ejaculation and, possibly, premature ejaculation. Dopamine agonists such as apomorphine and cocaine have been reported to affect orgasm and SSRIs often delay ejaculation suggesting that abnormalities in serotonin and/or dopamine regulation may underlie premature ejaculation. Animal studies suggest that stimulation of the serotonin$_{1A}$ receptor decreases ejaculation latency (Ahlenius and Larsson, 1997). Usually ejaculations occur after several intromissions but in some animals the effects of serotonin$_{1A}$ stimulation are so pronounced that ejaculation occurs at the first intromission (Ahlenius *et al.*, 1981). It is feasible that the serotonin$_{1A}$ receptor is hypersensitive in men with premature ejaculation.

Kaplan (1974) proposed that men with premature ejaculation are more sensitive to erotic stimuli and thus become aroused and orgasm more quickly. They may also be less adept at perceiving the sensations leading to ejaculatory inevitability (i.e., the point when semen is in the base of the urethra and ejaculation cannot be stopped). Several studies have tested this hypothesis with mixed results. Spiess *et al.* (1984) found that men with and without premature ejaculation did not differ in how quickly they became aroused, the length of time it took for them to obtain their maximum erection, or their erectile response to an erotic film. Colpi *et al.* (1986) found that men with premature ejaculation had a more sensitive ejaculation reflex and Fanciullacci *et al.* (1988) found that men with premature ejaculation had larger areas of the somatosensory cortex devoted to the genital region compared to normal controls. Taken together, these studies suggest that some cases of premature ejaculation may be organic in nature.

Psychological Factors

Traditionally, anxiety has been implicated in the aetiology of premature ejaculation yet empirical studies have found conflicting evidence regarding its role. Strassberg *et al.* (1990) compared self-reported thoughts during sexual stimulation in men with and without premature ejaculation and found no differences in self-reported anxiety. Cooper and Magnus (1984) conducted a double-blind, placebo controlled, crossover study where men with premature ejaculation were randomly assigned to receive an anxiolytic medication versus placebo. Although the anxiolytic medication had the expected impact on anxiety (it reduced it), it did not affect ejaculation latency. Cooper *et al.* (1993) compared men with primary premature ejaculation (i.e., life-long premature ejaculation problem) to men with secondary premature ejaculation (i.e., developed the problem after a period of normal ejaculation latencies) and found that men with secondary premature ejaculation were significantly more likely to report anxiety during intercourse and scored significantly higher on a general measure of anxiety. This suggests that anxiety may play a role in secondary premature ejaculation but not primary premature ejaculation.

Treatment

Treatments for premature ejaculation include psychological as well as pharmacological interventions. The most common psychological treatment, the pause-and-squeeze technique, was introduced by Semens (1956) and popularized by Masters and Johnson (1970). This technique is fairly straightforward; the man is stimulated to a point close to orgasm, the stimulation is interrupted (pause) and firm pressure is placed under the glans of the penis (squeeze). The procedure is repeated several types (typically twice) before ejaculation is permitted. These behavioural strategies are often combined with other strategies aimed at increasing control over ejaculation such as increasing the range of sexual activities (i.e., other than intercourse) and increasing the awareness of physical sensations associated with approaching ejaculation (so that stimulation can be ceased prior to ejaculatory inevitability). Patients may also be encouraged to use sexual imagery and thoughts to slightly decrease arousal levels and thus help control ejaculation (e.g., mentally listing the players in a favourite sports team).

More recently, pharmaceutical agents that have the side-effect of delaying ejaculation have been used to treat premature ejaculation. These include antidepressants such as the SSRIs and tricyclic antidepressants, and anti-anxiety medications such as the benzodiazepines. These types of medications are often effective in delaying ejaculation, although the effectiveness can wear off after several weeks and some men do not experience any delay in ejaculation (often the men least likely to respond are also those who ejaculate the most quickly). In some people these medications can have unpleasant side effects such as gastrointestinal disturbance and headache. Topical creams that dull sensation, such as lidocaine, may also effectively delay ejaculation, although they are not appropriate for men who ejaculate prior to insertion, and the creams can be irritating to vaginal tissue (Metz and Pryor, 2000).

PAIN DISORDERS

Dyspareunia

The DSM-IV defines dyspareunia as recurrent or persistent genital pain associated with sexual intercourse. The diagnosis of dyspareunia is not given if the pain decreases or is eliminated by adequate vaginal lubrication.

Although dyspareunia is currently classified as a psychiatric disorder, experts contend that it may be better classified as a pain syndrome that results in sexual dysfunction rather than a sexual dysfunction (that involves pain). In a recent review, Binik *et al.* (2000) suggested that describing genital pain along several dimensions including location, quality, elicitors, course, intensity, and meaning could be useful in identifying the cause of the pain and directing the type and course of treatment. Some women report that the pain is localized, generalized, or wandering while some women are not able to identify the location of the pain. The pain may have a 'sharp', 'burning', 'dull', or 'shooting' quality that may reflect the type of pathology. The pain may be specific to intercourse, or may follow other types of stimulation (e.g., oral sex). It may begin before, during, or after stimulation, and may be mild, moderate, severe, or excruciating. Women may attribute meaning to the pain — they may believe it is related to a medical condition or a psychological source. Meana *et al.* (1999) found that women who attributed their pain to a psychological source rated the pain as more severe in intensity.

Dyspareunia may be caused by anatomical, pathological, iatrogenic, or psychological factors. A rigid hymen would be an anatomical factor that could result in genital pain during intercourse. Infections in the genitals could produce genital pain during intercourse, as could endometriosis and non-malignant and malignant

tumours. Surgical procedures (e.g., episiotomy) could also result in dyspareunia. Following menopause, atrophy of the vulva and vaginal tissue can increase the likelihood of dyspareunia. No one disease is associated with dyspareunia and a disease or disorder can be quite extensive without causing sexual pain. A variety of psychological factors may also lead to dyspareunia. For example, it may develop as a result of attitudes and values passed down from parents that lead to fear and anxiety in sexual situations, traumatic events where sexual or non-sexual contact with the genitals was experienced as painful, or emotional or relational factors, such as depression or discord between partners (Meana and Binik, 1994).

Recent evidence suggests that some forms of dyspareunia may be associated with abnormalities in pain sensation. The sense of touch and pain was measured in women with vulvar vestibulitis and control women (vulvar vestibulitis is a condition characterized by severe pain upon attempted intercourse or vestibular touch — the vestibule refers to the area of tissue below the clitoris, between the labia minora, and the vaginal opening). Touch and pain thresholds were obtained by applying small amounts of force to the skin; touch threshold was defined as the minimum amount of force needed for the women to consciously detect the stimulation, and pain thresholds were defined as the minimum amount of force that was experienced as painful. The women with vulvar vestibulitis were more sensitive to light touch and pain than the control women suggesting greater tactile and pain acuity (Pukall *et al.*, 2000). Women with vulvar vestibulitis also had more densely packed sensory nerves in the vestibule, which may account for their increased sensitivity (Westrom and Willen, 1998).

Treatment

Regardless of the cause of dyspareunia, the symptoms are most effectively treated with cognitive-behavioural therapy. Even when the pain is a direct result of a medical condition, the pain often continues after medical intervention (Schover *et al.*, 1982). Psychological treatment typically involves one or more of the following techniques: vaginal exercises, vaginal dilation, systematic desensitization, and couples therapy (education regarding communication and sexuality). The goal is for the woman and her partner to learn, through education and direct experience, that sexual contact and intercourse do not necessarily produce pain. Vaginal exercises involve the voluntary contraction of the vaginal muscles, allowing the women to gain familiarity and greater control over her muscle contractions. Vaginal dilation involves inserting increasingly larger dilators into the vagina until the woman is able to insert one that is a similar size to her partner's penis, without experiencing pain or anxiety. Vaginal dilation is one form of systematic desensitization but systematic desensitization can also be performed by fantasizing about pain producing activities. The woman is first asked to list activities in order from least painful or anxiety provoking to most painful and anxiety provoking. She is then instructed to fantasize about the least painful activity until she is able to picture it without discomfort. Once she is able to do this, she moves to the next item on the list, until she is able to fantasize about the most painful and anxiety provoking item on the list without feeling discomfort.

Vaginismus

The DSM-IV defines vaginismus as repeated and persistent involuntary spasm of the vaginal muscles that interferes with intercourse. For many women, this difficulty is not specific to intercourse; they are often unable to insert even tampons into their vaginas and fear and avoid gynaecological exams. The condition is not necessarily a generalized sexual problem; many women with vaginismus are able to enjoy sexual stimulation and orgasm that does not involve penetration of the vagina. The prevalence of vaginismus is not known. Laumann *et al.* (1994) interviewed a random sample of 1749 women and found that 10–15% of women reported sexual pain, either dyspareunia or vaginismus. Approximately 12–17% of women seeking sexual therapy present with symptoms of vaginismus (Spector and Carey, 1990).

Although the DSM-IV indicates that vaginismus involves spasm of the musculature of the outer third of the vagina, this description is based almost exclusively on self-report rather than physical examination. One study found no difference in vaginal muscular activity (measured via EMG) between women with vaginismus and control women (van der Velde and Everaerd, 1996). No empirical studies have explored what specifically occurs to prevent penetration. It is not clear whether muscle contraction prevents penetration or makes penetration difficult or painful, or whether penetration is not attempted due to anticipatory pain. The DSM-IV does not include pain as a characteristic of vaginismus, yet some experts in the field argue that the pain, or the anticipation of pain, may be central to the disorder (Reissing *et al.*, 1999).

Vaginismus has traditionally been thought to result primarily from psychological factors. A review of the family histories of women with vaginismus reveals similar backgrounds. Often women with vaginismus were raised by parents with oppressive or authoritarian attitudes (Tugrul and Kabakci, 1997) and had parents who were engaged in frequent conflict (Silverstein, 1989).

Many women with vaginismus report having fathers who were domineering or threatening, alcoholic, seductive, or overprotective, and mothers who disliked sex or viewed sex as an obligation. Approximately 40% of women with vaginismus report a history of sexual trauma (Silverstein, 1989).

Medical conditions that could lead to vaginismus include: vaginal surgery, prolapse of the uterus, endometriosis, vaginal tumours, vaginal lesions, vaginal atrophy, congenital abnormalities, sexually transmitted diseases, abnormalities of the hymen, and pelvic congestion. In such cases, the condition may produce genital pain that develops over time into vaginismus. Medical conditions are associated with vaginismus in 23–32% of cases (Reissing *et al.*, 1999).

Treatment

Vaginismus is treated with cognitive-behavioural therapy targetted at eliminating the erroneous beliefs and the vaginal spasms. Therapy involves identifying faulty beliefs (e.g., 'my vagina is too small to accommodate his penis') and educating the woman and her partner regarding normal sexual anatomy and physiology (e.g., in the aroused and non-aroused state, the vagina is capable of accommodating even a large penis). Vaginal spasms are treated with vaginal muscle exercises and progressive vaginal dilation. The woman and her partner insert dilators into her vagina, starting with very small sized dilators, progressively increasing the size until she is able to insert a dilator that is as large as an erect penis, and finally, attempting intercourse. Few well-controlled treatment outcome studies have been conducted making it difficult to evaluate the effectiveness of therapy, but estimates suggest that 60–100% of vaginismus cases are successfully treated with this type of intervention (Reissling *et al.*, 1999).

PARAPHILIAS

According to the DSM-IV, in order to be diagnosed with a paraphilia, one must demonstrate the following features.

- "Recurrent, intense sexually arousing fantasies, sexual urges, or behaviours generally involving 1) nonhuman objects, 2) the suffering or humiliation of oneself or one's partner, or 3) children

or other non-consenting persons, that occur over a period of at least 6 months."
- The behaviour, sexual urges, or fantasies cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

The DSM-IV lists eight types of paraphilic disorders but in practice, individuals displaying one paraphilia very often also exhibit other paraphilic behaviours. Incarcerated paedophiles often report, for example, that they have also engaged in other paraphilic behaviours (e.g., exhibitionism, voyeurism) and that deviant sexual behaviours other than pedophilia are their primary interest. The presence of paraphilic behaviour may represent an underlying sexual impulsivity disorder that is characterized by sexual compulsivity and hypersexuality, and in some cases, aggression (Kafka, 1997).

Fetishism

According to the DSM-IV, fetishism involves "recurrent, intense sexually arousing fantasies, sexual urges, or behaviours involving the use of nonliving objects" as sexual stimuli (American Psychiatric Association, 1994). Most fetishists are male and nearly one in four are homosexual. Common fetish items include shoes and lingerie and common materials include rubber and leather. Fetishists become aroused by stealing the object, viewing the object, or masturbating with the object. Most fetishists are aroused by a number of different objects. The aetiology of fetishism is not known. Two reported cases of fetishism have been associated with abnormalities in the temporal lobe. In one case the patient had temporal lobe epilepsy and in the other the fetish behaviour was linked to the development of a temporal lobe tumour (Wise, 1985). Some evidence suggests that fetishism may be a learned behaviour that results when a normal sexual stimulus is paired with the fetish item. Seven heterosexual males free from any prior fetish were repeatedly shown erotic stimuli paired with a slide of a black knee-length women's boot. When the slide of the boot was later shown alone, five of the seven men demonstrated penile erection, indicating that a boot fetish had been conditioned. The conditioned fetish was shown to generalize to other types of shoes in three of the men. That is, the men also became aroused when shown a slide of a high-heeled black boot and a low-heeled black shoe. They did not become aroused to a slide of a short brown boot, a brown string sandal, or a golden sandal, suggesting that the fetish only generalized to similar types of shoes (Rachman and Hodgson, 1968). A similar study was conducted in women to determine whether women could also be conditioned to become sexually aroused to a stimulus. Subjects were randomly assigned to repeatedly view an erotic film paired with a light stimulus versus an erotic film alone. No significant differences were found in physiological sexual arousal between the experimental and control groups when a light stimulus was later presented alone (Letourneau and O'Donohue, 1997). Meston and Rachman (1994) tried to condition sexual arousal to the sound of a male's voice. Even after repeated pairings of erotic video clips and the male's voice, later presentation of the male's voice alone did not produce sexual arousal. This suggests that sexual arousal is not readily classically conditioned in women and may explain why, like other paraphilias, fetishism occurs almost exclusively in men.

Transvestic Fetishism

Transvestic fetishism is diagnosed in heterosexual males who experience "recurrent, intense sexually arousing fantasies, sexual urges, or behaviours involving cross-dressing" (American Psychiatric Association, 1994). A distinction is drawn between transvestism (cross-dressing) and transvestic fetishism. A variety of people cross-dress but the behaviour is not considered a fetish unless the cross-dressing is associated with sexual feelings. For example, transsexuals, or people who feel that their external sex does not match their internal gender identity, may cross-dress in order to feel more congruent with their gender identity but do not find the cross-dressing sexually arousing. Similarly, homosexual males may cross-dress (e.g., drag-queens), but the cross-dressing is not considered to be a fetish unless it is sexually arousing.

Very few studies have been published regarding transvestic fetishism and those that have often grouped transvestic fetishists with transvestites who experienced little to no sexual arousal from cross-dressing. Doctor and Prince (1997) surveyed 1032 male transvestites between 1990 and 1992. They found that 40% of respondents found cross-dressing 'often' or 'nearly always' sexually exciting but only 9% described themselves as a "fetishist [who] favoured women's clothing". While keeping in mind that it is unclear what percentage of subjects would meet DSM-IV criteria for transvestic fetishism, the following characteristics were reported. Respondents ranged in age from 20 to 80 years of age, lived throughout the United States, and reported a range of religious affiliations (24% were Catholic, 38% were Protestant, 3% were Jewish, 10% were agnostic, and 25% were with other religious affiliations). The majority of respondents were well educated (65% had at least a BA), in committed relationships, and had children. Of those currently married, 83% reported that their wives were aware of their transvestic tendencies at present, but only 28% accepted the behaviour. The vast majority reported a heterosexual orientation (87%) although 29% reported having had homosexual experiences. The majority of respondents began cross-dressing before age 10 (66%) or between age 10 and 20 (29%), had been raised by both parents (76%), and reported that their father "provided a good masculine image" (76%).

A few cases have been reported of men with transvestic fetishism who had fathers or brothers who also cross-dressed. Since so few cases of familial co-occurrence have been reported in the literature, and because the occurrence of transvestic fetishism in the general population is not known, it is not clear whether family environment and/or genetics contributes to the likelihood of developing a cross-dressing fetish. Transvestic fetishism is associated with learning disabilities, and a few cases of transvestic fetishism have been associated with temporal lobe abnormalities (Zucker and Blanchard, 1997).

A number of studies have been published examining psychosocial causes of transvestic fetishism but most have serious methodological flaws that limit drawing confident conclusions. Some such studies suggest that adolescents with transvestic fetishism tendencies may have a history of separation from and hostility towards their mothers. The cross-dressing may serve as a means to make a connection with females, even if that connection often involves some expressions of anger and hostility (Zucker and Blanchard, 1997).

Pedophilia

Pedophilia is defined as intense and repeated sexually arousing fantasies, urges, or behaviours involving sexual activity with children, typically less than 14 years old (American Psychiatric Association, 1994). Since few paedophiles are likely to openly admit their preference, it is difficult to estimate the prevalence of pedophilia in the general population. Furthermore, individuals who feel sexual attraction to children may resist the temptation due to societal pressures, yet may nonetheless experience sexual fantasies involving children. Recent evidence suggests that pedophilia may be associated with homosexuality, mental retardation, and high maternal age. Homosexuality in the general population is estimated at 2% while homosexuality in paedophiles is estimated at up to 40%. When sexual orientation, intellectual functioning, and maternal age were measured in 991 male sex offenders, high maternal age and low intellectual functioning were significantly

associated with homosexual pedophilia. The association between low intelligence and pedophilia suggests that pedophilia may reflect a developmental disorder. The association between high maternal age and pedophilia is unclear, although it may reflect differences in birth order as homosexuality is associated with being later born (discussed below under gender identity disorder) (Blanchard *et al.*, 1999).

Some researchers have speculated that a childhood history of sexual abuse contributes to an adult preference for sexual activity with children. In a large sample of men who were child sex offenders, Freund *et al.* (1990) found that heterosexual and homosexual paedophiles were significantly more likely to report childhood sexual abuse by a male abuser (versus female abuser) as compared to controls. Freund and Kuban (1994) classified child sex offenders according to whether they demonstrated phallometric (increased penile volume) preference to photographs of nude children versus adults. They found that child sex offenders who demonstrated preference for children were significantly more likely to have a childhood history of sexual abuse. It should be noted that although reports indicate approximately 49% of paedophiles have a history of childhood sexual abuse, very few people with a history of childhood sexual abuse become paedophiles (Freund and Kuban, 1994).

Paedophiles may have difficulty with gender differentiation. Freund *et al.* (1991) showed slides of nude male and female children and adults to paedophiles and controls, and measured penile volume changes. The paedophiles demonstrated less differentiation between stimuli containing males versus females as compared to non-paedophiles. Although this pattern of undifferentiated arousal has also been noted in a case study of a 20-year-old woman with multiple paraphilias (Cooper *et al.*, 1990), few cases of female pedophilia have been reported in the literature.

Paedophiles may differ from non-paedophiles on several physiological dimensions as well. Baseline plasma cortisol, prolactin, and body temperature were significantly higher in paedophiles than controls. When both groups were administered a serotonin agonist, mCPP, versus placebo, plasma cortisol levels were more elevated and remained elevated longer for paedophiles compared to controls. The paedophiles reported experiencing side effects (e.g., dizzy, restless) of mCPP administration while the controls did not. Consistent with these findings, some researchers have speculated that pedophilia may be associated with disturbances in serotonin-related aggression and impulsivity (Maes *et al.*, 2001). It has also been suggested that pedophilia may be a subtype of obsessive–compulsive disorder; a problem that is marked by repetitive, irrepressible behaviour associated with serotonin dysregulation (Balyk, 1997).

Sexual Masochism and Sexual Sadism

The DSM-IV defines sexual masochism as "recurrent, intense sexually arousing fantasies, sexual urges, or behaviours involving the act (real, not simulated) of being humiliated, beaten, bound, or otherwise made to suffer" (American Psychiatric Association, 1994). In 1886, Krafft-Ebing coined the term, masochist, after Leopold von Sacher-Masoch, who wrote novels depicting men being humiliated and bound by females. Sexual sadism is characterized by "recurrent, intense sexually arousing fantasies, sexual urges, or behaviours involving acts (real, not simulated) in which the psychological or physical suffering (including humiliation) of the victim is sexually exciting to the person" (American Psychiatric Association, 1994). The term, sadism, was derived from writings of the Marquis de Sade, an 18th century author who wrote stories depicting sexual torture and brutality. A distinction is drawn between minor versus major sexually sadistic acts. Minor sexually sadistic acts would include, for example, humiliation and bondage of a willing sexual masochist while major sexually sadistic acts would involve acts such as sexual torture and rape of an unwilling participant. The key distinction here is whether the victim was consenting or not.

The practice of sadomasochism (referred to as S&M), or the consensual participation between sexual sadist and sexual masochist, involves carrying out predetermined sexual scenarios. These scenarios commonly involve several themes: flagellation (usually on the buttocks), bondage, 'water sports' (urophilia — attraction to urine, coprophilia — attraction to feces, and mysophilia — attraction to filth), and penis and nipple torture (Arndt, 1991). Sadomasochists interviewed in New York and San Francisco between 1976 and 1983 reported S&M activities that included elements of dominance and submission, role-playing (e.g., master and slave), consensuality (i.e., both participants were willing), and were of sexual context (i.e., the role-playing was sexual) (Weinberg *et al.*, 1984). Commonly reported S&M role-play themes include: "severe boss and the naughty secretary", "the queen and many slaves", "the male barber and his customer", and "arrest scenes and military training" (Sandnabba *et al.*, 1999). Although the sexual sadist appears to be in control, often the degree of domination and humiliation is agreed upon earlier, and it is the sexual masochist who indicates with a predetermined cue when he/she has reached his/her limit (Arndt, 1991).

Female sexual masochists and sadists are outnumbered by male sexual masochists and sadists and in many cases, the females are prostitutes who specialize in sadomasochism. One study found that approximately a quarter of female sexual sadists are prostitutes (Breslow *et al.*, 1985). Approximately 80% of sadomasochists reported that they were regularly engaging in sadomasochistic activities by age 30 years (Sandnabba *et al.*, 1999). Spengler (1977) obtained questionnaire data from 245 male sadomasochists recruited through S&M magazine advertisements and via S&M clubs. The majority of respondents reported that they met partners through sadomasochism advertisements, clubs, or bars. The sample contained 30% heterosexual sadomasochists, 31% bisexual sadomasochists, and 38% homosexual sadomasochists. The respondents came from all ages, socioeconomic backgrounds and levels of education. In most cases, the families knew little if anything about the respondents' S&M activities; 41% of married respondents ($n = 109$) reported that their wives knew nothing about the sadomasochistic activity.

When queried whether they thought the sadomasochistic behaviour was acceptable, 70% indicated acceptance of the behaviour, 85% reported that they "want to do it again", "it was fun" (84%), and "sexually satisfying" (79%). Although many of the respondents reported that they enjoyed non-sadomasochistic sexual activity, they reported being more likely to orgasm with sadomasochistic activity (79%) than without (45%). About a third of respondents reported fetishisms (e.g., boots and leather).

Very few studies have been conducted examining sexual sadists who target unwilling victims. Seto and Kuban (1996) examined penile volume changes in seven sadistic rapists compared to 14 non-sadistic rapists and 20 controls. The subjects were presented audiotapes depicting five different scenarios: (1) nonviolent, non-sexual interaction with a female; (2) consensual sexual activity with a female; (3) non-sexual violence against a female; (4) rape; and (5) violent rape. Compared to controls, the sadistic rapists and non-sadistic rapists were equally aroused by the different types of sexual contact — they were less likely to differentiate between consensual sexual activity, rape, and violent rape.

A subset of sexual sadists may have abnormal endocrine activity although hormone levels typically do not differ between sexual sadists and controls. In a review of individual cases, one sexual sadist had unusually high levels of luteinizing hormone (stimulates progesterone secretion) and follicle-stimulating hormone (stimulates estradiol in women and sperm development in men), another had low testosterone levels and another Klinefelter's syndrome (XXY chromosomes rather than the typical XY male pattern). Gross examination of brain functioning revealed no differences between sexual sadists and controls, but more careful examination

revealed a subtle but significant difference in the right temporal lobe. Forty-one percent of the sexual sadists had a slightly dilated right temporal horn, compared to 13% of controls. One sexual sadist had a slow growing tumour in the left frontal-temporal lobe, likely present since childhood. Another had enlargement of the ventricles, a condition typically associated with schizophrenia and suggestive of overall brain atrophy. In short, temporal lobe abnormalities may be implicated in sexual sadism, but more information is needed before any strong conclusions can be made (Langevin *et al.*, 1988).

Serial killing, which is often reported in the media and dramatized in movies, may reflect comorbid sexual sadism and antisocial personality disorder. Geberth and Turco (1997) examined records of 387 serial murderers within the United States and found that 248 had sexually assaulted their victims. These included famous cases of serial killing, such as Theodore (Ted) Bundy and the Green River Killer. Of these, they determined that 68 met DSM-IV criteria for both sexual sadism and antisocial personality disorder (in other cases, sufficient data were not available to make a determination). These 68 individuals displayed a pattern of behaviour characterized by childhood aggressiveness and antisocial behaviour, and a pattern of killing involving sexual violence, humiliation, domination and control. Examination of their records suggests that these 68 individuals engaged in sexual violence and killing because they derived pleasure from it.

Courtship Disorders: Voyeurism, Exhibitionism, and Frotteurism

Voyeurism, exhibitionism, and frotteurism may be different behavioural expressions of a single underlying courtship disorder. The overt behaviours differ, but can also be conceptualized as different stages on a continuum — different degrees of proximity to the victim. Voyeurism involves viewing the victim from a distance, exhibitionism involves approaching the victim, and frotteurism involves physically touching the victim. The preference for rape over consensual sexual activity (termed the preferential rape pattern) may represent the fourth phase in the courtship disorders (Freund *et al.*, 1983). A common aetiological factor has not been identified although evidence indicates that the courtship disorders are associated with a preference for eliciting an alarmed reaction from an unfamiliar target rather than any lack of interest in intercourse (Freund and Watson, 1990). A high degree of comorbidity exists between these disorders and even when no overt comorbid behaviour is present, some evidence suggests that presence of one disorder predisposes to another such disorder (Freund *et al.*, 1983).

Voyeurism

The DSM-IV defines voyeurism as "recurrent, intense sexually arousing fantasies, sexual urges, or behaviours involving the act of observing an unsuspecting person who is naked, in the process of disrobing or engaging in sexual activity" (American Psychiatric Association, 1994). Most men, if given the opportunity to view a woman disrobing, would not avert their eyes. A man who engages in an opportunistic 'peep' is not a voyeur, the peeping must be recurrent and the urges to do so intense. Voyeurs tend to be the youngest child in the family. Compared to other sex offenders and controls, voyeurs have fewer sisters, have a good relationship with both parents, but have parents who do not have a good marital relationship. Voyeurs are often underdeveloped socially and sexually. They tend to engage in sexual activity later than other groups, and are less likely to marry than controls and other sex offenders (Smith, 1976). The more sexually experienced a voyeur, the more frequently he is likely to engage in peeping behaviour (Langevin *et al.*, 1985). Some evidence suggests that voyeurs may be predisposed to other paraphilias as well (e.g., sadomasochism, zoophilia) (Langevin *et al.*, 1985).

Although voyeurism is rare in women, some evidence suggests that women have similar 'peeping' urges as men. Friday (1975) interviewed women from all ages (teen to retirement) and walks of life and found that women expressed fantasies about peeping and, in some cases, engaged in actual peeping behaviour.

Learning theorists have suggested that voyeurism develops when the subject is provided a voyeuristic opportunity, and then subsequently masturbates while fantasizing about the experience. Some evidence supports this hypothesis; 50% of voyeurs reported that prior to the onset of their peeping behaviour they believed that normal sexual relations were not likely to be an option for them, and so they fantasized about scenarios they believed to be more obtainable, such as peeping. In addition, 75% of voyeurs reported that the sexual scenario they envision while masturbating reflected their first peeping experience (Smith, 1976).

Exhibitionism

Exhibitionism is defined as "the exposure of one's genitals to an unsuspecting stranger" (American Psychiatric Association, 1994) and involves some form of sexual gratification. Exhibitionism occurs almost exclusively in men. Very few cases of female exhibitionists have been reported in the literature, but the characteristics of these women differed from typical male exhibitionists. Male exhibitionists tend to be timid and unassertive men who have underdeveloped social skills and who are uncomfortable with angry or hostile feelings. Some studies suggest that exhibitionists were more likely to have been raised in a sexually puritanical background. The few female exhibitionists described in the literature, and studies examining female strippers, would suggest that the majority of female exhibitionists gain no pleasure from exposing their genitals but do so either to gain money or attention (Blair and Lanyon, 1981).

Behavioural theory proposes that exhibitionism develops as a result of a learned behaviour that is subsequently reinforced. This theory has been applied successfully to the treatment of exhibitionism (i.e., a learned behaviour can be replaced with a more socially acceptable behaviour) but it is not clear whether this reflects the actually aetiology of exhibitionism. Attempts to identify a physiological cause of exhibitionism have thus far been unsuccessful.

Frotteurism

Frotteurism involves "intense sexually arousing fantasies, sexual urges, or behaviours involving touching and rubbing against a non-consenting person" (American Psychiatric Association, 1994). The majority of published articles on this disorder group frotteurism with other paraphilic disorders or report cases of men with multiple paraphilias, including frotteurism. Abel *et al.* (1987) examined 62 males diagnosed with frotteurism, as well as other paraphilic disorders, and found that, at the time of the interview, they had committed an average of 849 frottage acts. Rooth (1973) interviewed 561 nonincarcerated men with paraphilias and found that of those exhibiting frotteurism, 79% had other paraphilias, with an average of 4.8 paraphilias each.

It is unclear whether true frotteurism in women exists, perhaps in part because of the decreased likelihood that male victims would view the behaviour as unwelcome or threatening. A handful of case reports of sexual molestation of men by women have been reported in the literature. The molestation typically occurred subsequent to erectile failure or inhibited desire (Sarrel and Masters, 1982). Although these cases do not represent female frotteurism, they suggest that it is feasible that rare cases of female frotteurism may exist, but are rarely reported.

Treatment of Paraphilias

In the mid 1900s, some European countries used castration as a means of treating exhibitionism, pedophilia, and other forms of sexual crimes. In West Germany, psychosurgery, which involved removing the nucleus ventromedialis of the hypothalamus, was used as a treatment for male sex offenders. Published reports of these practices rarely provided sufficient information to determine whether this intervention was successful in eliminating the inappropriate sexual behaviour. Of course there are serious consequences to performing such extreme and permanent techniques.

Cognitive–behavioural therapies, such as aversion therapy, are often used to treat paraphilias. The arousing stimulus is paired with an aversive stimulus such as a shock or noxious odour until the paraphilic behaviour no longer produces sexual arousal. A review of the handful of studies and case reports published suggests that aversion therapy alone is effective in reducing arousal, but that relapse rates are high (Kilmann *et al.*, 1982). More recently, other forms of cognitive–behavioural therapy such as covert sensitization or orgasmic reconditioning, are being used. Orgasmic reconditioning involves fantasizing about the paraphilic behaviour while masturbating, and at the moment just before orgasm, switching the fantasy to a more acceptable stimulus, such as one's partner. The belief is that orgasm, being an intensely pleasurable sensation, will serve to reinforce the more accepted sexual fantasy. Few well-controlled treatment outcome studies have been published, however, making it difficult to determine whether these types of interventions are effective. Covert sensitization involves fantasizing about the paraphilic behaviour followed by imagining a noxious scenario, such as vomiting, or an undesirable consequence such as being discovered by one's family. It is not yet clear how successful these techniques are in eliminating the behaviour although a few reports indicate that they can be highly successful for some patients.

Pharmacological interventions include hormonal supplements or psychotropic medications. Hormonal treatments are designed to inhibit deviant sexual behaviour by reducing sexual drive and sexual arousal. They include the following: (1) oestrogen; (2) medroxyprogesterone acetate (MPA), which lowers plasma testosterone and reduces gonadotropin secretion; (3) luteinizing hormone-releasing hormone agonists (LHRH agonists), which produce the pharmacological equivalent of castration by significantly inhibiting gonadotropin secretion; and (4) antiandrogens such as cyproterone acetate (CPA), which blocks testosterone uptake and metabolism. Treatment outcome studies suggest that these treatments are effective in reducing deviant sexual behaviour provided that the treatment regimen is maintained, although more well-controlled treatment outcome studies are needed before the true effectiveness of these treatments can be determined. Psychotropic medications that affect the serotonin systems have recently been used to treat paraphilias. Clinical studies suggest that SSRIs such as Prozac are effective in reducing paraphilic arousal and may be effective in reorienting arousal to more socially acceptable scenarios. The effectiveness of SSRIs in reducing paraphilic fantasies and behaviours suggests that these disorders may have an obsessive–compulsive component, as SSRIs are often used to treat obsessive–compulsive disorders. As with hormone treatments, however, more well-controlled treatment outcome studies must be conducted before the true effectiveness of these treatments can be determined (Bradford, 2000).

GENDER IDENTITY DISORDER

The DSM-IV describes gender identity disorder as a persistent and strong cross-gender identification and a persistent unease with one's sex. Gender identity disorder is not diagnosed if these symptoms co-occur with a physical intersex condition. As with the sexual disorders, a diagnosis is only made if the symptoms produce marked distress or impairment. According to the DSM-IV, gender identity disorder can occur in childhood, adolescence, and adulthood. Sexually mature individuals may be heterosexual, homosexual, bisexual, or may feel little sexual attraction to either men or women (American Psychiatric Association, 1994). Gender identity disorder is often confused with transvestism (cross-dressing) although the two are distinct.

When biological males and females feel a cross-gender identification, it is termed male-to-female transsexualism (MF) and female-to-male transsexualism (FM), respectively. Prevalence estimates suggest that MF transsexualism is more common than FM transsexualism although a few studies have found a 1 : 1 ratio. Prevalence estimates range from 1 : 10,000 to 1 : 100,000 for MF and 1 : 30,000 to 1 : 400,000 for FM (Cohen-Kettenis and Gooren, 1999; Zucker and Green, 1992).

Studies examining the biological causes of gender identity disorder have typically examined the effects of prenatal hormones on prenatal brain development. During normal prenatal development, the presence of testosterone leads to the development of external male genitalia and to a male differentiated brain. It is hypothesized that for individuals with gender identity disorder, a discrepancy may exist between prenatal genital differentiation and brain differentiation such that the external genitals develop, for example, as male while the brain develops as female. The evidence to support this hypothesis is mixed. Genetic females exposed to high levels of testosterone *in utero* (e.g., congenital adrenal hyperplasia), rarely develop gender identity disorder. Similar prenatal exposure to antiandrogenic, androgenic, and oestrogenic drugs rarely leads to gender identity disorder in either genetic females or males although some of these individuals display abnormal gender role behaviour (Cohen-Kettenis and Gooren, 1999). The strongest evidence to suggest that abnormal prenatal brain differentiation may lead to gender identity disorder comes from a recent study examining hypothalamic brain nuclei in men with gender identity disorder. Zhou *et al.* (1995) found that the central subdivision of the bed nucleus of the stria terminalis (a region of the hypothalamus) was smaller in MF transsexuals compared to normal males but similar in size to normal females, a difference that was not accounted for by hormone therapy. Sadeghi and Fakhrai (2000) recently reported a case of 18-year-old monozygotic female twins requesting gender reassignment surgery. The twins had a childhood history of cross-dressing. Unfortunately they were lost to follow up after the initial evaluation but this case suggests that gender identity disorder may have a genetic component.

Recent studies indicate that, compared to controls, MF transsexuals have more older brothers (but not more older sisters) and a later birth order (Blanchard *et al.*, 1995; Zucker *et al.*, 1997). Conversely, FM transsexuals are more likely to have several younger sisters but not brothers compared to controls (Zucker *et al.*, 1998). The histocompatibility-Y antigen (H-Y antigen), which is responsible for the development of the male testes and brain differentiation, may be implicated in this process for males. With progressive male births, mothers may become immunized to the H-Y antigen, leading to increased production of H-Y antibodies, and a disruption in normal brain differentiation (Blanchard *et al.*, 1998).

Social, parental, or familial factors have been associated with mild gender disturbance. MF transsexuals often report over controlling, rejecting fathers. FM transsexuals often report mothers and fathers who were rejecting and mothers who were over protective. It is feasible, however, that these differences may have been the result of abnormal gender development, rather than the cause (Cohen-Kettenis and Gooren, 1999).

Childhood gender identity disorder may, in some cases, predict adult gender identity disorder. Fifty-five feminine boys with gender

identity disorder were followed into early adulthood. Five of the feminine boys were diagnosed with gender identity disorder, one as a transvestite, 21 as homosexual, 14 as heterosexual, and 14 that were not rated. This suggests that childhood gender identity disorder reflects a high likelihood of either adult gender identity disorder or homosexuality (Green, 1987).

In cases where gender identity disorder is present, if the individual displays only a mild tendency, displays serious psychopathology, or is not functioning well socially, psychotherapy rather than sex reassignment surgery may be advised. For those with extreme symptoms of gender identity disorder, who are free of from psychopathology, and who are functioning well in society, sex reassignment surgery is still not permitted until the person has lived full time as the preferred gender, often for a period of 2 years. During this period, candidates may be required to change their name, inform their family, boss, and co-workers, cross-dress full time, and receive hormone treatment. This period is considered to be essential for determining whether surgery is appropriate. The candidates have the opportunity to experience what it is like to live as the other gender and to determine whether they are fully prepared for and fully comprehend the impact of living the remainder of their lives as the other sex (Cohen-Kettenis and Gooren, 1999).

A review of sex reassignment surgery outcome studies suggests that in most cases, surgery resolves the gender identity disorder. Depending on the study, between 71% and 97% of subjects were successfully treated with surgery and less than 1% later took steps to reverse the sex reassignment. Factors that predict a poor outcome include: misdiagnosed transvestism, poor surgery outcome, poor social or work functioning, suicidal tendencies, and sex reassignment surgery late in life. This suggests that the current procedure for determining appropriateness of sex reassignment surgery is effective, when applied strictly (Cohen-Kettenis and Gooren, 1999). Male to female transsexuals who are attracted to men (MF homosexuals) seem to have a better post-surgery outcome compared to MF transsexuals who are attracted to women (MF heterosexuals). MF heterosexuals may have a poorer post-surgery outcome because of the added stigma of becoming homosexual after surgery, and because they typically present for surgery much later in life than MF homosexuals and thus are likely to have more male-role investments (e.g., husband, father). FM transsexuals in general have better post-surgery outcome than MF transsexuals (Cohen-Kettenis and Gooren, 1999).

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XXIII

Eating Disorders

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XXIII-1

Animal Models of Eating Disorders

Jeanette E. Johansen and Martin Schalling

INTRODUCTION

Eating disorders such as obesity, anorexia and bulimia are complex disorders displaying a variety of symptoms apart from an abnormal eating behaviour. Like many other motivated behaviours, feeding requires the integration of internal and external signals and it is not clear if the physiological correlates observed in these disorders are causes or effects of the altered eating behaviour. A good understanding of the physiology underlying feeding behaviour is therefore essential. This chapter deals with some of the many animal models that are being used to study feeding behaviour.

Early animal models of eating disorders include experimental studies of the effect of anorectic drugs on the amount of food consumed by rats. However, while successful in rats, pharmacological treatment of obesity is generally unimpressive in terms of weight loss in humans for a number of reasons.

That tumours in the region of the hypothalamus can cause obesity has been known for a long time. In 1940, Hetherington and Ranson confirmed the importance of the hypothalamus in the control of feeding and body weight. By performing electrolytic lesions in the hypothalamus of rats, they observed: 'A condition of marked adiposity characterized by as much as a doubling of body weight and a tremendous increase of extractable body lipids. . . ' (Hetherington and Ranson, 1940). The damaged regions included the dorsomedial and ventromedial hypothalamic nuclei (DMH and VMH), the arcuate nucleus (Arc), the fornix, the lateral hypothalamic area (LHA) ventral to the fornix and possibly also the ventral premammillary nucleus. They also noted that lesions in the adjacent lateral hypothalamus could lead to decreased food intake. Anand and Brobeck pursued this observation and showed that bilateral electrolytic lesions of the LHA caused loss of feeding and even death by starvation (Anand and Brobeck, 1951). Thus, the concept arose of the LHA serving as a 'feeding centre' and the VMH as a 'satiety centre' — the dual centre model. This hypothesis has been widely questioned, and among the observations speaking against the dual centre model are findings that damage outside the hypothalamus can produce syndromes similar to those seen after lesions of the LHA or VMH. As cell-specific lesion methods emerged the focus was once again put on the VMH and LHA, and several studies showed that the LHA indeed could have a phagic function (Saper, 1985; Saper *et al.*, 1986; Bittencourt, 1992). Today we know that hypothalamic cell populations and nuclei play important and specific roles in the regulation of food intake and other motivated behaviours.

Most of the population practices weight control, but in spite of that, weight seems to be stable in both lean and obese individuals. Dieting is usually not successful in the long run and most obese individuals eventually regain the lost weight (Wadden, 1993). The relative stability of weight in individuals indicates that there is a feedback loop controlling energy balance and maintaining constancy of total body energy stores. In 1953, Kennedy introduced his theory on a lipostatic mechanism that maintained energy homeostasis (Kennedy, 1953). He suggested that the size of the fat depots were sensed by a lipostat, which would regulate and adjust food intake and energy metabolism accordingly, to maintain body weight at a set point. He also proposed that an impaired lipostatic mechanism could lead to obesity. Further support for this hypothesis came from a study by Hervey (1958). He performed a series of parabiosis experiments, where the circulations of two animals are surgically joined, and showed that lesions in the VMH in one of the members in a parabiotic rat pair caused the lesioned rat to become obese as the unlesioned rat starved to death (Hervey, 1958). He suggested that the obese lesioned rat produced excessive amounts of a satiety factor that was transferred to the unlesioned rat, causing it to starve itself.

Over the past five years there has been a tremendous increase in the understanding of the genetic regulation of food intake and energy expenditure, using monogenic rodent models of obesity. Several genes have been cloned that, when mutated, cause obesity in the mouse and rat (Schalling *et al.*, 1999; Barsh *et al.*, 2000). These genes and their products have unravelled biochemical pathways involved in obesity. Some of these genes have been shown to be important for the regulation of food intake and/or metabolism also in humans. Crosses of mouse, rat, pig or chicken strains that are informative with regard to body mass or body fat have produced a number of quantitative trait loci (QTL) (Chagnon *et al.*, 2000) that have opened the door to polygenic approaches in the study of obesity in animals. As with the monogenic rodent models, these QTLs can be applied to a human genetic obesity map by identifying the syntenic chromosomal regions in the human.

There has been less of a focus on genetic models of anorexia. One reason might be that there are more genetic animal models that shift the regulation of food intake, satiety or metabolic turnover towards obesity than anorexia. A possible explanation for this could be that even a relatively mild anorectic phenotype could lead to malnutrition and/or death by starvation early enough in life to affect the number and viability of the offspring (see the anorexia mouse, the dopamine-deficient mouse and the HNF-3α deficient mouse below). A number of starvation, dehydration and chemically induced models have been developed and an example of each is discussed below. There are also numerous models of anorexia induced by either infection or cancer. Anorexia, or cachexia, is a frequent complication of malignant tumours and infectious and inflammatory diseases and is contributing significantly to the mortality of these disorders (Kotler *et al.*, 1985; Tisdale, 1997; Larkin, 1998). This type of anorexia will not be dealt with in this chapter.

There are very few animal models of bulimia or binge eating. The models that exist are all based on cycles of food restriction

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and refeeding. The lack of animal models for bulimia and binge eating may be related to the opinion of many investigators that an animal model should mimic all the important aspects of human syndromes.

Because of the enormous toll on human health taken by eating disorders and their related disorders, there is a need for a better understanding of the underlying mechanisms for regulation of body weight and food intake. This chapter attempts to review some of the available animal models for studying eating disorders and the mechanisms behind the control of food intake. Because of the considerable uncertainty regarding the biological basis of eating disorders in humans, and the possibility that they are symptoms of personality traits, the development of animal models is complicated. Furthermore, for the same reasons validation of existing animal models of eating disorders is compromised. For face validity animal models of eating disorders should involve a change in body weight. However, there are many possible reasons why an animal lose or gain weight without having an altered eating behaviour. Therefore, measures of food intake are also required. One should also keep in mind that reduced or increased food intake is not necessarily the result of a change in appetite. Moreover, eating disorders in humans often occur under conditions where food is freely available and therefore it may be inappropriate to use models with restricted access to food as is often the case in pharmacological studies of rat feeding behaviour. Lack of potent pharmacological agents for treatment of human eating disorders make it difficult to assess predictive validity for animal models. Despite substantial research efforts, the nature of the underlying behavioural mechanisms for eating disorders remains unclear. Construct validity is therefore extremely hard to determine. A better description of the molecular mechanisms involved in human eating disorders would be invaluable to the animal modeller and paradoxically this valuable information may be gained from the study of animal models. Even though the animal models described in this chapter may not mimic all of the aspects of human eating disorders they may provide useful information on underlying causes and how to treat at least some of the symptoms. In fact, several animal models have been used as hypothesis generators and in some cases they have been most helpful in revealing the nature of specific human eating disorders.

A brief description of three genetic and three physiological models of anorexia, and one model of bulimia will be followed by a more comprehensive review of genetic models of obesity.

MODELS OF ANOREXIA

Genetic Models of Anorexia

The Anorexia Mouse

The autosomal recessive *anx* mutation arose at the Jackson laboratory (Bar Harbor, ME, USA) in 1976. Mutant mice (*anx/anx*) are characterized by poor appetite. Stomach contents are reduced compared to normal littermates at about post-natal day 5 and continue so until death (Maltais *et al.*, 1984). Interestingly, although the amount of food ingested is reduced, the daily pattern of food intake of *anx/anx* mice is very similar to that observed in normal littermates from birth to 20 days of age (Maltais *et al.*, 1984). These data indicate that *anx/anx* mice fail to properly regulate the amount of food consumed rather than failing to eat for other reasons. Other characteristics are reduced body weight, emaciated appearance and abnormal behaviour including body tremors, headweaving, hyperactivity and uncoordinated gait. The animals die at the age of 3 to 5 weeks depending on the genetic background. No organ abnormalities have been found using routine stained sections. Total RBC, hematocrits, haemoglobin and mean cell volume are within normal range (Maltais *et al.*, 1984). However, there are several dramatic alterations of peptide distributions in the hypothalamus of *anx/anx* mice, relevant to the regulation of feeding (Broberger *et al.*, 1997, 1998, 1999; Johansen *et al.*, 2000).

Abnormalities of feeding-related peptides have been described in the *anx/anx* mouse, particularly in the arcuate nucleus of the hypothalamus, which contains neuronal populations producing orexigenic as well as anorexigenic substances. The feeding stimulatory peptides neuropeptide Y (NPY) and agouti gene-related protein (AGRP) are distributed in a pattern suggestive of accumulation in the cell bodies in the arcuate nucleus of *anx/anx* mice instead of axonal transportation to their respective targets (Broberger *et al.*, 1997, 1998). Conversely, the pro-opiomelanocortin (POMC) derived peptides and cocaine- and amphetamine-regulated transcript (CART) peptides, which decrease food intake (Fan *et al.*, 1997; Kristensen *et al.*, 1998), are both decreased in the arcuate nucleus of the *anx/anx* mouse (Broberger *et al.*, 1999; Johansen *et al.*, 2000). In addition, morphological characteristics of POMC neurons are altered, as seen with immunohistochemical markers (Broberger *et al.*, 1999). Taken together these studies suggest that the reduced food intake and weight loss seen in the *anx/anx* mouse may be related to neurochemical alterations in the hypothalamus and particularly in the arcuate nucleus.

The abnormal behaviour of head weaving, body tremors, uncoordinated gait and hyperactivity of *anx/anx* mice is affected by serotonin (Maltais *et al.*, 1984). When treated with the serotonin precursor 5-hydroxy-DL-tryptophan (5-HTP) 15-day-old normal mice display the same type of abnormal behaviour as *anx/anx* mice. Similarly, 15-day-old anorexic mice show body tremors and head weaving typical for anorexic mice 18 days of age or older, when treated with 5-HTP. Conversely, treating a 20-day-old anorexic mouse with the serotonin neurotoxin 5,7-dihydroxytryptamine, diminishes the severity of the neurological symptoms (Maltais *et al.*, 1984). *anx/anx* mice have also been shown to have an increased number and density of serotonergic fibres in the forebrain and the arcuate nucleus (Son *et al.*, 1994; Jahng *et al.*, 1998), which would be consistent with the experimental data on eating and motor behaviour.

Taken together these results suggest that the abnormal feeding behaviour observed in *anx/anx* mice is caused by a CNS defect.

The Dopamine Deficient Mouse

Dopamine (DA) is a classical neurotransmitter in the central nervous system (CNS) that has been implicated in the regulation of food intake, but the neural pathways are not yet established. Some pharmacological reports suggest a stimulatory role for DA agonists, and an inhibiting role for DA antagonists in the control of food intake (Phillips and Nikaido, 1978; Dourish, 1983; Salamone *et al.*, 1990). However, opposite results have been obtained in other studies (Sanghvi *et al.*, 1975; Cooper and Al-Nasar, 1993).

Dopamine (DA) deficient mice (*DA*-/-) were created by disruption of the dopamine synthesizing enzyme tyrosine hydroxylase (TH) gene specifically in dopaminergic neurons (Zhou and Palmiter, 1995). *DA*-/- mice are born normal but gradually become hypoactive and aphagic and die prematurely at about three weeks of age (Zhou and Palmiter, 1995; Szczypka *et al.*, 1999). When *DA*-/- mice are born, they initiate suckling behaviours and nurse, however, after approximately 2 weeks, when normal mice begin to explore other sources of food, the *DA*-/- mice become lethargic and fail to eat and drink. Daily treatment with L-DOPA

normalizes feeding behaviour and restores locomotor activity in *DA-/-* mice (Szczypka *et al.*, 1999). It is unlikely that the aphagia is secondary to motor deficits as *DA-/-* mice can grasp and swallow food when put in their mouth (Zhou and Palmiter, 1995). Also, *DA-/-* mice can execute behaviours necessary to seek and ingest food (Szczypka *et al.*, 1999). The *DA-/-* mice provide further evidence for the importance of dopamine for normal feeding behaviour.

That dopamine plays an important role in the regulation of food intake is a conclusion that was reached many years ago by several investigators. Studies by Anand and Brobeck (1951) showed that bilateral electrolytic lesions of the LHA in rats produced aphagia and akinesia. Evidence that DA could be a critical player in the LHA-lesion syndrome came from the demonstration that most of the symptoms of LHA ablation could be reproduced by introducing bilateral lesion of midbrain dopaminergic pathways in rats by the use of 6-hydroxydopamine (6-OHDA) (Ungerstedt, 1971; Zigmond and Stricker, 1972; Fibiger *et al.*, 1973). Furthermore, multiple connections between LHA and midbrain DA neurons have been identified (Bunney and Aghajanian, 1976; Phillipson, 1979; Wright *et al.*, 1980). It is remarkable that the phenotypes resulting from 6-OHDA and LHA lesions are so similar to the genetic model described here, given that the damage produced by a lesion greatly exceeds that resulting from the removal of one enzyme.

The Hepatocyte Nuclear Factor 3α-Deficient Mouse

The hepatocyte nuclear factor 3 (HNF-3) family of transcription factors include three different genes designated HNF-3α, HNF-3β and HNF-3γ, which have been suggested to play a critical role in pancreatic islet function (Duncan *et al.*, 1998). HNF-3α has been shown to be of importance for pancreatic alpha-cell function, including glucagon gene expression (Kaestner *et al.*, 1999). Mice lacking HNF-3α expression, due to targetted disruption of this gene, develop a complex metabolic syndrome characterized by abnormal feeding behaviour, progressive starvation, hypoglycaemia, wasting and neonatal mortality between days 2 and 14 (Shih *et al.*, 1999). The molecular and physiological mechanisms by which HNF-3α regulates food intake are currently not understood. However, HNF-3α is highly expressed in the hypothalamus (Shih *et al.*, 1999). Thus, it cannot be ruled out that the abnormal feeding behaviour observed in HNF-3α deficient mice is caused by a CNS defect.

Conclusion

There is increasing evidence suggesting that anorexia nervosa is genetic in nature (see Chapter XXIII-9: 'The evolving genetic foundations of eating disorders' by Klump *et al.*). The genetic mouse models described here probably have their strength in being hypothesis generators more than being models mimicking the human situation. The diagnostic criteria for anorexia nervosa are body weight <85% of expected weight, amenorrhea, intense fear of weight gain and inaccurate perception of own body size, weight or shape (DSM-IV, 1994). The latter two criteria are impossible to measure in animals, resulting in a low face validity for any model of anorexia. In the three models described above the face validity will be even lower as they all die prematurely before reaching puberty and amenorrhea can thus not be detected. Furthermore, the low food intake observed in the described models, is most likely caused by a reduced appetite for food. This is not the case in the human situation where the reduced food intake seems to be driven by an intense fear of weight gaining. Thus, construct validity also scores low in these models. Nevertheless, these models have their strength in allowing the identification of novel pathways involved in regulation of food intake. Genes in these pathways could certainly serve as targets for future therapeutic interventions.

Physiological Models of Anorexia

Starvation Induced Hyperactivity in Rat

If young rats are restricted to 90 minutes access to food every day, they will adapt and eat sufficient amounts to survive in good health. However, if a running wheel is available and food is restricted, the rats will start to run (Epling *et al.*, 1983). The running distance increases rapidly day by day and at the same time food consumption decreases and the rats begin to lose weight. Running may exceed 15 000 m per day, while food consumption decreases to 1 g per day or less (Epling *et al.*, 1983). In this model starvation and hyperactivity mutually reinforce each other resulting in a vicious circle where the rats starve and run themselves to death. There are major differences between male and female rats. Female rats develop running activity much faster and their activity is modulated by the menstrual cycle (Pirke *et al.*, 1993). Eventually, the menstrual cycle disappears in female rats. The reproductive function is impaired in both male and female rats, as a consequence of the starvation-induced hyperactivity (Pirke *et al.*, 1993).

It has been suggested that endogenous opioids play a role in this excessive activity (Boer *et al.*, 1990). However, data on central endorphin turnover and more detailed studies of endorphin agonists and antagonists are needed to clarify the role of opioids in this animal model. Leptin (an adipocyte derived satiety factor) has also been implicated to play a role in starvation-induced hyperactivity. Interestingly, it has been shown that leptin suppresses starvation induced hyperactivity in rats (Exner *et al.*, 2000). Also, patients with anorexia nervosa rank their motor restlessness higher when hypoleptinaemic, in the emaciated state, than after reaching maximal leptin levels, after treatment (Exner *et al.*, 2000). Thus, it is possible that hypoleptinaemia may contribute to the hyperactivity associated with anorexia nervosa. It should, however, be noted that it is not known whether the patients are restless because of the emaciation or the hypoleptinaemia.

This experimental protocol provides an animal model for the human activity anorexia syndrome (Epling *et al.*, 1983; Epling and Pierce, 1985). One should, however, keep in mind that the interpretation of the hyperactive behaviour seen in patients with anorexia nervosa has been discussed. Some argue that anorectics increase their activity for the purpose of burning more energy although this cannot explain their total weight loss, other investigators believe that the increased activity could be biological and involuntary. In 1994, Davis *et al.* showed that anorectic patients have an increased urge to be active during accelerated weight loss. This animal model, where starvation and hyperactivity mutually reinforce each other, supports the view that the increased activity is involuntary. However, there is no clear evidence for the applicability of this model in human anorexia nervosa.

Dehydration-Associated Anorexia

Dehydration is a homeostatic challenge resulting in a series of well-characterized endocrine, autonomic and behavioural motor responses. These are directed towards minimizing the impact of dehydration as rapidly as possible. Such responses are for example modifications of the ingestive behaviours so that water seeking behaviours increase and food seeking behaviours decrease (Hsaio, 1967). Thirst is the most obvious effect of dehydration, but when prolonged, dehydration also generates anorexia (Watts *et al.*, 1999). Dehydration can be induced by replacing the drinking water with 2.5% saline. By pair-feeding non-dehydrated rats with the same

Table XXIII-1.1 Animal models of anorexia

Model	Phenotype	Cause	Face validity	Construct validity
anx/anx mouse	Reduced food intake, gait disturbance, hyperactivity, premature death.	Recessive mutation in the *anx* gene.	++	++
DA-/- mouse	Aphagic, hypoactive, motor deficits, premature death.	Knockout of the TH-gene in dopaminergic neurons.	+	+
HNF3α-/- mouse	Progressive starvation, hypoglycaemia, neonatal mortality.	Knockout of the hepatocyte nuclear factor 3α.	++	−
Hyperactivity anorexia in rats	Increased activity and reduced food intake.	Starvation-induced hyperactivity.	+++	++
Dehydration-associated anorexia	Decreased food seeking behaviour.	Dehydration induced.	++	−
TCDD anorexia	Reduced food intake, wasting, permanent inhibition of weight gain.	Induced by TCDD.	++(+)	+

Lack of potent pharmacological agents makes it difficult to assess predictive validity for the models described. The genetic models of anorexia have their strength in allowing the identification of novel pathways involved in regulation of food intake serving as targets for future therapeutic interventions. Perhaps the most useful approach would be to combine the results from studies of genetic, physiological and biochemical models of anorexia, despite the weaknesses of each individual model. The 'Hyperactivity anorexia in rats' model has gained popularity as it mimics many aspects of human anorexia.

amount of food eaten by dehydrated rats and compare the neuronal and endocrine effects, one can distinguish between mechanisms causing anorectic behaviour from those occurring as a consequence of anorexia (Watts, 2000; Watts *et al.*, 1999).

This model is not applicable to human anorexia but may very well serve as a hypothesis generator and provide new insights in the complex regulation of food intake.

TCDD Induced Anorexia and Wasting Syndrome

The common environmental trace contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most potent anorexigens known (Pohjanvirta *et al.*, 1994). TCDD causes a starvation-like or wasting syndrome with food intake refusal and consequent body weight loss (Pohjanvirta and Tuomisto, 1994; Unkila *et al.*, 1993). A sublethal dose can cause permanent inhibition of body weight gain and the new body weight gain is defended against external manipulations (Seefeld *et al.*, 1984; Pohjanvirta and Tuomisto, 1990). This is suggestive of a specific effect of TCCD on body weight regulatory systems. Despite extensive studies, the physiological mechanisms behind this wasting syndrome are unknown.

Humans suffering from anorexia will gain weight once they start eating again. Thus, this model has little validity as a model for anorexia. However, the regulation of a 'body weight set point' is unknown and this model may provide valuable information on the mechanisms behind this complex system.

Conclusions on Models of Anorexia

As mentioned several times already all the models above have their strength as hypothesis generators. Taken together these models may be very informative despite the weaknesses of each model individually. Perhaps the most useful approach at this point in time would be to combine the results from studies of genetic, physiological and biochemical models of anorexia. With such a comprehensive approach it may be possible to gain insight into many, but not all, aspects of reduced food intake as a disease. We have attempted to evaluate these models comparatively in Table XXIII-1.1.

MODELS OF BULIMIA AND BINGE EATING

Not many animal models of binge eating and/or bulimia have been described; the few existing models are all based on cycles of food restriction and refeeding. In the model described by Hagan and Moss (1997) rats were subjected to 12 restriction-refeeding cycles of 6–8 days during an 84-day period. One group of animals was cycled in a bulimic-like pattern with restriction followed by palatable refeeding and one group was cycled through a non-bulimic-like pattern. Postcycling eating behaviour was tested under conditions of hunger, satiety and availability of palatable food in both groups. Rats with a history of cycles of restriction followed by hyperphagia (bulimic-like feeding) continued to exhibit persistent binge-eating behaviour even after a 30-day period of normalization (full feeding, no restriction). This effect was shown particularly with access to palatable food in sated conditions. This model implicates restriction and overeating as biological determinants of binge-eating behaviours.

Restriction alone cannot account for bulimia. Not everyone who restricts becomes bulimic. However, restriction does seem to be a major factor in the development of binge eating, an important feature of bulimia. This model may provide clues to possible mechanisms involved in the genesis of a bulimic episode.

MODELS OF OBESITY

The Leptin System

Obese (*ob*) and diabetes (*db*) are two recessive mutations in mouse that lead to hyperphagia, decreased energy expenditure and morbid obesity (Coleman, 1978). Parabiosis experiments with lean (wild-type) mice and obese (*ob/ob*) mice suppressed weight gain in the *ob/ob* mice, while parabiosis of lean (wild-type) mice and obese (*db/db*) mice caused hypophagia and weight loss in the former (Hausberger, 1959; Coleman and Hummel, 1969; Coleman, 1973, 1978). These results were interpreted as meaning that the *ob* mutation disrupted a circulating satiety factor. In the parabiosis experiment the normal lean mouse would deliver the satiety factor to the *ob/ob* mouse with subsequent weightloss. Similarly, the *db* mutation was believed to disrupt a component required for the response to a satiety factor. Through lack of feedback inhibition the *db/db* mouse was thought to over-express the satiety factor that it could not respond to. Consequently, excess satiety factor would be delivered to the normal mouse (with a fully functioning response) leading to profound weightloss in the normal mouse in the parabiosis experiment between *db/db* and normal mouse.

The *ob* gene was positionally cloned and named leptin (*Lep*) (Zhang *et al.*, 1994). There are two mutations of the *Lep* gene in mouse, Lep^{ob} and Lep^{ob2J}. The Lep^{ob} mutation results in a premature stop codon and synthesis of a truncated protein incapable of being secreted (Zhang *et al.*, 1994; Rau *et al.*, 1999). In the Lep^{ob2J} homozygous mouse mutant, a transposon inserted into the first intron of the *Lep* gene prevents the synthesis of mature *Lep* mRNA (Zhang *et al.*, 1994). In addition to obesity, the *ob* mutations in mouse also cause hyperinsulinemia, hypoglycaemia, hypercorticism, hypothalamic hypogonadism and hypothermia (Charlton, 1984). Mutations in the human *Lep* gene are rare but there are a few cases reported (Montague *et al.*, 1997; Strobel *et al.*, 1998; Rau *et al.*, 1999). The human *ob* mutations cause hyperphagia, obesity and hypothalamic hypogonadism, but unlike the *ob/ob* mice, hyperinsulinemia, hypoglycaemia, hypercorticism and hypothermia have not been reported in leptin deficient humans. This implies that although there are many similarities in the regulatory pathways, there are also distinct differences no doubt relating to the development of regulatory systems during the evolution of the respective species. This selection process will have to be taken into account when validating leptin mutations and their role as models for obesity.

The *db* mutation phenotypically mimics the *ob* mutation and was proposed to be a mutation in an *ob* receptor gene (Coleman, 1978). The cloning of the *db* gene confirmed that *db* indeed was a mutation in the leptin receptor gene (*Lepr*) (Tartaglia *et al.*, 1995; Lee *et al.*, 1996). Similar mutations in the *Lepr* gene have been shown to underlie the obese phenotypes of the *fatty* Zucker rat and the *corpulent* Koletsky rat (Chua *et al.*, 1996; Takaya *et al.*, 1996). There are five alternatively spliced forms of the receptor, Ob–Ra, Ob–Rb, Ob–Rc, Ob–Rd and Ob–Re (Lee *et al.*, 1996). Ob–Rb (or Ob–RL) is the longest form and has a long cytoplasmic region containing several motifs required for signal transduction (Chen *et al.*, 1996; Lee *et al.*, 1996). The other forms lack some or all of these motifs and may function as transport proteins (Lee *et al.*, 1996) or act in a buffering system for free, circulating leptin. Ob–R mutations are extremely rare in humans. There is, however, one report on a family with three obese sisters with a mutation in the *Lepr* gene (Clement *et al.*, 1998).

The Melanocortin System

Pro-opiomelanocortin (POMC) is a neuropeptide precursor molecule that is cleaved post-translationally in the hypothalamus to yield multiple peptides including α-melanocyte-stimulating hormone (α-MSH) (Smith and Funder, 1988). Melanocortin peptides have been demonstrated to inhibit food intake and recent data suggest that they may play a role in energy expenditure as well (Fan *et al.*, 1997; Haynes *et al.*, 1999).

α-MSH is the principle agonist of the neuronal melanocortin receptor isoforms, MC-3R and MC-4R (Gantz *et al.*, 1993a, 1993b). Both isoforms influence body weight, but they act through distinct and complementary mechanisms. MC-4R knockout mice ($Mc4r^{-/-}$) eat excessively resulting in maturity onset obesity syndrome associated with hyperphagia, hyperinsulinemia and hypoglycaemia (Huszar *et al.*, 1997). MC-3R knockout mice ($Mc3r^{-/-}$) show an increased body fat mass at the expense of a decreased lean muscle mass (Chen *et al.*, 2000). This is not caused by increased food intake, instead $Mc3r^{-/-}$ mice gain more fat per calorie of food consumed (Chen *et al.*, 2000). Mice lacking both receptor isoforms are more obese than mice lacking just MC-3R or MC-4R, probably because the double mutants not only eat more but also store ingested calories more efficiently (Chen *et al.*, 2000). Mutations in the MC-4R have also been reported to be associated with dominantly inherited pediatric obesity and hyperphagia in humans (Vaisse *et al.*, 1998; Yeo *et al.*, 1998).

The agouti gene was the first obesity gene cloned (Bultman *et al.*, 1992; Miller *et al.*, 1993). There are five known dominant mutations in the agouti gene that result in obesity and yellow pigmentation in mice. Normally agouti mRNA is expressed exclusively in the skin of neonatal mice, but mice carrying a dominant yellow allele have a ubiquitous expression pattern (Michaud *et al.*, 1994). The agouti protein is an antagonist of MC-4R in the brain and blocking of the receptor leads to increased food intake (Lu *et al.*, 1994; Fan *et al.*, 1997). This discovery led to the cloning of an agouti homologous protein normally expressed in the brain, the agouti-related protein (AGRP) (Shutter *et al.*, 1997). AGRP was shown to be an endogenous antagonist of MC-3R and MC-4R and overexpression causes obesity in mice (Fong *et al.*, 1997; Graham *et al.*, 1997; Ollmann *et al.*, 1997). No mutations in the AGRP gene have been found in humans.

The Fat Mouse

The fat phenotype includes obesity that develops between 6 and 8 weeks of age, hypoglycaemia and hyperinsulinemia (Coleman and Eicher, 1990). The hypoglycaemia only occurs in males and is transient whereas the hyperinsulinemia is consistent throughout life and associated with hypertrophy and hyperplasia of the islets of Langerhans in the pancreas (Coleman and Eicher, 1990). The syndrome does not progress to diabetes. This phenotype is the result of a missense mutation in the carboxypeptidase E (*Cpe*) gene (Naggert *et al.*, 1995). CPE is a secretory granule enzyme involved in dibasic cleaving of proproteins and prohormones including intermediates derived from proinsulin and pro-opiomelanocortin (Naggert *et al.*, 1995). The fat mutation (Cpe^{fat})results in a loss of CPE activity and proinsulin processing is aberrant in fat mutants (Naggert *et al.*, 1995). No mutations in the human *Cpe* gene have been reported. However, a mutation in the endopeptidase, prohormone convertase 1 (*PC1*) have been found in a patient with extreme childhood obesity (Jackson *et al.*, 1997). *PC1* acts proximally to *Cpe* in the pathway of post-translational processing of prohormones and neuropeptides. Thus, it has been suggested that defects in prohormone conversion may represent a generic mechanism for obesity both in humans and rodents.

The Tubby Mouse

The recessive tubby mutation (*tub*) causes maturity-onset obesity, including insulin resistance, accompanied by retinal and cochlear degeneration (Coleman and Eicher, 1990; Ohlemiller *et al.*, 1995; Ohlemiller *et al.*, 1997). The obesity of *tub/tub* mice is relatively mild and late in onset, resembling the weight gain in human populations more closely than that observed in the *ob/ob* and *db/db* mice. Weight gain occurs slowly and *tub/tub* mice reach about twice the weight of unaffected siblings. Tubby mice are not sterile but as they develop obesity they become infertile.

The tubby gene was identified by positional cloning (Kleyn *et al.*, 1996; Noben-Trauth *et al.*, 1996). The tubby phenotype is caused by a point mutation in a donor splice site resulting in a substitution of 44 amino acids in the carboxyterminal part of the Tub protein with 24 different amino acids encoded by the unspliced intron (Kleyn *et al.*, 1996). The aberrant transcript is expressed at elevated levels in tubby mice but expression at the protein level has not been investigated. The expression pattern of *tub* mRNA appears to be specific for the nervous system (Kapeller *et al.*, 1999) but the biochemical function remains unclear. However, structure-based functional analysis suggests that Tub is a bipartite transcription factor (Boggon *et al.*, 1999). It has not been established whether all tubby phenotypes (obesity, insulin resistance and retinal and cochlear degeneration) are attributable to the *tub* gene. It has not been ruled out that some features of the phenotype could be caused

by a tightly linked, but yet unidentified gene. No human examples of this mutation have been identified yet.

The Adult Mouse

The adult mutation (*Ad*) causes adult obesity and diabetes in a semidominant fashion (Wallace and MacSwiney, 1979). The obesity can be recognized at the age of 4 to 6 months and it is greater in homozygotes than in heterozygotes and it is more penetrant in heterozygote males than females (Wallace and MacSwiney, 1979). The mice are hyperinsulinemic but have normal blood glucose levels. The gene has not been identified and hence there are no known human examples of this mutation.

The 5-HT2CR Deficient Mouse

Serotonergic drugs are known to modulate appetite and serotonergic agonists with affinity for the serotonin (5-hydroxytryptamine, 5-HT) 2C receptor (5-HT2CR) such as mCPP, act as appetite suppressants (Kennett and Curzon, 1991; Kitchener and Dourish, 1994). Mice lacking 5-HT2CR, generated by the introduction of a nonsense mutation in the 5-HT2CR gene, are overweight as a result of overeating (Tecott *et al.*, 1995). 5-HT2CR transcripts have been detected in the paraventricular nucleus of the hypothalamus (Hoffman and Mezey, 1989), supporting the hypothesis of a central role for serotonin in the control of food intake. 5-HT2CR deficient mice provide a tool for elucidating some of the neurochemical pathways that underlie the regulation of food intake.

Psammomys Obesus

The sand rat *Psammomys obesus* (*P. obesus*) is a polygenic model of obesity and type II diabetes. In their native environment these animals remain lean and free from diabetes but when put on normal laboratory rodent diets a proportion of the animals develop metabolic abnormalities. These abnormalities include obesity, hyperinsulinemia, glucose intolerance, hyperleptinemia and diabetes (Barnett *et al.*, 1994a, 1994b; Collier *et al.*, 1997; Walder *et al.*, 1997). The spectrum of metabolic responses to obesity and the variability in the susceptibility to develop obesity in *P. obesus* makes this model more analogous to human obesity than the single gene obesity models.

The New Zealand Obese Mouse: A Model of the Metabolic Syndrome

New Zealand obese (NZO) mice exhibit a polygenic syndrome of hyperphagia and obesity associated with a type II diabetes-like syndrome of hyperinsulinemia and hypoglycaemia (Bielschowsky and Bielschowsky, 1953; Crofford and Davis, 1965; Veroni *et al.*, 1991). It has been shown that the NZO mouse has elevated levels of leptin in both adipose tissue and serum. Furthermore NZO mice fail to respond to peripheral recombinant leptin infusions with reduced food intake, indicating that they are leptin insensitive (Igel *et al.*, 1997). However, NZO mice respond to intracerebroventricular infusions of leptin with sensitivity similar to wild-type mice (Halaas *et al.*, 1997). It has therefore been suggested that the leptin resistance seen in NZO mice is the result of a diminished transport of leptin into the Cerebrospinal fluid (CSF) (Halaas *et al.*, 1997). Decreased levels of CSF leptin have been observed in some obese patients and the NZO mouse could thus have important implications for understanding the pathogenesis of human obesity (Schwartz *et al.*, 1996).

NZO mice also show symptoms of hypertension and hypercholesterolaemia (Ortlepp *et al.*, 2000). In humans, obesity associated with insulin resistance, dyslipidemia and hypertension is called the metabolic syndrome. The pathophysiological links between the different components of this syndrome is not fully understood and the NZO mouse presents a model for the study of these interactions.

Quantitative Trait Loci for Obesity

Quantitative trait loci (QTL) are produced by statistical analysis of genetic information derived from the offspring of crosses between informative strains with regard to, for example, body mass or fat content. It is believed that this will be a way of identifying susceptibility genes for complex disorders such as obesity. QTL

Table XXIII-1.2 Animal models of obesity

Model	Phenotype	Cause	Face validity	Predictive validity	Construct validity
ob/ob mouse	Obesity, hypoglycaemia, hypothermia, hyperinsulinemia, hypercorticism.	Recessive mutation in the *Lep* gene.	+++	+++*	+(+++)*
db/db mouse	Obesity, hypoglycaemia, hypothermia, hyperinsulinemia, hypercorticism.	Recessive mutation in the *Lepr* gene.	+++	n.d.	+(+++)*
fatty Zucker rat	Obesity, hyperphagia.	Recessive mutation in the *Lepr* gene.	+++	n.d.	+(+++)*
corpulent Koletsky rat	Obesity, hyperphagia.	Recessive mutation in the *Lepr* gene.	+++	n.d.	+(+++)*
Mc3r-/- mouse	Increased body fat mass.	Knockout of the *Mc3r* gene.	++	n.d.	+
Mc4r-/- mouse	Maturity-onset obesity, hyperphagia.	Knockout of the *Mc4r* gene.	++	n.d.	+(+++)*
Agouti yellow mouse	Obesity, yellow pigmentation.	Dominant mutations in the agouti gene.	++	n.d.	+
fat/fat mouse	Obesity, hyperinsulinemia.	Mutation in the *Cpe* gene.	+++	n.d.	+(+++)*
tub/tub mouse	Maturity-onset obesity, insulin resistance.	Recessive mutation in the *tub* gene.	+++	n.d.	+
Adult mouse	Adult obesity, diabetes.	Dominant mutation in the *Ad* gene.	+++	n.d.	+
5-HT2C-/- mouse	Obesity, hyperphagia.	Introduced nonsense mutation in the *5-HT2C-/-* gene.	++	n.d.	+
P. obesus sand rat	Obesity, type II diabetes.	Polygenic.	+++	n.d.	n.d.
NZO mouse	Obesity, type II diabetes.	Polygenic.	+++	n.d.	n.d.

*Excellent predictive and/or construct validity as models for subgroups of human obesity cases with related mutations. n.d.: not determined. Although there are several cases described where single gene mutation models have counterparts that cause obesity in the human, polygenic animal models will most likely best mimic the human situation. The monogenic models will probably have their strength as hypothesis generators and in the cases of the *ob, db, Mc4r* and *fat* mutations they have been most helpful in revealing the nature of specific human eating disorders.

analysis has become a method of choice for mimicking more closely the complex obesity in human. A total of 98 animal QTLs, from mouse, rat, pig and chicken, linked to body weight or body fat were reported in the 1999 update of 'The human obesity gene map' (Chagnon *et al.*, 2000). This number is constantly increasing as more QTL crosses are analysed. The main task of identifying and cloning the QTLs remains. Some of these QTLs will turn out to be more important than others and several will, in all likelihood, be proven to be false positives.

Conclusions on Obesity Models

Human obesity is a complex disorder influenced by both environmental as well as physiological and genetic factors. Although there are several cases described where single gene mutations cause obesity in the human the polygenic animal models will most likely mimic the human situation best. Table XXIII-1.2 contains a summary and a comparative evaluation of the models described.

CONCLUDING REMARKS

When producing valid animal models of eating disorders one is faced with two major obstacles, first the lack of accurate descriptions of the disorders and second the lack of effective treatments for these disorders. The central mechanisms of hunger and satiety are under complex physiological control. A good understanding of these mechanisms is necessary in order to understand the underlying causes for eating disorders. In this chapter we have reviewed some of the available animal models of eating behaviour that may provide clues to possible mechanisms involved in the regulation of food intake.

Given that eating disorders have become a major health concern, the combination of gene variants, mutations and environmental factors that contribute to these disorders need to be defined. Irrespective of the value of animal models for the understanding of the causes of human eating disorders, it is very probable that most of the molecules identified, using animal models, will be involved in biochemical processes regulating food intake and/or metabolism also in human. As such, these molecules constitute targets for the development of new classes of small drug pharmaceuticals that may be used to treat human eating disorders. There are thus great expectations that studies of animal models will result in new tools for the treatment and/or prevention of a major health hazard in the western world.

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XXIII-2

Transmitter Systems in the Eating Disorders

Timothy D. Brewerton

INTRODUCTION

Investigations into the role of neurotransmitters and other neuromodulators in the eating disorders have been quite productive in the last few years, especially during the last decade. The eating disorders occur in a relatively small cohort of people, primarily young women and girls, across many cultures, and they are known to be associated with significant mortality and morbidity, both medical and psychiatric (Walsh and Devlin, 1998; Becker *et al.*, 1999). Despite popular beliefs, there is no convincing evidence that cultural factors alone cause eating disorders. Recent data clearly identify strong genetic factors in the aetiology of both anorexia nervosa (AN) and bulimia nervosa (BN), which appear to share common genetic vulnerabilities (Lilenfeld *et al.*, 1998; Strober *et al.*, 2000) possibly linked to obsessionality, perfectionism, anxiety and/or behavioural inhibition (Halmi *et al.*, 2000; Kaye *et al.*, 1999a, 1999b). Such information implicates primary neurotransmitter disturbances in both disorders. One powerful piece of evidence to support monoamine involvement in the eating disorders is the observation that antidepressant medications can be beneficial in controlled studies, not only in BN patients, but also in recovered AN patients (Kaye *et al.*, 1998b).

However, it is also clear that a number of secondary disturbances come about as a result of the profound eating disturbances characteristic of these disorders (Brewerton, 1995), which then in turn only worsen or perpetuate signs and symptoms (Pollice *et al.*, 1997). This perspective, taken together with the disorders' consequences, challenges and costs, compels us towards a better understanding of the biological mechanisms underlying all stages and types of eating disorders. The identification of the psychobiological underpinnings of these conditions may be useful in many ways, including the development of improved medical and psychopharmacologic interventions, improved education and psychotherapy for patients and their families, and improved prevention efforts at a primary level.

It must be emphasized that most measurements of neurotransmitter function provide only a glimpse into the state of the organism at that moment in time. Sorting out what is trait- and what is state-related has been a challenging focus of neurotransmitter research in the eating disorders.

MONOAMINES

The classical monoaminergic neurotransmitter systems, including serotonin (5-HT), norepinephrine (NE), and dopamine (DA), have been fairly extensively studied in the eating disorders using available techniques in biological psychiatry. Most of these studies have been conducted during the disease state, which has the disadvantage of being confounded by severe nutritional compromise. Dieting and/or semi-starvation clearly deplete central monoamines and lead to altered neurotransmitter levels and receptor sensitivity in animals and humans (Cowen *et al.*, 1996; Cowen and Smith, 1999; Goodwin *et al.*, 1987a, 1987b, 1990). To avoid this problem a more recent strategy has been to study 'recovered' eating disorder patients, i.e., AN and BN patients who have attained normalization of eating and weight, resumption of menses and/or normalization of gonadal hormone levels, and abatement of typical cognitive features to sub-clinical levels. This strategy attempts to minimize starvation-state-related effects and to reveal potential trait-related disturbances or vulnerabilities. However, the long-term effects of chronic malnutrition and disordered eating behaviours on the brain (similar to substance use disorders) should not be underestimated. Studies of transmitter function in at-risk pre-morbid individuals as well as non-affected identical and fraternal twins, siblings, and other first-degree relatives of ED patients, could begin to confirm trait-related disturbances.

Neurotransmitter function in patients with EDs have been investigated using a variety of existing techniques and methodologies, each of which has its own advantages and disadvantages. Studies of cerebrospinal fluid (CSF) concentrations of the major metabolites have been a popular strategy and include measures of 5-hydroxyindoleacetic acid (5-HIAA) for serotonin (5-HT), 3-methoxy-4-hydroxyphenylglycol (MHPG) for NE, and homovanillic acid (HVA) for DA. Some studies have also examined actual concentrations of 5-HT and NE, but not DA. Such studies measure transmitter metabolism of the whole brain and spinal cord and lack any anatomical specificity.

Neuroendocrine and other psychobiological response measures have been studied following acute challenges with various agents, including amino acid precursors, e.g., L-tryptophan (L-TRP) and 5-hydroxytryptophan (5-HTP) for 5-HT, pre-synaptic receptor agonists, e.g., dl-fenfluramine (dl-FEN) or d-fenfluramine (d-FEN) for 5-HT, post-synaptic receptor agonists, e.g., m-chlorophenylpiperazine (m-CPP) for 5-HT and isoproterenol (ISOP) for NE. Longer term challenges with receptor antagonists, e.g., antipsychotics for DA and 5-HT, and antidepressants, especially the serotonin-specific reuptake inhibitors (SSRIs), also illuminate the role of neurotransmitters in the eating disorders. Acute amino acid precursor depletion, most notably of L-TRP (Delgado *et al.*, 1990; Weltzin *et al.*, 1995; Smith, Fairburn and Cowen, 1999; Kaye *et al.*, 2000), has been another important source of information about the role of central 5-HT function in eating and related disorders.

Platelet (PLT) or leukocyte studies are possibly reflective of central neurotransmitter function but are always at least step removed from the nervous system, e.g., platelet 5-HT reuptake, ^{3}H-imipramine binding, ^{3}H-paroxetine binding, platelet MAO, platelet 5-HT content, as well as platelet receptor mediated aggregation (5-HT_2 and alpha-adrenergic).

Plasma concentrations of neurotransmitter precursors, e.g., L-TRP, L-tyrosine (L-TYR), and their competing large neutral amino acids (LNAA), neurotransmitters themselves, e.g., NE, DA,

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and whole blood serotonin (WBS), as well as the usual metabolites, MHPG, HVA, and 5-HIAA.

Brain imaging-receptor-binding studies are a promising avenue but remain relatively unexplored in the eating disorders.

For each neurotransmitter, the results from controlled studies in humans will be reviewed and summarized for both AN and BN. Where applicable, comparisons between restricting AN patients, bingeing/purging AN patients, and normal-weight BN patients will be made.

NOREPINEPHRINE

There are a number of reasons to suspect NE involvement in the eating disorders. Most notably, NE pathways at the level of the hypothalamus are known to be involved in the initiation of feeding (Rowland, Morien and Li, 1996). Disturbances in these pathways may therefore be involved in the pathophysiology of the profoundly altered feeding behaviours classically associated with the eating disorders. In addition, NE's role in the modulation of mood, anxiety, neuroendocrine control, metabolic rate, sympathetic tone, and temperature make it a likely candidate of study (Jimerson *et al.*, 1987; Kaye *et al.*, 1988b, 1990a, 1990c; Lesem *et al.*, 1989; Pirke, 1996). It has been well recognized for quite some time that low-weight anorexic patients, and to some degree bulimic patients, have reduced body temperature, blood pressure, pulse and metabolic rate (Gross *et al.*, 1979; Lesem *et al.*, 1989; Obarzanek *et al.*, 1991). Investigations in this area have shown that low-weight AN patients have reduced measures of plasma, urinary and CSF MHPG (Gross *et al.*, 1979; Halmi *et al.*, 1978; Johnston *et al.*, 1984; Kaye *et al.*, 1984a, 1984b). In contrast, reports of plasma NE levels in the eating disorders has been more variable (Luck *et al.*, 1983; Kaye *et al.*, 1988a, 1988b), and this appears to be linked not only to weight but to the stresses associated with the illness (Lesem *et al.*, 1989). AN patients tend to have higher plasma NE levels at admission, which then decrease as treatment and weight gain progresses (Pahl *et al.*, 1985; Lesem *et al.*, 1989).

When ill, BN patients demonstrate lower values of plasma NE at baseline (Jimerson *et al.*, 1987; Obarzanek *et al.*, 1991) and in response to abstinence (Kaye *et al.*, 1990b), standing (Pirke *et al.*, 1985), testmeal challenge (Pirke *et al.*, 1992), and mental challenge (Pirke *et al.*, 1992). They also have other evidence of blunted sympathetic activation in response to mental stress (Koo-Loeb, Pedersen and Girdler, 1998). However, despite low baseline plasma NE levels, BN patients show normal responses to exercise (Pirke *et al.*, 1989) but reduced responses to orthostasis (Lonati-Galligani and Pirke, 1986).

In AN patients, depression has been found to be significantly worse in those patients with the lowest delta change in plasma NE concentrations to orthostasis (Pirke *et al.*, 1988). Reduced urinary MHPG levels have also been related to the presence of comorbid major depression (Biederman *et al.*, 1984a, 1984b; Halmi *et al.*, 1978). It is therefore important in such studies to control for psychiatric comorbidity.

Like the plasma NE studies, CSF NE levels have been reported to be no different in AN patients compared to controls at low weight and after short-term weight gain, but then significantly lower after weight recovery of at least 6 months (Kaye *et al.*, 1984a, 1984b, 1985; Pirke, 1996). In BN patients, reduced CSF NE levels have been reported during the active state of the illness (Kaye *et al.*, 1990a, 1990b). However, upon long-term recovery, concentrations of CSF MHPG have been reported to normalize in both AN and BN (Kaye *et al.*, 1999a, 1999b) despite earlier reports of lower levels (Kaye *et al.*, 1985). Given that CSF NE concentrations have not yet been reported in long-term (>1 year) recovered AN or BN patients, the extent to which adrenergic alterations seen in the eating disorders are trait-related is unclear. Nevertheless, available evidence suggests exquisite sensitivity of this system to malnutrition or stress.

Challenge studies using the beta-adrenergic agonist isoproterenol in underweight anorexic patients revealed erratic secretion of plasma NE in response to increasing doses (Kaye *et al.*, 1990a, 1990b). Bulimic patients demonstrated significantly increased chronotropic responses to isoproterenol (George *et al.*, 1990). Challenge studies with adrenergic agents in recovered patients have not been reported.

The number of platelet alpha-2-receptors has been reported to be reduced in both AN and BN compared to controls (Luck *et al.*, 1983; Heufelder, Warnhoff and Pirke, 1985), thereby suggesting increased post-synaptic receptor sensitivity which is probably secondary to dieting or semi-starvation. In summary, peripheral and central sympathetic nervous activity is reduced in both AN and BN, although it tends to normalize with recovery. Taken together, the preponderance of the evidence so far leads to the conclusion that these changes are a result of chronic starvation or intermittent dieting (Pirke, 1996). However, a trait-related disturbance of the adrenergic system cannot be ruled out at this time (Kaye *et al.*, 1990a, 1990b).

Studies of adrenergic receptors on human leukocytes have been another strategy to investigate adrenergic function in the eating disorders. Buckholtz *et al.* (1988) reported altered beta-adrenergic receptor affinity on circulating lymphocytes of BN patients compared to those of controls. However, in a similar study of a mixed group of eating disorder patients, Lonati-Galligani and Pirke (1986) reported lower receptor number (B_{max}) but normal affinity (K_d) in low-weight AN patients, while both measures were no different from controls in the BN patients and the weight-recovered AN patients. Gill and colleagues (1992) reported differential changes in alpha- and beta-adrenoceptor linked [$^{45}Ca^{2+}$] uptake in platelets from patients with anorexia nervosa, further documenting an adrenergic disturbance in eating disorder patients. However, the issue of cause vs. effect remains unanswered in platelet and leukocyte studies.

DOPAMINE

Dopamine (DA) is also suspect in the neuropathophysiology of the eating disorders given its reported involvement in the regulation of feeding, mood, activity, perception, sexual/social behaviour, hormone and peptide release and to some extent aggression (Hoebel, 1985; Jimerson *et al.*, 1992; Engstrom *et al.*, 1999; Kaye *et al.*, 1999a). Notably, DA is involved in the hedonic reward responses to eating and its maintenance as well as to other pleasurable activities (Hoebel, 1985; Hoebel *et al.*, 1989; Jimerson *et al.*, 1992).

The majority of studies of DA metabolism in the eating disorders have consistently shown that low-weight AN patients have reduced measures of peripheral and central DA activity, including decreased plasma (Gross *et al.*, 1979) and CSF HVA (Kaye *et al.*, 1984a, 1984b). In BN patients, reduced CSF HVA levels also have been reported in BN patients with frequent binge–purge episodes (Kaye *et al.*, 1990a; Jimerson *et al.*, 1992) but not in those less severely ill. Furthermore, binge frequency was inversely correlated with CSF HVA levels in one study (Jimerson *et al.*, 1992). Upon long-term recovery, normal concentrations of CSF HVA have been reported to normalize in BN (Kaye *et al.*, 1998a, 1998b), while a trend for decreased CSF HVA levels persisted in six restricting AN patients compared to controls and to bingeing and/or purging AN patients (Kaye *et al.*, 1999a). This suggests a possible trait-related disturbance specific to restricting AN, although this finding needs replication given the small sample size. These results could also

still be due to nutritional factors given that the patients in this study weighed significantly less than the BN group and may still have been at the low end of the normal weight range.

Anecdotal reports of the successful use of dopaminergic antagonists (typical anti-psychotic agents) in the treatment of AN patients (Dally and Sargant, 1960) have been generally followed by equivocal results in controlled studies (Vandereyken and Pierloot, 1982; Vandereyken, 1984). Atypical anti-psychotic agents may show more promise in the adjunctive treatment of AN given their combined anti-dopaminergic and anti-serotonergic effects (Hansen, 1999; Jensen and Mejlhede, 2000; LaVia, Gray and Kaye, 2000), but the results of placebo-controlled studies remain to be seen.

Genetic investigations into the role of DA have been limited to the Bal I DRD3 receptor polymorphisms in which no differences were found between AN patients and controls (Bruins-Slot *et al.*, 1998). However, the polymorphisms of other genes coding for DA receptors could be tested. Interestingly, Corcos and colleagues (1999) reported significantly lower IgG and IgM autoantibodies to DA in BN patients compared to controls. There was also a trend for lower levels of IgM autoantibodies to DA in the eating disordered group. The relevance of these findings to the pathophysiology of the eating disorders remains uncertain, but invokes possible autoimmune mechanisms.

SEROTONIN

There are several lines of reasoning that point to disturbances of serotonin (5-HT) function in the pathophysiology and neuropsychopharmacology of the EDs (Kaye and Weltzin, 1991b; Brewerton, 1995; Kaye *et al.*, 1999a, 1999b), including its role in feeding (Dourish *et al.*, 1988; Leibowitz, 1999), satiety (Brewerton *et al.*, 1994a, 1994b; Leibowitz and Alexander, 1998), dieting/fasting (Cowen *et al.*, 1996; Cowen and Smith, 1999), mood regulation (Delgado *et al.*, 1990), anxiety (Anderson and Mortimore, 1999), obsessive–compulsiveness/perfectionism/behavioural inhibition (Kaye *et al.*, 1984a), harm avoidance (Brewerton, Hand and Bishop, 1993; Waller *et al.*, 1993) impulsivity/aggression (Linnoila *et al.*, 1983; Coccaro *et al.*, 1989), motor activity (Brewerton *et al.*, 1995a; Epling and Pierci, 1988), gender (Carlsson *et al.*, 1985; Goodwin, Fairburn and Cowen, 1987a), seasonality (Brewerton, 1989; Brewerton *et al.*, 1988a, 1994a, 1994b), body image/perception (Goldbloom and Olmsted, 1993), and social status (Raleigh *et al.*, 1984, 1985; McGuire and Raleigh, 1985) (see Table XXIII-2.1).

Decreases in a variety of 5-HT parameters have been consistently reported in low-weight AN patients. Although no significant differences have been found in absolute plasma L-TRP levels (Russell *et al.*, 1967; Coppen *et al.*, 1976; Hassanyeh and Marshall, 1991), the plasma L-TRP/LNAA ratio is reduced in the low-weight state (Askernazy *et al.*, 1998; Kaye *et al.*, 1984a; Johnston *et al.*, 1984) but normalizes upon short-term weight recovery (Johnston *et al.*, 1984; Kaye *et al.*, 1988b). In BN, Gendall and Joyce (2000) reported that the L-TRP/LNAA ratio inversely correlated with the desire to binge eat. In addition, symptomatic bulimic relapse or worsening of symptoms has been reported following acute L-TRP depletion in BN (Weltzin *et al.*, 1995; Smith, Fairburn and Cowen, 1999; Kaye *et al.*, 2000).

Other significant findings include decreased CSF L-TRP levels (Gerner *et al.*, 1984) and decreased CSF 5-HIAA levels (Kaye *et al.*, 1988b, 1984a; Gillberg, 1983) during low-weight status with normalization of these levels with short-term weight recovery (STWR, goal weight maintenance $\geq$3 weeks). Following long-term weight recovery (LTWR, goal weight maintenance $\geq$6–12 months), Kaye and colleagues (1991a, 1999b) have reported increased CSF 5-HIAA levels, which is associated with obsessionality and behavioural inhibition.

In BN, reduced levels of CSF 5-HIAA are consistently reported only in the subgroup of patients with more frequent binge–purge frequencies (Kaye *et al.*, 1990a, Jimerson *et al.*, 1992). In addition, binge frequency was inversely correlated with CSF 5-HIAA concentrations (Jimerson *et al.*, 1992). In a small pilot study, Brewerton and colleagues (1995a, 1995b) have reported no difference in CSF 5-HT levels between BN patients and controls. However,

Table XXIII-2.1 Monoamine involvement in the phenomenology of the eating disorders

	Norepinephrine	Dopamine	Serotonin
Feeding Initiation/Hunger	X		
Feeding Maintenance/Hedonic Reward		X	
Feeding Termination/Satiety			X
Fasting Effects	X	X	X
Activity/Exercise	X	X	X
Impulsivity/Aggression		X	X
Novelty/Sensation Seeking		X	
Mood Regulation	X	X	X
Anxiety	X		X
Harm Avoidance			X
Obsessive–Compulsiveness/Perfectionism			X
Behavioural Inhibition			X
Body Image/Perception		X	X
Social Hierarchy/Rank			X
Metabolic Rate	X		
Temperature	X		X
Blood Pressure/Pulse	X		X
Gender Differences			X
Seasonality/Light Effects			X
Circadian Rhythmicity			X
Age/Developmental Effects			X
Trauma Effects	X		X
Sexual Behaviour		X	X
Hormone Regulation	X	X	X
Neuropeptide Regulation	X	X	X

upon recovery for at least one year, BN patients have been reported to have elevated CSF 5-HIAA levels compared to healthy controls (Kaye *et al.*, 1998b). As in AN, this finding has been linked to obsessive–compulsive personality traits, perfectionism and behavioural inhibition.

Decreased prolactin (PRL) responses following m-CPP (Brewerton *et al.*, 1990; Brewerton and Jimerson, 1996; Hadagan *et al.*, 1996), L-TRP (Brewerton *et al.*, 1990; Brewerton and Jimerson, 1996), and fenfluramine (FEN) (Halmi *et al.*, 1993; Monteleone *et al.*, 1998b) have been reported in AN and indicate an anatomically specific alteration in 5-HT receptor sensitivity at the level of the hypothalamus, which could conceivably also occur in other brain pathways (Brewerton, 1995). Blunting of PRL following m-CPP persist into short-term weight recovery, although there are trends toward normalization with refeeding and weight gain (Brewerton and Jimerson, 1996). With at least a year of recovery, neurohormonal responses to m-CPP normalize in restricting AN patients (Kaye *et al.*, 1999b). Apparently, full normalization of PLR responsivity to serotonergic agents does occur after full weight restoration, normalization of hypothalamic-pituitary-gonadal function, and abatement of overt eating disorder symptoms (Kaye *et al.*, 1999b). However, the appetite-suppressing effect of FEN is significantly diminished in recovered AN patients despite normalization of hormonal release (Ward *et al.*, 1998).

In BN, there is a consistent pattern of PRL blunting following m-CPP (Brewerton *et al.*, 1992b, 1992c; Brewerton, 1995; Levitan *et al.*, 1997), fenfluramine (Halmi *et al.*, 1993; Jimerson *et al.*, 1997; McBride *et al.*, 1991; Monteleone *et al.*, 1998a), and 5-hydroxytryptophan (5-HPT) (Goldbloom *et al.*, 1996), but not L-TRP (Brewerton *et al.*, 1992b, 1992c; Brewerton, 1995). PRL responses following L-TRP are low only in the BN patients with concurrent major depression, again emphasizing the need to control for comorbidity. PRL responses following m-CPP are inversely correlated to baseline cortisol (CORT) (Brewerton, 1995). Self-reported binge frequency also has been reported to be inversely correlated to PRL responses following m-CPP (Brewerton, 1995) and fenfluramine (Jimerson *et al.*, 1997; Monteleone *et al.*, 1998a) in BN patients. Given that this presumed alteration in hypothalamic post-synaptic 5-HT functioning normalizes with recovery from BN (Kaye *et al.*, 1998a, 1998b; Wolfe *et al.*, 2000), these serotonergic abnormalities are likely a *result* of bingeing, purging, and/or dieting rather than a *cause* of these behaviours, although other vulnerabilities of the 5-HT system may also exist and interact with these psychosomatic behaviours. Dieting, bingeing and vomiting all may affect central 5-HT synthesis (Fernstrom, 1985; Goodwin *et al.*, 1987a, 1987b; Kaye *et al.*, 1988a, 1989) and could conceivably result in downregulation of post-synaptic 5-HT receptors and blunted PRL responses. In addition, these behaviours may involve activation of the HPA axis, which in turn appears to dampen 5-HT receptor sensitivity (Brewerton *et al.*, 1992b, 1992c; Brewerton, 1995).

Taken together, research findings from plasma, CSF, and pharmacologic challenge studies suggest reduced 5-HT synthesis, uptake, and turnover, as well as altered post-synaptic 5-HT receptor sensitivity during the active phases of both AN and BN. Consequently, most reported alterations in 5-HT function appear to be state-dependent, although they may play important biological roles in the perpetuation of symptoms, particularly the mood dysregulation, increased anxiety, obsessionality, impulsivity, self-aggression, and perhaps the resistance to and difficulty in learning healthier coping strategies (Riedel *et al.*, 1999).

Interestingly, other findings suggest heightened 5-HT receptor sensitivity in non-recovered eating disorder patients. Brewerton (1995) has reported enhanced temperature and migraine headache responses to m-CPP, but not L-TRP in BN patients (regardless of the comorbid presence of AN or MD) (Brewerton, 1995; Brewerton *et al.*, 1988b, 1992c). As discussed in detail elsewhere (Brewerton *et al.*, 1992c), the enhanced migraine-like HA responses in the BN patients may indicate enhanced 5-HT_2 receptor sensitivity in CNS vascular tissues. Enhanced 5-HT mediated platelet aggregation, a 5-HT_2 receptor mediated phenomenon, has also been reported in BN (Spigset *et al.*, 1999) and AN (Halmi *et al.*, 1993; McBride *et al.*, 1991; Spigset *et al.*, 1999) and lends further support to this hypothesis. The normal cortisol responses following m-CPP and L-TRP in AN and BN are compatible with this view given the involvement of both 5-HT_1 (facilitative) and 5-HT_2 receptors (inhibitive) in cortisol secretion. These presumed alterations in 5-HT receptor sensitivity, whether primary or secondary, demonstrate that 5-HT receptor sensitivity can be both decreased and increased in the same subjects depending on anatomical location of the receptor as well as receptor subtype. Brewerton (1995) has argued in favour of a dysregulation hypothesis of monoamine dysfunction in the eating and related disorders in which there is a failure in transmitter regulation in the face of a variety of psychobiological perturbations potentially affecting monoamine function, including dieting, fasting, purging, substance abuse, excessive exercising, medical illnesses, family stresses or losses, sociocultural pressures, traumatic events, puberty, other developmental tasks/challenges, and changes in the seasons.

A number of other platelet (PLT) studies contribute to the demonstration of serotonergic dysfunction in the eating disorders. Significant increases/reductions in PLT imipramine (IMI) binding (Weizman *et al.*, 1986b), but not PLT 5-HT uptake (Weizman *et al.*, 1986a; Zemishlany *et al.*, 1987) or PLT MAO content (Biederman *et al.*, 1984a, 1984b), have been reported in low-weight AN patients. However, a more recent study reported decreased PLT MAO in AN (Diaz-Marsa *et al.*, 2000), which was inversely correlated with impulsivity and positively correlated with persistence. In BN, platelet studies indicate reduced PLT IMI binding (Marazziti *et al.*, 1988) and PLT MAO (Hallman, Sakurai and Oreland, 1989). PLT 5-HT uptake has been reported to be increased in one study (Goldbloom, Hicks and Garfinkel, 1988) but not another (Hallman, Sakurai and Oreland, 1989). Steiger *et al.* (2000) reported reduced PLT paroxetine binding in a group of BN patients compared to healthy controls regardless of the presence of borderline personality disorder. Whether these changes reflect a central trait-related dysfunction remains unclear and awaits further studies in recovered patients.

In a novel study of indole metabolism, Finocchiaro and colleagues (1995) reported altered phytohaemagglutinin stimulated, light-induced [3H]thymidine incorporation into the DNA of peripheral blood mononuclear leukocytes in AN patients compared to controls. The authors concluded that the white cells of AN patients show a failure in the regulation of 5-HT and melatonin metabolism in response to light.

Genetic investigations into the role of 5-HT in the eating disorders have been more numerous and more promising than other monoamines. Collier *et al.* (1997) reported a statistically significant 5-HT2A-1438G/A receptor gene polymorphism in a group of restricting AN patients compared to healthy controls. This finding has been replicated in at least two other studies in AN (Nacmias *et al.*, 1999; Enoch *et al.*, 1998) as well as in OCD (Enoch *et al.*, 1998), but not in BN (Enoch *et al.*, 1998). Nacmias *et al.* (1999) reported that other serotonergic polymorphisms of the 5-HT2A as well as those of the 5-HT2C receptors showed no differences in AN patients compared to controls. Likewise, no differences between AN patients and controls have been reported for serotonin transporter gene linked polymorphisms (5-HTTLPR) (Hinney *et al.*, 1997; Sundaramurthy *et al.*, 2000), tryptophan hydroxylase polymorphisms (Han *et al.*, 1999), and 5-HT1Dbeta and 5-HT7 gene polymorphisms (Hinney *et al.*, 1999). 5-HT2C receptor polymorphisms showed no differences in allelic variations in a group of patients with BN or binge-eating disorder (BED) (Burnet *et al.*, 1999).

It is well known that serotonin-specific antidepressant medications can be beneficial in controlled studies of BN patients (Fluoxetine Bulimia Nervosa Collaborative Group, 1992), but not in low-weight AN patients (Attia *et al.*, 1998; Strober *et al.*, 1999). More recent data indicate a prophylactic effect of fluoxetine following weight gain in recovered AN patients (Kaye *et al.*, 1998). SSRIs don't work during the low-weight state, presumably because of central depletion of 5-HT and other monoamines with starvation. There is significantly less 5-HT centrally to inhibit the reuptake of.

Finally, recent evidence indicates significant anti-bulimic responses to 5-HT3 antagonists, such as ondansetron (Faris *et al.*, 1998, 2000). Although the authors attribute this therapeutic response to the drug's ability to reduce vagal tone, the role of the 5-HT3 receptor remains intriguing given its anti-anxiety effects (Roychoudhury and Kulkarni, 1997). These findings opens important new arenas for future research involving possible serotonergic–cholinergic mechanisms, which has been a relatively unexplored area in the eating disorders.

MAO/ISATIN

Isatin, or tribulin, is an endogenous indole associated with stress, which inhibits monoamine oxidase (MAO) (Glover *et al.*, 1988). Brewerton *et al.* (1995b) reported significantly higher CSF concentrations of isatin in BN patients compared to healthy controls. There was also a trend for CSF isatin concentrations to be inversely correlated with CSF concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) ($n = 14$, $\rho = -0.51$, $p = 0.06$), although CSF isatin levels were not significantly correlated with CSF MHPG or HVA. The increase in isatin levels has been hypothesized to be in response to the resultant monoamine depletion secondary to the effects of the illness on monoaminergic function. As noted previously, PLT MAO has been reported to be decreased in BN (Hallman, Sakurai and Oreland, 1989) and in AN (Diaz-Marsa *et al.*, 2000). This decrease may represent a compensatory change in response to monoamine depletion during the active state of the disorders.

RELATIONSHIP TO OTHER SYSTEMS

Neurotransmitter systems do not exist in a vacuum but are exquisitely interdependent with other brain and body systems and the environment as well. It is important to think about systems (e.g., 5-HT) and their subsystems (pre-synaptic, post-synaptic, receptor subtypes) in the context of larger systems (brain, environment) and interacting systems/subsystems (e.g., NE, DA, neurohormones, neuropeptides) with complex feedback and counter-feedback mechanisms at multiple anatomical levels. An extensive discussion of this rather far-reaching topic is beyond the scope of this chapter but is discussed in more detail elsewhere (Brewerton, 1995).

CONCLUSIONS

Taken together, available findings implicate abnormalities of all monoamine neurotransmitter systems during the active phases of both AN and BN. Upon normalization of weight and neurohormonal function, most transmitter abnormalities normalize, or at least improve. The strongest data show that dysregulation of 5-HT systems appears to persist and may be related to trait-related psychological characteristics found in both AN and BN that extend beyond classical eating disorder signs and symptoms, such as obsessionality, perfectionism, high harm avoidance and behavioural inhibition. Some evidence suggests prolonged alterations in NE metabolism, but this is most likely due to persistent low-grade dietary restraint following recovery. Preliminary data indicate a DA deficit in restricting AN patients, but this result remains to be replicated in larger samples. Recent findings also emphasize the importance of neurotransmitter precursor substrate availability to normal brain function and especially to the process of recovery from an eating disorder. Future research directions will include further exploration of neurotransmitter-related gene candidates, *in vivo* receptor imaging studies, and improved psychopharmacological interventions based on biological alterations characteristic of the different stages and features of these dangerous disorders.

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XXIII-3

Neuroendocrinology

Frances Connan

INTRODUCTION

Although over a single day food intake is poorly matched to energy expenditure, longer term food intake and energy expenditure can be extremely well balanced such that weight may remain remarkably stable over time (Edholm, 1977). However, the systems governing energy homeostasis appear to function more effectively to defend against starvation than they do in defence of overweight. Indeed, it has been argued that the capacity of the system to maintain healthy weight in the face of high availability of energy dense food and low physical activity levels is poor: there is a marked tendency to over consume, resulting in a high prevalence of obesity (Pinel *et al.*, 2000). In addition to the significant morbidity associated with obesity in its own right, a tendency toward overweight may be a vulnerability factor for the most common of the eating disorders, bulimia nervosa (BN) and binge-eating disorder (BED). These disorders are associated with elevated subjective reward value of food (Karhunen *et al.*, 1997a; Wisniewski *et al.*, 1997), reduced subjective sense of satiety (Kissileff *et al.*, 1996) and premorbid or family history of obesity (Fairburn *et al.*, 1997, 1998).

At the other end of the weight spectrum, anorexia nervosa (AN) is characterized by reduced food intake, but the question of whether appetite is impaired remains controversial and poorly researched. Studies employing subjective assessment have consistently reported reduced hunger and desire to eat and enhanced satiety and sensation of fullness in people with AN (e.g., Halmi and Sunday, 1991; Robinson, 1989). Furthermore, the subjective reward value of food is reduced (Drewnowski *et al.*, 1987; Sunday and Halmi, 1990), and the rate of eating is slow (Halmi and Sunday, 1991). Some authors argue that these findings reflect tight cognitive control of normal appetite (Palmer, 2000). However, relative to healthy comparison women, those with AN show reduced salivation (LeGoff *et al.*, 1988) and a heightened autonomic response to food (Leonard *et al.*, 1998). Images of food elicit fear and disgust (Ellison *et al.*, 1998). These objective data suggest that appetite may indeed be impaired in AN (Pinel *et al.*, 2000), although some capacity to respond to hunger and satiety cues clearly remains (Cugini *et al.*, 1998; Rolls *et al.*, 1992). A predisposition to leanness may be a risk factor for AN (Hebebrand and Remschmidt, 1995), supporting the notion that heritable risk for the disorder may be exerted through the biological systems regulating appetite and weight.

If we are to understand the neuroendocrinology of appetite and weight regulation in eating disorders, we must first understand the normal function of these systems and their responses to changes in body weight. It is well recognized that starvation causes profound changes in neuroendocrine systems and thus many of the findings associated particularly with AN, are liable to be consequence rather than cause of the disorder. Accordingly, the first section provides an overview of current models for understanding the neuroendocrine regulation of appetite and weight. Subsequent sections examine neuroendocrine data relating to each of the eating disorders in turn before presenting a synthesis and considering the implications for treatment and future research.

SECTION 1: REGULATION OF APPETITE AND WEIGHT

Leptin — A Peripheral Energy Sensor

The discovery of leptin in 1995 led to dramatic advances in the understanding of central pathways regulating appetite and weight. Leptin is a 146 amino acid protein that is synthesized and secreted by fat cells. When energy balance is stable, leptin concentration is proportional to fat mass (Considine *et al.*, 1996). However, acute changes in energy balance modulate leptin expression, such that fasting is associated with a greater fall in leptin than might be expected for the change in fat mass and conversely, overeating results in an enhanced postprandial rise in circulating leptin (Ahima *et al.*, 1996; Kolaczynski *et al.*, 1996a). The leptin system therefore responds to potential weight change before fat mass actually changes.

The importance of leptin as a signal of energy balance is demonstrated by genetic strains of mice lacking functional leptin (*ob/ob* mice) or its receptor (*db/db* mice). These animals are hyperphagic, hypothermic, hyperinsulinaemic and obese (Chua-SC *et al.*, 1996; Lee *et al.*, 1996; Zhang *et al.*, 1994). Although rare, genetic deficits of leptin signalling in humans are associated with a similar phenotype (Clement *et al.*, 1998; Montague *et al.*, 1997). Exogenous leptin administration reduces food intake and weight in normal and leptin deficient individuals (Farooqi *et al.*, 1999; Halaas *et al.*, 1997; Pelleymounter *et al.*, 1995). Thus, despite the obese phenotype, genetic leptin deficiency is actually a model of starvation: reduced leptin levels signal reduced fat mass and appetite is appropriately elevated.

Leptin enters the brain via an active transport mechanism and its functional long-arm receptors are located in areas of the hypothalamus, such as the arcuate, ventromedial, paraventricular and dorsomedial nuclei (Elmquist *et al.*, 1998): areas important for the function of neuroendocrine systems, including those regulating appetite and weight. The leptin system is therefore an excellent candidate for a peripheral signal of energy homeostasis and adequacy of fat stores to central systems coordinating the adaptive response to starvation.

Other Peripheral Signals of Energy Homeostasis

Several other peripheral hormones play a significant role in the central regulation of appetite. Insulin was one of the first hormonal signals known to be secreted in proportion to adipose mass. Insulin is actively transported across the blood–brain barrier and both insulin receptor mRNA and specific insulin binding sites

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have been demonstrated in regions of the brain important in the control of appetite such as the arcuate nucleus of the hypothalamus (Werther *et al.*, 1987). Chronic central infusion of insulin dose-dependently reduces daily food intake (Chavez *et al.*, 1995; Foster *et al.*, 1991). This effect is not immediate and is maximal only after 24 hours. Central administration of insulin not only reduces appetite but also activates brown fat thermogenesis and increases energy expenditure (Muller *et al.*, 1997). Animals that are insulin deficient show marked hyperphagia and this effect can be reversed by central administration of insulin (Sipols *et al.*, 1995). Central administration of anti-insulin antibodies enhances appetite and weight gain in normal animals (Strubbe and Mein, 1977).

Reciprocal regulatory effects have been demonstrated between leptin and insulin. Insulin has a delayed stimulatory effect on adipocyte leptin production (Kolaczynski *et al.*, 1996b; Utriainen *et al.*, 1996). Conversely, the hyperglycaemia, hyperinsulinaemia and insulin resistance of leptin deficient mice (*ob/ob* mice) is reversed by leptin replacement (Pelleymounter *et al.*, 1995), suggesting that leptin may modulate insulin expression and sensitivity. Recent evidence from the study of diabetic hyperphagia suggests that the role of leptin is more critical to energy homeostasis than that of insulin (Sindelar *et al.*, 1999).

Whilst leptin and insulin inhibit appetite, the recently identified peptide ghrelin stimulates appetite (Tschop *et al.*, 2000). This 28 amino acid peptide was identified in 1999 having been purified from rat stomach and subsequently cloned in man. Ghrelin is the endogenous agonist of the growth hormone secretagogue receptor (GHS-R), which is present in the hypothalamus. Recent work has demonstrated that serum ghrelin concentrations increase with fasting and fall with refeeding whilst intraperitoneal and intracerebroventricular injection of ghrelin increases food intake and body weight in rats and mice (Asakawa *et al.*, 2001; Tschop *et al.*, 2000). In obese humans, ghrelin levels are low and inversely correlated with serum leptin and insulin concentrations (Tschop *et al.*, 2001).

Hypothalamic Regulation of Appetite

First Order Neurones of the Arcuate Nucleus

The hypothalamus has long been recognized as a key component of the pathways regulating food intake. For example, destruction of the arcuate nucleus (ARC) causes hyperphagia and obesity (Olney, 1969). The ARC lies outside the blood–brain barrier and expresses receptors for leptin, insulin and ghrelin (Elmquist *et al.*, 1998; Tschop *et al.*, 2000; Werther *et al.*, 1987). It is therefore ideally placed to communicate peripheral signals to central pathways for energy regulation. Indeed, microinjection of leptin into the ARC reduces food intake (Satoh *et al.*, 1997) and if the arcuate nucleus is destroyed, there is no response to leptin (Dawson *et al.*, 1997).

A variety of orexigenic and anorexigenic peptides are expressed in the ARC. Neuropeptide Y (NPY) is perhaps the most powerful of the orexigenic peptides. Centrally administered NPY is a potent stimulant of feeding and reduces energy expenditure, but repeated administration is needed to elicit significant weight gain (Billington *et al.*, 1991; Stanley *et al.*, 1986). Agouti gene related peptide (Agrp) has a less potent, but more prolonged orexigenic activity, increasing feeding for up to a week after single central administration (Hagan *et al.*, 2000). NPY and Agrp are co-localized to a sub-population of neurones located in the medial ARC that up-regulate expression of both peptides in response to fasting (Broberger *et al.*, 1998; Hahn *et al.*, 1998).

The melanocortin system is perhaps the most important anorexigenic system of the hypothalamus. Mutations of the melanocortin (MC4) receptor gene in both mice (Huszar *et al.*, 1997) and humans (Vaisse *et al.*, 1998; Yeo *et al.*, 1998) are associated with a hyperphagic, obese phenotype, suggesting that activity of this receptor tonically inhibits appetite. Alpha melanocyte stimulating hormone (αMSH), cleaved from pro-opiomelanocortin (POMC), is an endogenous agonist at MC4 receptors (Mezey *et al.*, 1985), whilst Agrp exerts its orexigenic effect by antagonizing the effect of αMSH at this receptor (Ollmann *et al.*, 1997).

Cocaine- and amphetamine-regulated transcript (CART) is thought to be a second contributor to the anorexigenic system of the ARC. Intraventricular administration inhibits both normal and starvation induced feeding (Kristensen *et al.*, 1998). CART is co-expressed with αMSH in a sub-population of neurones in the lateral ARC (Elias *et al.*, 1998) and, as might be predicted, activity of these POMC/CART neurones is inhibited by fasting (Vrang *et al.*, 1999). More recently however, microinjection studies have demonstrated that whilst CART inhibits feeding when injected into the third ventricle, it has the opposite effect when administered directly into the ARC or other hypothalamic nuclei involved in the regulation of appetite (Abbott *et al.*, 2001). It appears therefore that POMC/CART neurones may have mixed anorexigenic and orexigenic activity.

Leptin receptors are expressed on both NPY/Agrp and POMC/CART neurones (Baskin *et al.*, 1999; Cheung *et al.*, 1997). Leptin inhibits the expression of NPY and Agrp (Mizuno and Mobbs, 1999; Stephens *et al.*, 1995) whilst stimulating activity in POMC/CART neurones (Elias *et al.*, 1998). Furthermore, if the fasting induced fall in leptin is prevented by administration of exogenous leptin, the rise in NPY expression is also prevented and the rate of weight gain during refeeding is blunted (Ahima *et al.*, 1996). Insulin also inhibits the expression of NPY mRNA (Schwartz *et al.*, 1992) whilst fasting induced ghrelin elevates NPY and Agrp expression (Asakawa *et al.*, 2001; Kamegai *et al.*, 2000). Thus the fasting induced fall in the peripheral signals leptin and insulin and rise in ghrelin stimulates NPY/Agrp neurones and inhibits POMC/CART neuronal pathways of the arcuate, generating an appropriate appetitive response.

Second Order Hypothalamic Pathways

NPY/Agrp and POMC/CART neurones of the ARC project to other areas of the hypothalamus thought to play a role in the regulation of appetite and weight, including the lateral hypothalamus (LH) and paraventricular nucleus (PVN) (Broberger *et al.*, 1998; Dall *et al.*, 2000; Elias *et al.*, 1999). Lesion studies indicate that bilateral ablation of the LH causes anorexia and weight loss (Stellar, 1954) whilst destruction of the PVN results in hyperphagic obesity (Weingarten *et al.*, 1985). Furthermore, almost all known orexigenic and anorexigenic peptides modulate appetite when injected into the PVN. Schwartz *et al.* (2000) have therefore proposed a two stage model of hypothalamic pathways regulating appetite: second order PVN and LH neurones function as down-stream effectors for the first order ARC neurones terminating in these regions.

Melanin concentrating hormone (MCH) is an orexigenic peptide (Herve and Fellmann, 1997) for which the LH is a major site of expression in the human brain (Viale *et al.*, 1997). The recently identified MCH receptor (MCH-R/SLC-1) (Shimomura *et al.*, 1999) is widely distributed in the CNS, including hypothalamic sites such as the ventromedial, dorsomedial and arcuate nuclei (Chambers *et al.*, 1999; Saito *et al.*, 2001). Expression of MCH and MCH-R is up-regulated by fasting, an effect that is blocked by central administration of leptin (Kokkotou *et al.*, 2001; Tritos *et al.*, 2001). If these are second order neurones, co-expression of NPY, MC4 and CART receptors should be demonstrable but this remains to be proven.

The orexins, first identified in 1998 (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998), also stimulate feeding, albeit less potently than NPY and MCH (Edwards *et al.*, 1999). These peptides are

expressed in a distinct population of LH neurones that lie in close proximity to MCH neurones and NPY/Agrp nerve terminals (Broberger *et al.*, 1998). As with other orexigenic peptides, a fasting induced rise in the expression of orexin and its receptor can be prevented by the administration of leptin (Lopez *et al.*, 2000). Although orexin has been hypothesized to contribute to second order appetitive signalling, recent evidence suggests a role upstream of NPY: NPY-Y1 receptor antagonists block the feeding effect of exogenous orexin (Jain *et al.*, 2000). In addition to a role in appetite regulation, orexin increases arousal, locomotor activity and metabolic rate (Hagan *et al.*, 1999; Lubkin and Stricker-Krongrad, 1998). Orexin deficient mice exhibit narcolepsy, hypophagia and late onset obesity (Hara *et al.*, 2001). Orexin's primary role may therefore be regulation of the sleep–wake cycle and the modulation of appetite and energy expenditure in accordance with state of arousal (Willie *et al.*, 2001).

One last orexigenic peptide for consideration here is galanin (Smith *et al.*, 1994). Although preprogalanin mRNA is present in both ARC and PVN (Gundlach *et al.*, 1990), microinjection studies suggest that the orexigenic action of galanin may be restricted to the PVN (Kyrkouli *et al.*, 1990). Once more, expression is decreased by central administration of leptin (Sahu, 1998), as predicted for an orexigenic peptide.

The PVN is also rich in anorexigenic peptides, including thyrotrophin releasing hormone (TRH) and corticotrophin-releasing hormone (CRH). TRH expression is reduced by fasting, an effect that is mediated by low leptin levels and dependent upon an intact ARC (Legradi *et al.*, 1997; Legradi and Lechan, 1999; Seoane *et al.*, 2000). Leptin inhibits TRH expression directly via leptin receptors co-expressed on TRH neurones, and indirectly via regulation of αMSH and NPY which respectively stimulate and inhibit TRH expression (Fekete *et al.*, 2001; Nillni *et al.*, 2000).

The anorectic effect of leptin is at least partially mediated by CRH (Gardner *et al.*, 1998; Okamoto *et al.*, 2001; Uehara *et al.*, 1998) and several studies demonstrate enhanced expression of CRH in response to leptin (Costa *et al.*, 1997; Morimoto *et al.*, 2000; Schwartz *et al.*, 1996). However, leptin may also inhibit the expression of CRH (Arvaniti *et al.*, 2001; Heiman *et al.*, 1997) suggesting an indirect mechanism for leptin regulation of CRH. This hypothesis is supported by the finding that the fasting induced fall in CRH expression is mediated at least in part by reduced αMSH activity (Fekete *et al.*, 2001). These data are consistent with a putative role for TRH and CRH as second order anorectic effectors (Schwartz *et al.*, 2000). In addition to the anorectic effect of these neuropeptides, each also plays a role in peripheral energy regulation, via the thyroid and adrenal axis respectively (see below).

Integration of Anorexigenic and Orexigenic Signals within the PVN

GABA interneurones in the PVN are thought to be the substrate for functional integration of anorexigenic and orexigenic pathways of the hypothalamus (Cowley *et al.*, 1999). Since GABA is an inhibitory neurotransmitter, this model is consistent with studies demonstrating an inability to adequately restrain eating following bilateral PVN ablation. Electrophysiological and immunohistochemical studies indicate that NPY/Agrp and POMC/CART neurones converge on GABA interneurones expressing both MC4 and NPY receptors. NPY receptor activation inhibits adenylate cyclase activity via the Gi subunit of the plasma membrane G protein. Reduced availability of intracellular cyclic AMP results in decreased GABA release, which in turn reduces inhibition of feeding. Conversely, MC4 receptors are coupled to the Gs subunit. αMSH therefore stimulates adenylate cyclase, activating GABA neurones. Agrp inhibits GABA activity via prolonged antagonism of MC4 mediated activation of adenylate cyclase. Thus when food intake is restricted, reduced leptin signalling gives rise to increased NPY/Agrp activity and reduced POMC activity which in turn inhibits the activity of GABA interneurones to enhance appetite (see Figure XXIII-3.1). It is not yet known whether CART inhibits or stimulates GABA interneurone activity, but if CART is an orexigenic peptide an inhibitory effect would be predicted.

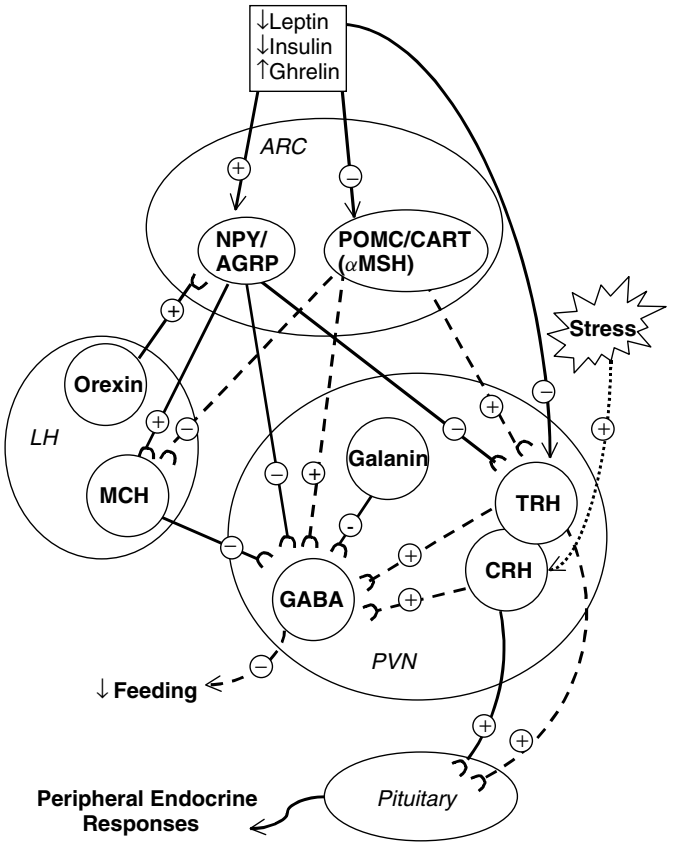

Figure XXIII-3.1 Proposed Model for the Neuroendocrine Response to Starvation. In response to acute food restriction, leptin levels fall below that expected for body fat mass, facilitating an early response to reduced energy availability. If food intake does not increase, falling body mass maintains this low leptin response. Reduced leptin signal to the ARC enhances activity of orexigenic NPY/Agrp neurones, whilst inhibiting anorexigenic αMSH activity. The appetitive effects of CART remain to be clarified. NPY/Agrp and POMC/CART neurones in the ARC project to the PVN and LH where putative second order orexigenic peptides MCH and galanin are up-regulated and anorexigenic peptides such as CRH and TRH are inhibited. Stress-induced CRH release may enhance anorexigenic activity in the PVN. Orexin appears to function upstream of NPY, perhaps providing a link between sleep–wake cycles and feeding behaviour. Orexigenic and anorexigenic signals may be functionally integrated via G protein coupled receptors on GABAergic interneurones in the PVN. When the leptin signal is low, inhibition of GABA activity reduces inhibition on feeding. Additionally, low leptin levels modulate the function of hypothalamic–pituitary endocrine axes such that energy is mobilized from peripheral stores and energy expenditure for growth and reproduction is minimized. In the case of the HPA axis, although fasting may inhibit CRH release in the PVN, a fasting induced increase of NPY expression stimulates CRH release in the median eminence, giving rise to enhanced HPA axis activity. (ARC = arcuate nucleus; LH = lateral hypothalamus; PVN = paraventricular nucleus; NPY = neuropeptide Y; Agrp = agouti related peptide; POMC = pro − opiomelanocortin; αMSH = alpha melanocyte stimulating hormone; CART = cocaine amphetamine related peptide; MCH = melanin concentrating hormone; TRH = thyrotrophin releasing hormone; CRH = corticotrophin releasing hormone. ——— = enhanced pathways; - - - - = suppressed pathways)

The effector neurones upon which GABA interneurones act have yet to be elucidated, but it is likely that at least some of the hypothesized second order neurones function in this way (Schwartz *et al.*, 2000). However, MCH receptors are also coupled to the inhibitory G protein subunit and CRH receptors are coupled to the Gs subunit. It is therefore possible that activity within the proposed second order anorexigenic and orexigenic pathways can also be functionally integrated at the GABA interneurone (Cowley *et al.*, 1999). Further studies are clearly needed to test these hypotheses.

Multiple orexigenic and anorexigenic peptides therefore contribute to the appetitive pathways of the hypothalamus, giving rise to a degree of redundancy within the system. NPY knockout mice, for example, are of normal weight (Erickson *et al.*, 1996), indicating that other neuropeptides, such as Agrp, compensate for this genetic deficit. However, it seems likely that our current model for understanding is an over-simplification of a highly complex network in which the different neuroendocrine systems contribute unique links with important higher functions. For example, the orexin system appears to provide a link between energy regulation and arousal, whilst the CRH system provides a link with stress responsivity. CRH inhibits the expression of NPY (Van Huijsduijnen *et al.*, 1993) and mediates stress-induced anorexia (Krahn *et al.*, 1986; Shibasaki, 1988). Thus even if undernourished, appetite may be impaired in the context of CRH hyperactivity (Schwartz *et al.*, 1995). This hypothesis may be particularly relevant to eating disorders, given their association with premorbid stressful life events and difficulties (Schmidt *et al.*, 1997) and impaired coping (Troop and Treasure, 1997).

Effector Systems

Second order PVN neurones provide a link between leptin and peripheral effectors that mediate adaptations to altered energy availability. One such mechanism involves the hypothalamic–pituitary hormonal axes. In humans, genetic deficits of leptin signalling are associated with widespread neuroendocrine abnormality of the type seen in starvation, including failure of puberty and reduced secretion of thyrotrophin and growth hormone (Clement *et al.*, 1998). Furthermore, if the fall in leptin associated with fasting is prevented by exogenous replacement, changes within the gonadal (HPG), adrenal (HPA) and thyroid hormone (HPT) axes are blunted (Ahima *et al.*, 1996).

Leptin suppresses HPA axis activity at both the central and adrenal level of the axis (Ahima *et al.*, 1996; Bornstein *et al.*, 1997; Heiman *et al.*, 1997), such that cortisol levels are elevated during fasting. Whilst the action of cortisol at high concentrations is catabolic in the periphery, generating glucose and ketone bodies from stored energy, at the central level, cortisol stimulates expression of NPY and is thus anabolic (Strack *et al.*, 1995). In turn, NPY further stimulates HPA axis activity via increased CRH expression in the median eminence, but not other hypothalamic regions (Haas and George, 1987). Thus a feed-forward system is generated in which cortisol and NPY are reciprocally positively regulated in order to maintain a robust central appetite response coupled with mobilization of peripheral energy stores. Schwartz *et al.* (1995) have suggested that persistently dysregulated CRH release in the PVN could impair this feed-forward system via inhibition of NPY expression.

During fasting, low leptin levels suppress prothyrotropin-releasing hormone expression, reducing peripheral thyroid hormone activity (Legradi *et al.*, 1997) and thus reducing energy expenditure. Low leptin levels also suppress growth hormone (GH) secretion during fasting (Carro *et al.*, 1997; LaPaglia *et al.*, 1998) and in genetic leptin deficiency (Clement *et al.*, 1998). This effect appears to be mediated by enhanced expression of NPY (Vuagnat *et al.*, 1998). Interestingly, the fasting induced fall in insulin-like growth factor (IGF-1) levels is not reversed by leptin (Carro *et al.*, 1997; LaPaglia *et al.*, 1998). In contrast to an acute fast, severe starvation is associated with elevated GH secretion, GH resistance and low IGF-1 and insulin levels (Soliman *et al.*, 2000). This pattern of GH-IGF-1 axis activity is thought to promote muscle catabolism, rather than growth, and is therefore adaptive to the starving state.

Leptin regulates gonadal axis activity via stimulation of both hypothalamic and pituitary components of the axis (Yu *et al.*, 1997) and plays a pivotal role in the onset of ovulation at puberty and cessation during starvation (Ahima *et al.*, 1997; Chehab *et al.*, 1996; Miller *et al.*, 1998). The effects of leptin upon gonadal and other anabolic hormone axes may be mediated by NPY (Catzeflis *et al.*, 1993). Since oestrogen enhances leptin production (Mannucci *et al.*, 1998), oestrogen deficiency arising from HPG axis suppression during starvation may amplify orexigenic signalling.

The adaptive response to leptin deficiency may therefore be as important as its role in the regulation of appetite. It is characterized by reduced energy expenditure, via suppression of reproductive and thyroid axis activity, and mobilization of stored energy via the actions of cortisol and altered GH-IGF-1 axis function. Leptin deficiency therefore defends against starvation by both increasing appetite and reducing energy expenditure (see Figure XXIII-3.1).

Similarly, excessive leptin production in the context of obesity may also elicit adaptive changes. POMC/CART neurones project to sympathetic preganglionic neurones in thoracic spinal cord (Elias *et al.*, 1998) suggesting these pathways may contribute to enhanced thermogenesis and energy expenditure when anorexigenic pathways are activated by elevated leptin. Pituitary endocrine axis changes in obesity include reduced GH and elevated cortisol release, which in this context may serve to promote growth and central adiposity. Excess leptin therefore appears to be somewhat less effective in defending healthy body weight than leptin deficiency. Central leptin resistance, perhaps arising from saturation of active leptin transport mechanisms, may contribute (Caro *et al.*, 1996). Indeed, the ratio of CSF to plasma leptin is reduced in obese subjects (Caro *et al.*, 1996; Schwartz *et al.*, 1996). These findings support the hypothesis that the primary role of leptin is to defend against starvation rather than obesity.

SECTION 2: NEUROENDOCRINOLOGY OF APPETITE AND WEIGHT IN EATING DISORDERS

Anorexia Nervosa

Leptin

The wealth of studies examining a putative role for leptin in the aetiology of AN have produced little evidence to support a functional abnormality. In acute AN, leptin levels are low and proportional to BMI and fat mass (Ferron *et al.*, 1997; Grinspoon *et al.*, 1996; Lear *et al.*, 1999; Mathiak *et al.*, 1999) except at extremely low BMI (Balligand *et al.*, 1998; Casanueva *et al.*, 1997; Eckert *et al.*, 1998; Pauly *et al.*, 2000). Even in the severely emaciated state, leptin diurnal rhythm and temporal relationship with insulin and IGF-1 are preserved (Casanueva *et al.*, 1997; Eckert *et al.*, 1998; Herpertz *et al.*, 2000), although the temporal relationship with cortisol is disrupted until weight is restored (Herpertz *et al.*, 2000). During weight gain, plasma leptin levels are relatively elevated for BMI (Hebebrand *et al.*, 1997; Mantzoros *et al.*, 1997) and this could at least partially explain the difficulty achieving full weight restoration during rapid refeeding. Similarly, leptin levels are greater than expected for BMI in those with the purging subtype of AN (Mehler *et al.*, 1999). An elevated CSF : plasma leptin ratio in acute AN (Mantzoros *et al.*, 1997) suggests that enhanced central leptin signalling could contribute to impairment of appetite. However, normalization of both plasma

leptin levels (Gendall *et al.*, 1999; Hebebrand *et al.*, 1997; Polito *et al.*, 2000) and the CSF : plasma ratio (Mantzoros *et al.*, 1997) following full recovery indicates that altered leptin dynamics are a state related phenomena.

Insulin and Ghrelin

Although studies of insulin and glucose dynamics in acute AN have not been entirely consistent, on balance, the evidence suggests that basal and fasting insulin levels are reduced in acute AN (Alderdice *et al.*, 1985; de Rosa *et al.*, 1983; Uhe *et al.*, 1992), consistent with the need to promote gluconeogenesis in the starving state. Delayed absorption and, in chronic cases, impaired pancreatic beta cell function (Nozaki *et al.*, 1994) may contribute to reduced insulin response to nutrients in AN (Alderdice *et al.*, 1985; Blickle *et al.*, 1984). Insulin sensitivity varies greatly between subjects (Kiriike *et al.*, 1990) and there may be a tendency toward increased glucose utilization rather than storage (Franssila-Kallunki *et al.*, 1991). These altered dynamics appear to resolve with full recovery from AN (Casper, 1996). The role of insulin as a central signal of peripheral energy balance has not been investigated in AN. Similarly, ghrelin has yet to be examined in AN.

Hypothalamic Regulation of Appetite and Weight

Several authors have postulated an imbalance between anorexigenic and orexigenic pathways of the hypothalamus in the aetiology of AN (Kaye *et al.*, 1989; Menzaghi *et al.*, 1993). Despite obvious methodological problems limiting the scope of available data, there is some indirect evidence to support this hypothesis. An association between an Agrp gene polymorphism and AN raises the possibility that this variant could be a less effective MC4-R antagonist, conferring susceptibility to AN via reduced ability to inhibit anorexigenic pathways (Vink *et al.*, 2001). NPY levels are elevated in CSF during the acute illness (Kaye *et al.*, 1990), consistent with a reduced leptin signal from the periphery. Levels normalize with full recovery, at which time CSF polypeptide Y (PPY) levels are also reported normal (Gendall *et al.*, 1999; Kaye *et al.*, 1990; Sapolsky, 1992). The NPY Y5 receptor mediates the orexigenic effects of NPY (Schaffhauser *et al.*, 1997) and there is no evidence for an association between receptor gene polymorphisms and AN (Rosenkranz *et al.*, 1998). However, low CSF galanin in women fully recovered from AN (Frank *et al.*, 2001) could reflect a functional impairment of orexigenic pathways of the hypothalamus in those vulnerable to AN.

One recent study found elevated plasma CART concentrations in those with the acute disorder, and normal concentrations in women who had fully recovered (Sarah Stanley, personal communication). This finding perhaps adds weight to the hypothesized orexigenic role of CART in the ARC, LH and PVN (Abbott *et al.*, 2001).

In terms of anorexigenic pathways in AN, there is no evidence of an MC4 receptor mutation or polymorphism associated with AN (Hinney *et al.*, 1999) and no other studies of the melanocortin system have yet been reported. CSF TRH levels are reduced at low weight and are not fully normalized at attainment of target weight (Lesem *et al.*, 1994). This is not inconsistent with an adaptive response to starvation. CSF CRH levels are elevated during the acute illness (Kaye *et al.*, 1987) and this appears to be in contrast to animal models of food restriction in which CRH activity in the PVN is reduced (Brady *et al.*, 1990).

Studies of HPA axis activity in AN suggest that an impairment of feedback inhibition at the level of the hypothalamus gives rise to elevated CRH activity. This is turn is thought to override intact feedback inhibition at the level of the pituitary (Kling *et al.*, 1993), giving rise to the HPA axis hyperactivity associated with AN (Kaye *et al.*, 1987; Licinio *et al.*, 1996). Elevated concentrations of CRH in the CSF (Kaye *et al.*, 1987) and blunting of the ACTH and β-endorphin responses to exogenous CRH (Brambilla *et al.*, 1996; Hotta *et al.*, 1986) are consistent. with this hypothesis. Although HPA axis arginine vasopressin (AVP) activity is up-regulated in the context of chronic stress in animals and depression in humans, there is no evidence of elevated AVP activity in acute AN (Connan *et al.*, 2001a, 2001b). This is significant because AVP modulates CRH release and sensitivity of the axis to feedback inhibition via altered glucocorticoid receptor activity (Felt *et al.*, 1984; Plotsky *et al.*, 1984). An abnormal HPA axis response to chronic stress may therefore contribute to persistently elevated CRH activity in AN. In addition to the inhibitory effect on appetite and feeding, the widespread behavioural and physiological effects of CRH include many of the features of AN including increased locomotor activity, cardiovascular changes, reduced social and sexual behaviour, impaired sleep and increased anxiety behaviours (Dunn and Berridge, 1990).

There is some evidence to suggest that a heightened cortisol response to stress and a blunted cortisol response to a meal may persist even after full recovery (Connan *et al.*, 2001c; Ward *et al.*, 1998). Subtle abnormalities of HPA axis regulation could therefore contribute to susceptibility to AN. Whilst genetic factors are likely to be important, the elevated prevalence of perinatal stress (Cnattingius *et al.*, 1999; Foley *et al.*, 2001) and insecure attachment (Ward *et al.*, 2000) in those vulnerable to AN suggest that early environmental modulation of HPA axis responsivity (Heim and Nemeroff, 1999) may also contribute.

Effector Systems

In acute AN, basal and fasting IGF-1 concentrations are reduced (Argente *et al.*, 1997; Fukuda *et al.*, 1999; Stoving *et al.*, 1999a) whilst GH (de Rosa *et al.*, 1983; Stoving *et al.*, 1999b) and cortisol concentrations are elevated. These findings are consistent with the need to promote gluconeogenesis in the starving state and resolve with weight gain (Casper *et al.*, 1988a; Casper, 1996; Golden *et al.*, 1994), suggesting that they arise as an adaptive response to prolonged negative energy balance.

GH hypersecretion in AN is associated with a disorganized pattern of both basal and pulsatile release (Stoving *et al.*, 1999b). Neuroendocrine challenge studies suggest that hypersecretion is attributable to impaired somatostatinergic inhibition rather than elevated GHRH activity (Ghigo *et al.*, 1994). Low T3 concentrations may also contribute (Valcavi *et al.*, 1990). Peripheral GH resistance, reflected in reduced availability of GH binding protein (GHBP), is characteristic of both AN and other forms of malnutrition (Stoving *et al.*, 1999a, for review). Since IGF-1 mediates many of the anabolic effects of GH, reduced levels in the starving state likely contribute to GH resistance. Reduced central IGF-1 feedback inhibition may also contribute to GH axis hyperactivity (Melmed *et al.*, 1996). It therefore appears that reciprocal interactions between IGF-1 and GH function effectively in AN, generating a feed-forward system of low IGF-1 and elevated GH. A paradoxical increase in the GH response to intravenous glucose in AN could reflect abnormality of hypothalamic appetite and satiety responses (Tamai *et al.*, 1991).

Altered thyroid function in acute AN does not differ from that associated with malnutrition (de Rosa *et al.*, 1983) and normalizes with maintenance of healthy weight and eating (e.g., Kiyohara *et al.*, 1987; Komaki *et al.*, 1992). Specifically, serum total and free thyroxine (T4) are low or normal, tri-iodothyronine (T3) is reduced and reverse T3 (rT3) is elevated (e.g., Boyar *et al.*, 1977; de Rosa *et al.*, 1983; Komaki *et al.*, 1992). Altered peripheral conversion of T4 to T3 contributes to these abnormalities. However, whilst TSH levels are normal, the TSH response to TRH is delayed and prolonged and T3 response to TRH is reduced, suggesting that

central HPT axis dysregulation may also play a role (Casper and Frohman, 1982; Kiyohara *et al.*, 1989).

In terms of the HPG axis, gonadotrophin releasing hormone (GnRH) release is reduced and gonadotrophin secretion exhibits a pre-pubertal pattern (e.g., van Binsbergen *et al.*, 1990), as would be expected in a low leptin state. Leptin levels are predictive of amenorrhoea in eating disordered patients (Kopp *et al.*, 1997) and restoration of normal levels is necessary, although not sufficient, for restoration of menses (Audi *et al.*, 1998). Similarly, leptin levels are positively correlated with HPG function in males recovering from AN (Wabitsch *et al.*, 2001). Although there is a critical BMI threshold for menstruation to return, the presence of anorexic attitudes and behaviours during recovery from AN appears to be more predictive of persistent amenorrhoea than BMI (Falk and Halmi, 1982). Interestingly, CSF NPY levels following weight gain were normal only in those women with return of menses (Kaye *et al.*, 1990), consistent with the hypothesized role of NPY in mediating the effects of leptin deficiency on HPG axis function.

Bulimia Nervosa

Leptin

The fall in leptin levels associated with fasting is blunted in those with BN (Monteleone *et al.*, 2000a). Additionally, although plasma leptin levels are positively correlated with BMI (Monteleone *et al.*, 2000b), leptin levels are lower than those of healthy comparison women, even after adjusting for low BMI (Brewerton *et al.*, 2000; Monteleone *et al.*, 2000b), a finding that persists even after recovery (Jimerson *et al.*, 2000). Duration of illness and severity of disorder are related to the reduced leptin levels in BN (Monteleone *et al.*, 2000a). Amongst obese binge eaters, lower leptin levels are associated with greater dietary restraint (d'Amore *et al.*, 2001) and restraint might similarly contribute to low leptin levels in those vulnerable to BN. These data suggest that whilst the body fat sensing function of leptin is intact, there is a tendency for a lower leptin secretion per fat mass and thus a higher settling point for weight in BN. A degree of restrained eating may therefore be necessary to maintain a healthy low weight in these individuals. The impaired capacity for the leptin system to respond to acute changes in energy intake could contribute to maintenance of disordered eating in BN.

Hypothalamic Regulation of Appetite and Weight

Following short-term abstinence from binge–purge behaviour, CSF levels of PYY and somatostatin are elevated relative to both acute BN and healthy controls (Kaye *et al.*, 1988, 1990). A relative excess activity in the orexigenic pathways of the hypothalamus might therefore be stimulating overeating in BN. However, levels of both PYY and NPY in CSF are normal after full recovery suggesting that this is unlikely to be a trait phenomenon (Gendall *et al.*, 1999). There are currently no published data regarding other orexigenic and anorexigenic neuropeptides in BN.

Effectors

Milder variants of HPG, HPT, HPA and GH axis changes associated with AN also occur in BN (Coiro *et al.*, 1992; Kennedy *et al.*, 1989; Levy, 1989; Pirke *et al.*, 1988). It is likely that these changes reflect undernutrition despite healthy weight, consistent with the hypothesis that women with BN are maintaining weight at a lower level than is necessary to switch off anabolic systems. The finding that a current weight of less than 85% of previous highest weight is predictive of impaired HPG function in BN (Weltzin *et al.*, 1994) further supports this. There is some suggestion of an association between polycystic ovary syndrome (PCO) and BN, but the nature and extent of such an association have been little studied (Michelmore *et al.*, 2001; Morgan, 1999). Raphael and colleagues (Raphael *et al.*, 1995) found a high prevalence of polycystic ovaries on ultrasound scan, but this was not associated with elevated levels of luteinizing hormone or the metabolic features of PCO. The ultrasound findings may therefore reflect multifollicular ovaries rather than true PCO (Treasure, 1988).

Women with BN exhibit reduced cortisol and sympathetic nervous system (SNS) responses to mental stress, relative to healthy comparison women (Laessle *et al.*, 1992), suggesting that in contrast to AN, BN may be associated with stress hyporesponsivity.

Binge-Eating Disorder

At the opposite end of the eating and weight spectrum from AN, binge-eating disorder (BED) is associated with obesity and overeating, in the absence of behaviours to compensate. The biology of BED is therefore likely to overlap with that of BN, but in the absence of the biological consequences of food restriction. When presented with food, those with BED experience a greater subjective desire to eat than women with simple obesity, although the salivary response to food exposure is reduced (Karhunen *et al.*, 1997a). Binge eating may therefore be driven more by emotional cues than hunger in BED.

The few available data examining biological contributions to dysregulated appetite and weight in obese binge eaters can be summarized as follows. This group do not differ from obese non-binge eaters in terms of resting energy expenditure, body composition, serum lipids, insulin and thyroid hormones (Adami *et al.*, 1995; Wadden *et al.*, 1993). Cephalic phase plasma insulin, free fatty acid and glucose levels also do not differ between binge- and non-binge-eating obese people (Karhunen *et al.*, 1997b). Leptin levels are elevated and proportional to BMI in women with BED (Monteleone *et al.*, 2000b), but it remains to be seen whether leptin levels differ between obese binge and non-binge eaters after adjustment for BMI. It is likely that obese binge eaters have a degree of leptin resistance, as occurs in simple obesity, but again, this remains to be demonstrated. Simple peripheral obesity is associated with elevated morning cortisol and high diurnal variability, whilst visceral obesity is associated with low morning cortisol and blunting of the diurnal rhythm, as well as reduced GH and HPG axis activity (Rosmond *et al.*, 1998). These endocrine changes are thought to be responsible for the metabolic and haemodynamic features of visceral obesity as well as reduced fertility in women (Bjorntorp and Rosmond, 2000a). Termed the Metabolic Syndrome by Bjorntorp and colleagues, an interaction between genetic factors, such as an associated glucocorticoid receptor polymorphism, and environmental stress in early and adult life is thought to contribute to aetiology (Bjorntorp and Rosmond, 2000b). Given the association between early adversity and BED (Fairburn *et al.*, 1998), one might predict that those with BED are more likely to exhibit visceral obesity and the associated metabolic syndrome than simple peripheral obesity.

Synthesis and Implications

Eating disorders can be conceptualized as stress related disorders. A severe life event or difficulty can be identified prior to onset in the majority of cases (Schmidt *et al.*, 1997) and there is evidence of abnormal coping (Troop and Treasure, 1997). Additionally, early adversity is a risk factor for each of the disorders, as it is for psychiatric disorders in general (Fairburn *et al.*, 1997, 1998, 1999). The capacity for adverse early life experience to modulate HPA axis development is now well recognized (Heim and Nemeroff, 1999)

and such experience may also affect appetite and weight regulation in adulthood (McIntosh *et al.*, 1999). Interestingly, feeding ameliorates the adverse effect of maternal deprivation upon HPA axis function (van Oers, de Kloet, and Levine, 1999), providing a potential precursor to emotion driven eating in later life. It seems likely that genetic and early environmental factors interact to generate specific vulnerabilities in the systems regulating appetite, weight and stress responsivity. For AN, the phenotype is characterized by impaired appetite and heightened stress responsivity. In contrast, the BN phenotype may be a tendency toward obesity, eating as a strategy to manage emotional experience and reduced stress responsivity.

In terms of the neuroendocrine data, leptin appears to signal low peripheral energy availability to hypothalamic networks effectively in AN, giving rise to elevated NPY expression and appropriate adaptations to the starving state. These include amenorrhoea, and enhanced metabolic efficiency arising from altered thyroid and IGF-1/GH axis function. Relatively elevated central leptin concentrations could play a role in maintenance of the disorder, whilst elevated leptin for BMI during rapid refeeding may contribute to difficulty restoring weight. There is some evidence to support a hypothesized imbalance between anorexigenic and orexigenic pathways of the hypothalamus. Specifically, impaired Agrp function and reduced galanin expression may impair orexigenic pathways, whilst hyper-reactivity of CRH expression may enhance activity in anorexigenic networks. These abnormalities could underlie the association between AN and premorbid tendency toward leanness. An aberrant HPA axis response to chronic stress, characterized by persistently elevated CRH activity, may be one factor that contributes to the onset and pathophysiology of AN. However, it is likely that other neuropeptide and neurotransmitter systems also play an important role. Indeed, the serotonin system may be particularly relevant in this regard because of the well recognized effects on appetite (Blundell, 1986; Dourish *et al.*, 1986) and reciprocal regulatory interactions with the HPA axis (Dinan, 1996; Grignaschi *et al.*, 1996; Lowry *et al.*, 2000). This may underpin some of the response to treatment. Preliminary evidence suggests that olanzepine, which acts on the serotonin system at multiple sites, may improve weight gain during refeeding in AN (Hanson, 1999; La Via *et al.*, 2000; Mehler *et al.*, 2000). There is also weak evidence to support the use of fluoxetine in preventing relapse after weight restoration (Kaye *et al.*, 2001). As in the treatment of depressive disorder, antidepressant modulation of GR levels, and thus HPA axis function, could be as important as serotonergic modulation for efficacy in AN.

In BN, a tendency toward overweight may arise from a variety of constitutional factors, including reduced leptin signalling from stored body fat. Overweight in the context of low self-esteem and emotional dysregulation increases the risk of engaging in weight reduction behaviours. Once initiated, food restriction gives rise to both hunger and dysphoric mood, which are key triggers for binge eating. At the central level, overactivity of orexigenic pathways in acute BN is in keeping with leptin levels that are relatively low for body mass. However, other factors are likely to contribute because orexigenic peptide levels in CSF return to normal after recovery, despite persistence of low leptin levels. The high reward value of food for those with BN may also be secondary to chronic food restriction and low leptin levels, and could serve to both establish and reinforce binge eating as a strategy for emotional regulation. Reduced satiety signalling from peripheral factors such as CCK (Devlin *et al.*, 1997) and the vagus nerve (Faris *et al.*, 2000) may impair the capacity to terminate binges, whilst the rapid fall in serum insulin and glucose associated with purging may play an important role in perpetuating binge–purge behaviour (Johnson *et al.*, 1994). However, there is no evidence currently to suggest that these are primary problems.

Understanding the biological components of appetite and weight regulation is vital if we are to develop better aetiological and treatment models for eating disorders. Functional neuroimaging, neuroendocrine and molecular genetic studies will help to further elucidate the central mechanisms maintaining low appetite and weight in AN, and the drive to overeat in BN and BED. However, emerging differences in the biology of restricting and binge–purge subtypes of AN, as well as between peripherally and centrally distributed obesity, highlight the need for good phenotypic description of study participants. A consensus definition of diagnostic subtypes, stage of illness and recovery is urgently needed.

Peptides that increase appetite and ultimately body weight have potential for the treatment of AN. Agrp analogues may be particularly interesting because of their long-lasting effect in promoting food intake and fat storage in animals. CRH antagonists may also prove fruitful therapeutic options for the treatment of AN. Safe and effective anti-obesity drugs that target central orexigenic pathways are likely to proliferate in the future, providing potentially useful treatments for BN and BED.

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XXIII-4

Neuroimmunology of Eating Disorders

Jan Pieter Konsman and Robert Dantzer

INTRODUCTION

Eating is a behaviour familiar to all of us. Despite this familiarity, regulation of eating in humans is complex and involves biological, psychological, social as well as cultural factors (Fischler, 2001). Most of our current knowledge concerning the biological factors involved in the regulation of food intake has been gained from investigations of eating behaviour in animals. The results obtained have recently been incorporated into a neurobiological model of the regulation of food intake (see for review Schwartz *et al.*, 2000) that most probably bears important implications for a better understanding of eating behaviour and disorders in humans.

In this paper, we will, therefore, first review the neurobiological basis of the regulation of food intake before addressing two of the most common causes of anorexia in the absence of lesions or dysfunction of the gastro-intestinal tract, namely anorexia associated with infectious diseases and anorexia nervosa. Factors inhibiting food intake will be discussed in more depth than signals promoting eating since the focus of this paper is on anorexia. Besides, our present day understanding of how feeding is initiated is limited and the prevailing model of the regulation of food intake is based on the assumption that ingestion of food generates signals that subsequently inhibit eating.

NEUROBIOLOGY OF EATING

Food intake needs to be regulated to assure the supply of amino acids and energy to cells throughout the body. All macronutrients, carbohydrates, lipids and proteins, can provide usable cellular energy, albeit with different efficiencies. Most cells are, however, capable of using carbohydrates in the form of glucose or lipids in the form of free fatty acids as energy substrates.

The early so-called depletion–repletion model of food intake postulated that organisms start eating when energy substrates, for example glucose levels, are low (depleted) and stop ingesting food as soon as energy substrate levels are replenished. According to this model a relationship should exist between the size of a meal and the time passed since the preceding meal. However, no such relationship is found when measuring meal size and the interval between meals in rats with free access to food (Le Magnen and Tallon, 1966). Instead, a relationship exists between the size of a meal and the time lag before the rat eats its next meal (Le Magnen and Tallon, 1966). These findings indicate that the ingestion of a meal generates satiety signals that suppress food intake.

Cholecystokinin Acts as a Satiety Signal Reducing Meal Size

During the 1970s and 1980s it became clear that the gut peptide cholecystokinin (CCK) constitutes a meal-generated satiety signal. CCK is synthesized in endocrine cells within the mucosa of the proximal small intestine (Buchan *et al.*, 1978) and secreted upon ingestion of food (Liddle *et al.*, 1985). Intraperitoneal administration of a synthetic peptide corresponding to the eight amino acids at the C-terminal portion of CCK (CCK-8) in rats just prior to food presentation causes a dose-dependent decrease in meal size, but not in water intake (Gibbs *et al.*, 1973). CCK-8 administered intravenously to humans also induces earlier satiation without reports or signs of sickness (Geary *et al.*, 1992).

Low concentrations of CCK-8 act on afferent fibres of the vagus nerves to reduce food intake in rats (Smith *et al.*, 1981). Vagal afferent fibres express CCK receptors (Lin and Miller, 1992) and terminate in the brain stem at the level of the nucleus of the solitary tract. Lesioning this brain structure attenuates the satiety effects of CCK-8 (Edwards *et al.*, 1986) indicating that it plays a role in the CCK-induced reduction of meal size. Although forebrain structures most probably influence brain stem circuits activated by CCK-8, it is important to note that brain stem circuits are sufficient to mediate the inhibitory effects of peripheral CCK-8 administration on sucrose intake (Grill and Smith, 1988).

More recently, with the development of CCK receptor antagonists, it has become possible to test the hypothesis that abdominal release of endogenous CCK constitutes a satiety signal. The expected effect of a CCK receptor antagonist would be to increase meal size. Indeed, administration of a CCK_A (CCK type A) receptor antagonist increases meal size by about 25% in rats (Brenner and Ritter, 1995). It is important to note here that these results were obtained with an antagonist that did not enter the brain, since CCK receptors are also present in the CNS. Altogether, these findings indicate that the peripheral release of CCK after ingestion of food constitutes a satiety signal suppressing eating by acting on the vagus nerve and activating hind brain circuits.

Leptin Constitutes an Adiposity Signal Inhibiting Long-Term Meal Size

Although CCK clearly inhibits the ingestion of food during a meal, systematic administration of CCK-8 to rats at the start of each spontaneous meal turns out to have no effect on body weight, since animals eat meals more frequently (West *et al.*, 1984). This indicates that other factors regulate eating over a longer time span to maintain energy stores. The hypothesis that the regulation of food intake is linked to the amount of energy stocked, for example in the form of fat, was first formulated in the 1950s by Kennedy (1953). This so-called lipostatic model of food intake and energy balance postulates that the organism eats to maintain a set point level of body adiposity. This stock of energy in the form of fat is used to meet the energetic demands of the organism. In this model the existence of adiposity signals reflecting the amount of energy stocked in the form of fat was postulated and proposed to act on

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the brain to inhibit feeding and body adiposity (Kennedy, 1953). These adiposity signals are integrated with other factors regulating food intake, such as satiety signals (see above), energy needs as well as the physiological state of the animal and food properties.

It was not until the mid 1990s that a factor proportional to body fat mass was identified in the form of leptin, also known as ob protein (Frederich *et al.*, 1995; Zhang *et al.*, 1994). Leptin is released mainly by subcutaneous and visceral adipose tissue. Leptin levels increase with feeding and quickly fall after starvation (Ahima *et al.*, 1996; Maffei *et al.*, 1995). Mice carrying mutations in the leptin gene or its receptor are obese (Chen *et al.*, 1996; Zhang *et al.*, 1994) indicating that leptin inhibits feeding and adiposity. Indeed, repeated Intraperitoneal administration of leptin reduces food intake and, in contrast to CCK (see above), induces weight loss in leptin deficient mice, but not in mice lacking the leptin receptor (Halaas *et al.*, 1995). Moreover, repeated subcutaneous administration of a stabilized form of leptin in rats reduces meal size through several days after the last injection and does not induce the compensatory overeating (Kahler *et al.*, 1998) that develops after repeated CCK administration (see above). Interestingly, injection of regular leptin into the lateral brain ventricle of the rat reduces meal size for at least 12 hours and at much lower doses than needed after peripheral injection (Flynn *et al.*, 1998). Moreover, neutralization of leptin in the CNS augments food intake in rats without any subsequent compensatory changes in eating patterns (Brunner *et al.*, 1997). These findings clearly show that leptin acts in the CNS to inhibit meal size in both the short and long term.

The Adiposity Signal Leptin Interacts with the Satiety Signal CCK, but Inhibits Long-Term Food Intake by its Action on the Hypothalamus

One of the predictions of the lipostatic model for the regulation of food intake is that adiposity signals, such as leptin, are integrated with satiety signals, such as CCK (see above). Leptin acts in the CNS to inhibit food intake (Brunner *et al.*, 1997) and its receptors are found at the level of the nucleus of the solitary tract (Elmquist *et al.*, 1998). Given that this brain structure is involved in the satiety effects of CCK (Edwards *et al.*, 1986), leptin might interact with the brain circuits mediating the effects of CCK to inhibit food intake. Indeed, intracerebroventricular administration of leptin at a dose that by itself does not affect food intake, increases the suppression of intake induced by Intraperitoneal injection of CCK-8 (Emond *et al.*, 1999). Moreover, combined leptin and CCK-8 treatment augments the number of activated neurons in the nucleus of the solitary tract compared with either leptin or CCK-8 treatment alone (Emond *et al.*, 1999) indicating that leptin and CCK signals interact at the level of the brainstem (see Figure XXIII-4.1).

However, intracerebroventricular injection of leptin still inhibits food intake in rats lacking the CCK_A receptor (Niimi *et al.*, 1999), the receptor responsible for the satiety effects of CCK (see above). In addition, intracerebroventricular leptin induces activation of hypothalamic structures in these rats (Niimi *et al.*, 1999) indicating that leptin inhibits food intake by acting on the hypothalamus independently from CCK. Strong expression of leptin receptors in the CNS is found in the arcuate nucleus at the basis of the hypothalamus in both humans and rodents (Couce *et al.*, 1997; Elmquist *et al.*, 1998). This hypothalamic structure that regulates long-term food intake and energy balance (Dawson and Lorden, 1981; Morris *et al.*, 1998) is pivotal in mediating the anorectic effects of leptin, since lesions of the arcuate nucleus prevent the inhibitory effects of leptin administered into the lateral brain ventricle (Tang-Christensen *et al.*, 1999). Moreover, intravenously injected leptin is taken up by the arcuate nucleus (Banks *et al.*, 1996) possibly by diffusion from the nearby median eminence which lacks a blood-brain barrier. Taken together, these results indicate that leptin acts as a hormone at the level of the arcuate hypothalamus to reduce food intake (see Figure XXIII-4.1).

The Arcuate Hypothalamus Contains Two Neuronal Populations Exerting Opposite Effects on Food Intake and Fat Tissue and Senses Adiposity Signals as well as Energy Needs

Leptin receptors in the arcuate nucleus of the hypothalamus are found on two populations of neuropeptide-expressing neurons (Hakansson *et al.*, 1998; Mercer *et al.*, 1996). The first population expresses neuropeptide Y and projects to the paraventricular nucleus of the hypothalamus (Baker and Herkenham, 1995). Local injection of neuropeptide Y into the paraventricular nucleus promotes food intake (Stanley and Leibowitz, 1985) and inhibits sympathetic output to fat tissue (Egawa *et al.*, 1991), thus inhibiting catabolic pathways (Schwartz *et al.*, 2000). Conversely, food intake is decreased by damaging arcuate NPY neurons immunologically (Burlet *et al.*, 1995) or by local administration of NPY antigens oligonucleotides (Akabayashi *et al.*, 1994).

The effects of NPY are countered by a second population of arcuate neurons that also project to the paraventricular hypothalamus, but express the neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) (Cowley *et al.*, 1999), a product of the pro-opiomelanocortin gene. α-MSH acts on so-called melanocortin receptors and intracerebroventricular injection of a melanocortin-4 receptor agonist, that mimics α-MSH action, inhibits food intake (Murphy *et al.*, 1998) and increases sympathetic nervous system outflow to adipose tissue (Haynes *et al.*, 1999). Conversely, administration of a melanocortin-4 receptor antagonist into the paraventricular nucleus stimulates feeding (Giraudo *et al.*, 1998).

Feeding increases metabolic rate by 25–40% (Shibata and Bukowiecki, 1987; Sims and Danforth, 1987), and decreases NPY contents in the paraventricular hypothalamus while increasing POMC expression in the arcuate nucleus (Hagan *et al.*, 1999; Kalra *et al.*, 1991). Food restriction, on the other hand, increases NPY, but decreases POMC expression in the arcuate nucleus (Brady *et al.*, 1990; Mizuno *et al.*, 1998; Schwartz *et al.*, 1993) and leads to a reduction in energy expenditure (Leibel *et al.*, 1995).

As might be predicted from its inhibitory effects on food intake, leptin decreases arcuate levels of neuropeptide Y (Stephens *et al.*, 1995; Wang *et al.*, 1997) (see insert Figure XXIII-4.1). Leptin receptors are also found on neurons expressing the pro-opiomelanocortin gene that gives rise to α-MSH in the arcuate nucleus (Cheung *et al.*, 1997) and leptin increases expression of pro-opiomelanocortin (Schwartz *et al.*, 1997). Furthermore, the anorectic effect of leptin directly injected into the brain ventricle is attenuated by administration of a melanocortin receptor antagonist (Seeley *et al.*, 1997) indicating that leptin inhibits food intake by stimulating α-MSH release from arcuate neurons (see insert Figure XXIII-4.1).

In summary, leptin inhibits food intake and promotes fat breakdown by stimulation of an α-MSH containing projection and inhibition of a neuropeptide Y-expressing projection from the arcuate to the paraventricular nucleus of the hypothalamus. In addition to sensing energy supplies via leptin receptors, the arcuate nucleus also contains receptors for growth hormone (Minami *et al.*, 1993), corticosterone (Fuxe *et al.*, 1987) and the pro-inflammator cytokine interleukin-1 (Ericsson *et al.*, 1995). These factors signal energy needs in conditions of growth, stress and disease. The presence of their receptors at the level of the arcuate nucleus indicates that the integration of adiposity signals and factors signalling energy needs, as postulated in the lipostatic model of the regulation of food intake (see above), occurs at the level of the arcuate hypothalamus.

Rewarding and Motivational Aspects of Feeding

In addition to its energetic value, food has rewarding properties. In fact, it has been suggested that the energetic value and rewarding

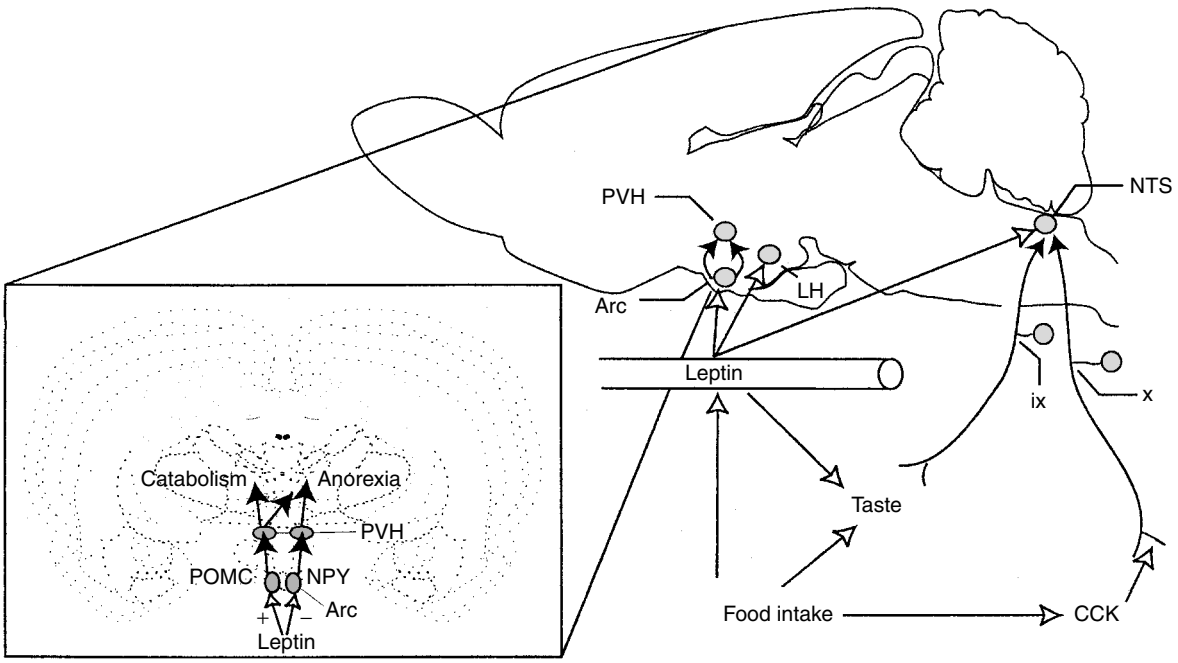

Figure XXIII-4.1 Saggital view of the rat brain to illustrate mechanisms by which leptin reduces food intake. Ingestion of food intake leads to an increase in intestinal cholecystokinin and circulating leptin. At the level of the nucleus of the solitary tract in the brainstem, leptin augments the anorectic signal induced by action of cholecystokinin on the vagus nerve. Leptin also acts at the level of the arcuate hypothalamic nucleus to inhibit the neuropeptide Y containing neuronal projection to the paraventricular hypothalamus which promotes food intake (see insert showing a coronal section at the level of the arcuate and paraventricular hypothalamus). At the same time, leptin increases the synthesis of α-melanocyte-stimulating hormone, a product of the pro-opiomelanocortin gene in neurons of the arcuate hypothalamus. These latter neurons project to the paraventricular hypothalamus to activates anorectic and catabolic pathways. Other mechanisms underlying the anorectic effects of leptin may include modulation of lateral hypothalamic (LH) circuits mediating the rewarding properties of food intake and inhibition of sweet taste sensitivity (see text). Open arrowheads indicate humoral or paracrine action, closed arrowheads neuronal projections. Abbreviations: ix: glossopharyngeal nerve, x: vagus nerve, Arc: arcuate hypothalamic nucleus, CCK: cholecystokinin, LH: lateral hypothalamus, NPY: neuropeptide Y, NTS: nucleus of the solitary tract, POMC: pro-opiomelanocortin, PVH: paraventricular hypothalamus. Drawings of rat brain sections were modified from Swanson (1998)

properties of a food are correlated in that highly palatable foods, such as chocolate, are more energy dense (Drewnowski, 1998). Palatability increases appetite and thus food intake and can be measured as the perceived pleasantness of a food or the intent to eat in humans (Drewnowski, 1998).

The term reward, which connotes pleasure, infers a subjective state and is therefore strictly spoken problematic when referring to animal experiments. Obviously, an animal cannot report the perceived pleasantness of a food or its intent to eat. However, since this term is widely used in the scientific literature, it will also be employed here. Despite this fundamental difficulty, there are ways to measure the rewarding properties of food in laboratory animals. One approach is to electrically activate the brain regions that mediate reward by a micro-electrode when the animal presses an operant lever and to study how natural stimuli like food affect the rate of lever pressing. It is well known that animals work vigorously in the form of lever pressing in order to trigger electrical stimulation of certain brain regions, for example the lateral hypothalamus, as if the stimulation had rewarding properties (Shizgal, 1997). When rats are forced to choose between brain-stimulation reward and a natural reward in the form of a sucrose solution, they choose sucrose when the electrical stimulation is weak. However, at higher stimulation frequencies, rats prefer brain stimulation over sucrose ingestion (Shizgal, 1997).

The rewarding properties of a stimulus also depend on the physiological state of the organism. A hungry animal will choose a more concentrated sucrose solution rather than a less concentrated solution, whereas a sated animal is likely to ingest both solutions with indifference, and an overfed animal chooses not to ingest at all. A hungry animal is also more sensitive to stimulation of the lateral hypothalamus (Abrahamsen *et al.*, 1995; Carr and Papadouka, 1994). Furthermore, mildly food-deprived rats choose to ingest sucrose and stimulate their lateral hypothalamus rather than apply brain stimulation alone (Shizgal, 1997). Since brain stimulation and natural stimuli compete with and add to each other, both signals are likely to activate the same brain circuits that mediate reward.

Interestingly, both food reward and self-stimulation of the lateral hypothalamus increase dopamine release in the nucleus accumbens (Hernandez and Hoebel, 1988). Dopamine containing terminals at the level of the nucleus accumbens are part of the so-called mesolimbic dopamine system that plays an important role in mediating reward (Berridge and Robinson, 1998). Finally, the findings described above are not restricted to the ingestion of sucrose, but also occur when a saline solution is provided to salt-deprived animals (Shizgal, 1997). Altogether, these data are in accordance with the hypothesis that self-stimulation of the lateral hypothalamus is an appropriate model to study reward mechanisms in animals.

From the experiments described above it is also clear that both the properties of a stimulus, such as sweetness, and the physiological state of the organism determine the rewarding properties of that stimulus and the motivational state of the animal. The animal's degree of motivation can be assessed by its capacity to work for a goal, for example by studying the number of times the animal is willing to press a lever in order to obtain a food pellet.

Leptin Modulates Brain Circuits Involved in the Rewarding Aspects of Feeding

According to the lipostatic model of the regulation of food intake, adiposity signals, such as leptin, should interact with the physiological state of the animal and food properties. Intracerebroventricular administration of leptin reduces intake of a sucrose solution from a bottle in rats (Ammar *et al.*, 2000) indicating that leptin is likely to act on those brain circuits that mediate the rewarding properties of food.

However, leptin also inhibits the sensitivity for sweet taste in peripheral taste cells of rodents (Kawai *et al.*, 2000). An alternative explanation would therefore be that leptin does not modulate brain circuits mediating reward, but merely alters the taste of a sucrose solution (see Figure XXIII-4.1). Although the modulation of taste by leptin most probably contributes to the reduction of sucrose intake, evidence exists to indicate that this is not the only factor. As discussed above, food deprivation enhances hypothalamic self-stimulation, an operant model to study reward in animals. Interestingly, intracerebroventricular injection of leptin inhibits the deprivation-induced enhancement of self-stimulation (Fulton *et al.*, 2000) indicating that leptin does indeed modulate brain circuits mediating reward (see Figure XXIII-4.1).

So, in addition to its interaction with brain circuits controlling energy balance at the level of the arcuate nucleus of the hypothalamus, leptin also interacts with CNS circuits mediating the rewarding properties of food. Although the brain circuits that mediate reward are not yet fully unravelled, leptin might act directly on the lateral hypothalamus as its receptors are expressed present in this structure (Elmquist *et al.*, 1998). In contrast to what occurs in the arcuate nucleus which is located close to the median eminence where the blood-brain barrier is absent, circulating leptin does not appear to reach the lateral hypothalamus by diffusion from the blood. Instead, an active transport system involving a shorter form of the leptin receptor might be responsible for leptin action on the lateral hypothalamus (Banks *et al.*, 1996).

ANOREXIA ASSOCIATED WITH INFECTIOUS DISEASES

Suppression of food intake is seen in humans and animals with a variety of systemic diseases as well as with more localized infections (Hart, 1988). By decreasing their food intake the organism reduces the chance of raising plasma concentrations of free iron, which is an essential element that many bacteria need to replicate (Weinberg, 1984). Low iron levels alone do not impair bacterial growth, but proliferation of bacteria is inhibited when iron levels are low and body temperature is elevated (Kluger and Rothenburg, 1979). This is probably due to decreased bacterial synthesis of iron-chelating compounds at temperatures above 37°C (Garibaldi, 1972). So, anorexia together with fever limits bacterial proliferation.

The low incidence of infections in iron-deficient humans is in accordance with this hypothesis. In the late 1970s Murray *et al.*, described a five-fold increase in the incidence of infectious episodes after treatment of iron-deficient nomads with iron (Murray *et al.*, 1978). Based on this finding and their experience with famines in Africa these authors proposed that therapeutic refeeding during infection can be harmful (Murray *et al.*, 1978). When this hypothesis was tested experimentally, it was indeed found that forced feeding of mice during acute bacterial infection reduces survival time and increases mortality (Murray and Murray, 1979), while food deprivation increases survival (Wing and Young, 1980). These findings indicate that anorexia upon acute infection is an adaptive response.

Anorexia during disease has long been thought to be either the consequence of fever or to result from a general weakness of the sick individual. However, hyperthermia alone does not suppress food intake (McCarthy *et al.*, 1984), indicating that anorexia during disease is not a necessary consequence of fever. Since muscle weakness and pain are common symptoms of infectious diseases (Hart, 1988), anorexia during disease might be due to difficulty or pain interfering with hoarding of food. Hoarding of food requires a high locomotor activity, but allows rodents to consume food safely and to anticipate food scarceness. When rats that are dependent for their food intake on the amount of food they hoard are injected with bacterial fragments, food hoarding decreases only by 22%, whereas food consumption drops by 70–75% (Aubert *et al.*, 1997). These findings indicate that the difficulty to move does not play a major factor in anorexia during infectious disease.

During infectious disease anorexia is accompanied by fever as a result of increased heat production in adipose tissue. However, normally when food intake diminishes, adipose tissue metabolism is decreased, a response mediated by the effects of low circulating leptin on the central nervous system (see above). The concomitant occurrence of fever and anorexia suggests, therefore, that the central nervous circuits controlling food intake and energy metabolism are altered during infectious disease.

Interleukin-1β Acts on the Vagus Nerve to Induce Anorexia

Soluble factors secreted by immune cells are proposed to act on the nervous system to induce anorexia during infectious disease (Hart, 1988). One of the soluble factors secreted by tissue macrophages upon detection of bacterial fragments is the pro-inflammatory cytokine interleukin-1β (IL-1β). Intraperitoneal administration of IL-1β induces a reduction in total caloric intake thus mimicking the effect of injection of bacterial fragments (Aubert *et al.*, 1995). To explain its anorectic effects, IL-1β was proposed to act on vagus nerve (Dantzer, 1994). The first evidence indicating that IL-1β affects functioning of the vagus nerve was obtained by Niijima, who showed an increase in firing frequency of the hepatic vagus nerve after injection of IL-1β into the hepatoportal system (Niijima, 1996).

Based on Niijima's initial findings, experiments were performed in our laboratory to study the role of subdiaphragmatic vagal branches in anorexia associated with infectious diseases. A modified Skinner box was used to study the effects of bacterial fragments or IL-1β on food-motivated behaviour in mice of which the vagus nerve was sectioned under the diaphragm. The depressing effects of Intraperitoneal administration of bacterial fragments or IL-1β on a food-motivated lever-pressing response were found to be abolished by subdiaphragmatic vagotomy (Bret-Dibat *et al.*, 1995) and were independent from the well-known anorectic effect of cholecystokinin on the vagus nerve (Bret-Dibat and Dantzer, 2000). These findings indicate that IL-1β acts directly on the vagus nerve to induce anorexia during infectious disease. The discovery of IL-1 receptors on cell bodies of the vagus nerve (Ek *et al.*, 1998) is in accordance with the idea of direct IL-1β action on the vagus nerve (see Figure XXIII-4.2).

The vagus nerve projects to the nucleus of the solitary tract in the brainstem which plays an important role in meal termination (Treece *et al.*, 1998, 2000). Peripheral administration of bacterial fragments (Gaykema *et al.*, 1995) or IL-1β (Ericsson *et al.*, 1994) activates in part the same neurons as the ingestion of a meal (Rinaman *et al.*, 1998) or a physiological dose of cholecystokinin (Rinaman *et al.*, 1993) (see above). However, an additional neuronal population is activated at the level of the nucleus of the solitary tract in response to immunological stimuli. This neuronal population contains the neuropeptide glucagon-like peptide 1 (GLP-1) and seems to mediate nausea during illness (Rinaman, 1999). Moreover, intracerebroventricular administration of an antagonist of GLP receptors attenuates anorexia induced by an Intraperitoneal injection

of bacterial fragments as well as activation of the paraventricular nucleus of the hypothalamus (Rinaman, 2000). This latter structure is important in the control of food intake and energy metabolism (see above), since neuropeptide containing neurons in the paraventricular nucleus of the hypothalamus have been proposed to give rise to pathways that stimulate breakdown of body fat and promote anorexia (Schwartz *et al.*, 2000). In addition to abolishing the anorectic effects of Intraperitoneal administration of bacterial fragments, subdiaphragmatic vagotomy prevents activation of neurons in the nucleus of the solitary tract as well as in the paraventricular nucleus of the hypothalamus (Konsman *et al.*, 2000). Altogether, these findings indicate that during infectious disease IL-1β acts on the vagus nerve to activate GLP-1 neurons in the nucleus of the solitary tract that signal nausea and by consequence induce anorexia.

Although subdiaphragmatic vagotomy prevents the decrease in food-motivated behaviour in response to Intraperitoneal injection of a low dose of IL-1β, it only attenuates the effect of a higher dose (Bret-Dibat *et al.*, 1995). Moreover, vagotomy does not affect activation of neurons in nucleus of the solitary tract and the paraventricular hypothalamus after intravenous injection of LPS or IL-1β (Ericsson *et al.*, 1997; Wan *et al.*, 1994). These observations suggest that still other pathways exist by which IL-1 can act on the central nervous system to induce anorexia during disease.

Interleukin-1β Acts in the Central Nervous System to Induce Anorexia

Observations by several authors show that IL-1 receptors are expressed in the central nervous system (Cunningham *et al.*, 1992; Ericsson *et al.*, 1995; Konsman *et al.*, 2000; Parnet *et al.*, 1994; Yabuuchi *et al.*, 1994), thus raising the possibility that IL-1β acts in the central nervous system to induce anorexia. An experiment was designed in our laboratory to study the role of brain IL-1 receptors in anorexia during infectious disease by infusing an IL-1 receptor antagonist into the lateral brain ventricle of mice after Intraperitoneal injection of bacterial fragments. Intracerebroventricular infusion of the IL-1 receptor antagonist was indeed found to attenuate the reduction in food intake induced by bacterial fragments (Layé *et al.*, 2000).

Although these findings clearly indicate that IL-1 acts in the central nervous system to mediate anorexia during infectious disease, they also give rise to the question as to how IL-1β enters the central nervous system. Blood-borne IL-1β, with its relatively high molecular weight and hydrophilic profile, cannot cross the blood-brain barrier passively. Therefore, circulating IL-1β can only enter the brain by active transport (Banks *et al.*, 1991) or by leakage at the level of circumventricular organs where the blood-brain barrier is non-functional. Circumventricular organs contain fenestrated capillaries that by consequence do not form a functional blood-brain barrier (Gross, 1992). Circulating IL-1β can thus leak from the blood stream into circumventricular organs and possibly into adjacent central nervous tissue. Circumventricular organs also contain phagocytic cells (Murabe *et al.*, 1981) and express receptors for bacterial fragments (Laflamme and Rivest, 2000) raising the possibility that these organs synthesize IL-1β during infectious disease. IL-1β immunoreactivity is, indeed, found in phagocytic cells of circumventricular organs after Intraperitoneal injection of bacterial fragments (Konsman *et al.*, 1999).

One of the circumventricular organs, the median eminence is juxtaposed to the arcuate nucleus of the hypothalamus, a structure involved in the integration of factors signalling energy stocks and needs (see above). The part of the arcuate nucleus of the hypothalamus just adjacent to the median eminence contains capillaries rich in vesicles suggesting transport of molecules through these cells (Gross, 1992). Furthermore, these capillaries are surrounded by perivascular spaces that are thought to be confluent with those around fenestrated capillaries in the median eminence (Gross, 1992), suggesting that circulating molecules reach at least this part of the arcuate hypothalamus. Physiological experiments using intravenous tracer injections in the rat revealed that the capillary and perivascular space available for blood-tissue exchange is four times larger and tissue penetration 34 times greater in the arcuate nucleus proximal to the median eminence compared to the distal arcuate nucleus (Shaver *et al.*, 1992). Since IL-1 receptor mRNA is expressed in the arcuate nucleus of the hypothalamus (Ericsson *et al.*, 1995; Konsman *et al.*, 2000), it is tempting to speculate that IL-1β acts on arcuate neurons to induce anorexia associated with infectious disease. Further research is necessary to establish which neuronal population expresses IL-1 receptors and to what extent IL-1β reaches its receptors in arcuate nucleus of the hypothalamus (see Figure XXIII-4.2).

In summary, IL-1β seems to induce anorexia during infectious disease by provoking nausea via its action on the vagus nerve and possibly by altering central nervous circuits regulating food intake and energy metabolism at the level of the arcuate nucleus of the hypothalamus. In contrast to leptin (see above), IL-1β does not modulate brain circuits mediating reward directly (Anisman *et al.*, 1998). However, both the administration of bacterial fragments and IL-1β do induce a rise in plasma levels of leptin at later time points (Faggioni *et al.*, 1998; Sarraf *et al.*, 1997). The role of leptin in anorexia associated with infectious diseases needs therefore to be addressed in future studies (see Figure XXIII-4.2).

ANOREXIA NERVOSA

Anorexia nervosa is an eating disorder common among teenage girls and characterized by self-starvation and weight loss. This disorder has a very poor prognosis with less than 50% chance of recovery and a mortality rate of 9% (Woodside, 1995). Despite intensive research and numerous hypotheses, the causes of this disorder remain at present unknown.

In view of the anorectic effects of pro-inflammatory cytokines during infectious and probably neoplasic diseases (see above), it was tempting to hypothesize that pro-inflammatory cytokines play a role in the aetiology of anorexia nervosa (Holden and Pakula, 1996; Vaisman and Hahn, 1991). Despite numerous studies aimed at measuring circulating cytokines or *in vitro* cytokine secretion by white blood cells, no clear-cut conclusion can be drawn from these studies (Marcos, 1997). Among the difficulties in interpreting these findings are the fact that cytokines may have paracrine or autocrine effects that do not result in elevated plasma levels and the fact that starvation alters cytokine production (Grimble, 1994). Besides, virtually all reported alternations of immune responses in anorexia nervosa patients improve with refeeding (Marcos, 1997) suggesting that they are a consequence rather than a cause of anorexia nervosa.

A closer look at the symptoms of anorexia nervosa and the non-specific symptoms of infectious disease indicates, however, that it is rather unlikely that pro-inflammatory cytokines play an important role in the aetiology of anorexia nervosa. Anorexia induced by bacterial fragments or interleukin-1 concerns mostly protein intake and not carbohydrates (Aubert *et al.*, 1995). On the contrary, the diet of a typical anorexia nervosa patient is deficient in carbohydrates, while relatively sufficient in protein and fat (Crisp and Stonehill, 1971; Russell, 1967). Another striking difference between anorexia nervosa and infectious diseases is that both infection and pro-inflammatory cytokines provoke depression of behavioural activity (Bluthe *et al.*, 1992), whereas anorexia nervosa is characterized by increased physical activity (Davis *et al.*, 1994). Taken together, these findings indicate that it is unlikely that pro-inflammatory cytokines play a causative role in anorexia nervosa.

Although it seems at first sight counter intuitive that starvation is accompanied by enhanced physical activity, the combination

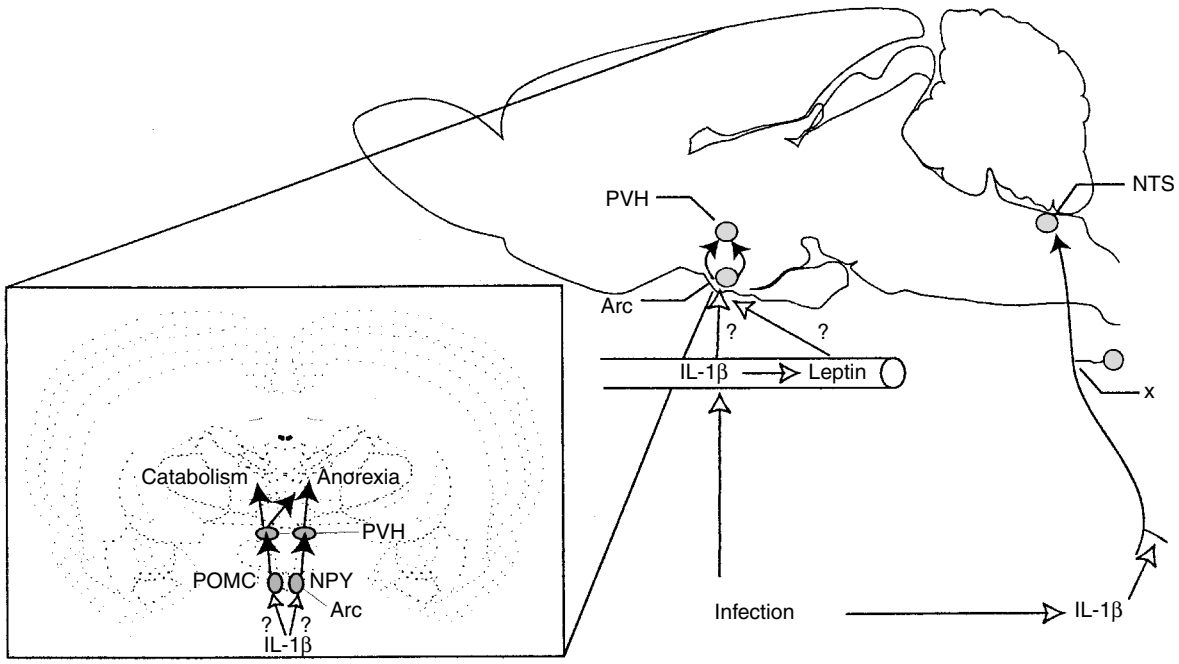

Figure XXIII-4.2 Saggital view of the rat brain to illustrate mechanisms by which interleukin-1β reduces food intake after infection. After detection of bacterial fragments by tissue macrophages, these cells produce interleukin-1β. Interleukin-1β produced by macrophages in the abdominal cavity then acts on the vagus nerve to induce anorexia. Interleukin-1β also acts directly in the brain to induce anorexia after infection. The mechanisms by which interleukin-1β acts in the brain are at present unknown. Circulating interleukin-1β or interleukin-1 produced by phagocytic cells in brain circumventricular organs may act at the level of the arcuate hypothalamus to inhibit the production of neuropeptide Y and to increase that of pro-opiomelanocortin. Interestingly, interleukin-1β induces a rise in circulating levels of leptin, raising the possibility that leptin also plays a role in mediating infection-induced anorexia. Abbreviations and arrowheads see Figure XXIII-4.1

of these two symptoms was considered to be most striking and distinctive in the eyes of Sir William Gull, who coined the disorder anorexia nervosa at the end of the 19th century (Bergh and Sodersten, 1996, 1998). Recent studies in both humans and animals suggest that enhanced physical activity may indeed play an important role in the development and maintenance of anorexia. Analysis of interviews with 32 anorexia nervosa patients revealed that 78% engaged in excessive exercise, 60% were competitive athletes prior to the onset of anorexia nervosa, 60% engaged in sports or exercise before dieting and 75% reported that their physical activity steadily increased during the period when food intake and weight loss decreased the most (Davis *et al.*, 1994). Moreover, earlier studies pointed out that both reducing food intake and exercise are rewarding and agreeable to the patient (reviewed in Bergh and Sodersten, 1996). Interestingly, when rats that have restricted access to food are given access to a running wheel, they also engage in excessive exercise, lose control over body weight and eventually even die (Morrow *et al.*, 1997). The findings both in humans and animals indicate that increased physical activity plays an important role in maintaining and perhaps even in the development of reduced food intake in anorexia nervosa.

The inverse relationship between food intake and energy expenditure indicates that, just like during infectious diseases, the inverse relationship between energy stocks reflected by leptin levels and food intake that normally regulates food intake is overridden in anorexia nervosa. In the case of anorexia nervosa this may be due to an increased release of endogenous opioids or of the neuropeptide corticotropin-releasing hormone. Interestingly, plasma endogenous morphine activities are increased in anorexia nervosa patients (Marrazzi *et al.*, 1997). Furthermore, injections of morphine can reduce food intake when animals are food deprived or have been engaged in intense physical activity (Gulati *et al.*, 1991; Sanger and McCarthy, 1980; White *et al.*, 1977). So, starvation and overactivity might potentate one another through the release of endogenous opioids. In view of the fact that morphine injection increases dopamine release in the nucleus accumbens (Rada *et al.*, 1991) which plays an important role in mediating reward (see above), it can be hypothesized that the rewarding properties of food restriction and exercise in anorexia nervosa are due to activation of brain circuits associated with reward (Bergh and Sodersten, 1996).

Another neuropeptide that may play a role in the association of reduced food intake and increased activity typical of anorexia nervosa is corticotropin-releasing hormone. This peptide controls the activity of the hypothalamus-pituitary adrenal axis, but is also involved in other manifestations of responses to stress (Dunn and Berridge, 1990). Interestingly, corticotropin-releasing hormone is involved in the reduction of food intake after exercise in rats (Rivest and Richard, 1990). In view of the elevated concentrations of corticotropin-releasing hormone in cerebrospinal fluid of anorexia nervosa patients (Kaye *et al.*, 1987), this neuropeptide may indeed play a role in maintaining reduced food intake in anorexia nervosa.

CONCLUSION

There is now ample evidence to suggest that pro-inflammatory cytokines are important mediators of the profound alterations in food intake and energy metabolism that develop during infection and probably during wasting syndromes associated with cancer and AIDS (Argilés and López-Soriano, 1999; Chang *et al.*, 1998). A role for cytokines in other pathological conditions such as geriatric cachexia is also likely especially in view of the increased production of pro-inflammatory cytokines that occurs with ageing (Yeh and

Schuster, 1999). In all these cases, however, the mechanisms of effects of cytokines have not yet been fully elucidated, and their exact targets within the central nervous system remain to be determined. Despite their potent anorectic and metabolic effects, cytokines do not appear to play a major role in anorexia nervosa.

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XXIII-5

Psychophysiology and Eating Disorders

Patricia P. Sanchez Gomez, Nicholas A. Troop and Janet L. Treasure

INTRODUCTION

For psychiatric disorders such as eating disorders in which psychological conflicts seem to be essentially played out on the body, psychophysiology is an important avenue of exploration. Apparently abnormal behaviours in relation to food and weight may be associated with an abnormal psychophysiological response to such stimuli.

One advantage of taking psychophysiological measures, is that this approach is relatively objective and may therefore overcome some of the characteristic biases of other techniques (Lattimore, Gowers and Wagner, 2000) such as the confounding in self-report measures caused by patients' motivational states (e.g. trying to make sense of the illness or even to punish/reward a therapist) and co-morbidity with mood or personality disorders. For example, several studies have shown elevated rates of alexithymia in individuals with eating disorders (Bourke *et al.*, 1992; Schmidt, Jiwany and Treasure, 1993). Alexithymia is a personality construct denoting difficulties in identifying and expressing feelings, an impoverished imaginative life, and an externally oriented cognitive style with thoughts characterized by pragmatic contents (Taylor *et al.*, 1996; Corcos *et al.*, 2000). Individuals displaying these characteristics may systematically misreport their inner states. Thus an objective assessment of emotional responses to food stimuli by monitoring of indices of autonomic arousal can be informative. Among these indices heart rate, blood pressure, skin conductance, finger temperature, respiration and eye movement variability are the most widely used (Léonard *et al.*, 1998; Parrott and Hertel, 1999).

There has been a relative lack of psychophysiological research in patients with either anorexia or bulimia nervosa. However, eating disorders can be set within a dimensional framework in which the clinical conditions merge on the continuum of eating behaviour from dietary restraint to overeating and on the continuum of body weight from emaciation to obesity. Therefore, in addition to studies using clinical groups of patients, we include in this review those studies that report on the psychophysiological responses of dieters and overeaters and in people of different weight groups.

Studies have generally used two types of provocative stimuli to elicit a physiological response. The first relates directly to the symptoms and includes food and body shapes (either real, pictorial or imagined). The second involves exposing participants to some kind of stressor such as a difficult cognitive task (such as unsolvable anagrams) or speech threat (where participants prepare a talk that they believe they will have to present to a group of judges). Below we review studies that measure psychophysiological responses to both of these kinds of stimuli. These studies are summarized in Table XXIII-5.1.

LIMITATIONS OF PSYCHOPHYSIOLOGICAL ASSESSMENT IN EATING DISORDERS

There are some limitations in using psychophysiological methods to assess the autonomic arousal in patients with eating disorders. In the first place, basal autonomic tone may differ from that of non-eating disordered women, particularly, for example, in those who are emaciated. Leptin levels are lower in those who are in a state of starvation and leptin is known to affect activity in the autonomic nervous system. Research has shown that patients with anorexia nervosa and bulimia report high levels of anxiety and depression (de Zwaan *et al.*, 1996; Phillips, Tiggeman and Wade, 1997; Godart *et al.*, 2000) and these variables should also be taken into account. Any differences in arousal between patients with eating disorders and controls could be partially due to their higher baseline levels of anxiety and depression (Léonard *et al.*, 1998; Staiger, Dawe and McCarthy, 2000).

In addition, it is often difficult to get patients with eating disorders to eat foods that they consider 'forbidden', let alone get them to consume as much as non-eating disordered groups (Williamson, 1988). Simple differences in the amount eaten may directly influence psychophysiological measures independently of any pathology. One way round this may be to ask participants to imagine or to look at pictures of food, rather than consume it. However, even here it is possible that patients with anorexia may use cognitive strategies such as avoidance.

Another variable to take into account is the individualization of food cues. As Staiger and co-workers (2000) suggest, an individual's favourite binge food may elicit a greater activation than a standard food. Although some studies have used individualized favourite foods (e.g. Bulik *et al.*, 1996; Staiger, Dawe and McCarthy, 2000) others have used standardized foods or meals (e.g. Léonard *et al.*, 1998). In studies on psychophysiological responses to stress it can also be questioned whether the stressors used approximate to the type and severity of problems that are associated with eating disorders outside of the laboratory. For example, it is severe events and difficulties (such as the ending of a relationship) that typically provoke the onset of an eating disorder rather than being taxed by mental arithmetic or preparing a brief speech. On the other hand, laboratory studies using tasks such as these have been shown successfully to disinhibit dietary restraint (e.g. Heatherton, Herman and Polivy, 1991).

Any or all of these reasons may account for why there is very little psychophysiological research in patients with anorexia nervosa or bulimia nervosa.

Furthermore, psychophysiological measurement is very complex, involving influences due to both situational and person factors. This creates variability in the data and one of the consequences is that only very salient effects can be established using psychophysiological methods. It is also difficult to control other variables that may influence results, such as the amount of food eaten prior to the

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Table XXIII-5.1 Psychophysiological responses to two types of stimuli

Authors	Stimuli	Participants	Psychophysiological measures	Results
Test meal				
Williamson *et al.* (1988)	Test meal	12 BN 12 obese 24 comparison women (12 ate test meal, 12 did not) Mean ages for all the groups ranged from 21.5 to 22.0 yrs. Weight ranges: obese subjects (mean percent overweight = 28%); the two normal groups had weights from −10% to +20% under/overweight of population norms.	Heart rate Vasomotor response Skin temperature Skin resistance Forearm electromyogram	After test meal, increase in heart rate and EMG in BN women. No other significant differences.
Léonard *et al.* (1998)	Standardized test-meal	14 AN women (Age: X = 23.9; BMI: X = 16.2) 10 BN women (Age: X = 27.8; BMI: X = 21.1) 18 comparison women (Age: X = 29.3; BMI: X = 21.1)	Skin conductance Heart rate ECG	Increase in skin conductance during meal in ANs only. No other significant differences between groups.
Food cues exposure and food consumption				
Bulik *et al.* (1996)	Exposure to individualized high-risk binge food	31 BN women (Age: X = 26; BMI<30 kg m^{-2})	Salivation Blood pressure Heart rate	Pre-treatment, no change in salivation after presentation of foods. Post-treatment, increased salivary reactivity. Salivation negatively related to blood pressure, not heart rate.
Vögele and Florin (1996)	Exposure and consumption of individualized binge food	30 female binge eaters (Age: X = 27.5; BMI: X = 23.4) 30 female non-binge eaters (Age: X = 24.3; BMI: X = 22.0)	Heart rate Blood pressure Electrodermal activity Respiration rate	Binge eaters show higher reactivity than comparison women. Heart rate during food exposure predicted amount subsequently consumed.
Overduin *et al.* (1997)	Slides of favourite binge food and subject's own body	11 restrained eaters (non-clinical but disinhibitive) (Age: X = 21.5; BMI: X = 21.8) 13 unrestrained eaters (controls) (Age: X = 20.3; BMI: X = 19.9)	Skin conductance Heart rate Startle eyeblink EMG	No significant differences between groups for any stimuli on any measure.
Nederkoorn *et al.* (2000)	Exposure and consumption of favourite foods	24 healthy students (Age: X = 20.1; BMI: X = 21.5)	Heart rate Blood pressure Peripheral pulse amplitude Electrogastrography Salivation Skin conductance level Temperature	Increases in salivation, temperature, heart rate, skin conductance, diastolic and systolic blood pressure during the exposure. Same changes for saliva, temperature, blood pressure, heart rate and gastric activity during food intake. Blood pressure related to restraint during exposure to food.
Staiger *et al.* (2000)	Sight and smell or sight, smell and taste of individualized binge/favourite and neutral food	17 BN women (Age: X = 21.8; BMI: X = 22.3) 17 comparison women (Age: X = 23.5; BMI: X = 21.1)	Salivation	No significant difference between groups.
Drobes *et al.* (2001) — Study 1	Emotional and food related pictures	105 male and female students	Heart rate Skin conductance Facial EMG	Food deprivation led to greater reactivity (an enhanced startle reflex and increased heart rate) in response to food stimuli but not emotion stimuli.
Drobes *et al.* (2001) — Study 2	Emotional and food-related pictures	76 female students: Binge eaters (Age: X = 19.1; BMI: X = 22.04)	Heart rate Skin conductance	Binge eater and food-deprived groups showed startle potentiation to food cues but not emotional stimuli.

Table XXIII-5.1 (*continued*)

Authors	Stimuli	Participants	Psychophysiological measures	Results
		Restrained eaters (Age: X = 18.5; BMI: X = 21.75) Food deprived (Age: X = 18.3; BMI: X = 21.00) Food non-deprived (Age: X = 18.7; BMI: X = 22.18)		
Stress stimuli				
Cattanach *et al.* (1988)	Interpersonal conflict vignette Audiovisual conflict task Speech delivery Social interaction vignette	15 BN students 15 non-BN students Mean age for both groups: ranging from 17 to 21 yrs.	Blood pressure Pulse	No significant group differences in psychophysiological responses.
Koo-Loeb *et al.* (1998)	Paced auditory task Serial addition task Interpersonal speech task	15 BN (Age: X = 25.3; Weight: X = 130.9 pounds; Height: X = 65.3 inches) 15 comparison women (Age: X = 24.4; Weight: X = 131.5 pounds; Height: X = 64.9 inches)	Blood pressure Heart rate Epinephrine Norepinephrine Systolic time interval Peripheral resistance	Blunted sympathetic activation in BN in response to mental stressors.
Tuschen-Caffier and Vögele (1999)	Achievement challenge Interpersonal conflict	27 BN women (Age: X = 24.5; BMI: X = 20.9) 27 women restrained eaters (Age: X = 26.5; BMI: X = 22.6) 27 women unrestrained eaters (Age: X = 24.6; BMI: X = 21.4)	Heart rate Blood pressure Skin conductance Respiration rate	No significant group differences in psychophysiological reactivity.
Lattimore *et al.* (2000)	Low and high conflict discussion tasks	20 AN (Age: X = 15.7; BMI: X = 17.1) and their mothers 14 psychiatric controls (Age: X = 15.2; BMI: X = 21.8) and their mothers	Heart rate Skin conductance	Higher arousal in AN patients than their mothers and psychiatric controls.

experimental session or the anticipatory anxiety due to participation in the study itself (Williamson *et al.*, 1988).

Another problematic aspect of these studies is that the appropriateness of some parameters to measure autonomic responses (e.g. heart rate, heart rate variability, blood pressure) is still uncertain and we do not exactly know what changes in these parameters mean (Nederkoorn, Smulders and Jansen, 2000). Most of the studies on cue reactivity have used non-specific autonomic responses (e.g. heart rate, blood pressure) that are multidetermined. Changes in these parameters may reflect a range of biological, affective and cognitive events that may or may not be related to the cue exposure (Bulik *et al.*, 1996).

Therefore, and although psychophysiological measures potentially offer an objective measure of emotional arousal, it is in fact likely that, due to the limitations noted above, other types of assessment in addition to psychophysiological measures should be included in the experimental procedure. Direct behavioural observation of participants throughout the experimental session, subjective ratings, and data from self-report inventories may be necessary to support psychophysiological data (Williamson *et al.*, 1988) in order that an overall picture of responses emerges. This is in line with current componential definitions of emotions which emphasize that an emotion includes a cognitive appraisal of the situation, a subjective feeling, an action tendency (or actual behaviour) as well as a psychophysiological response.

CUE REACTIVITY

Notable animal and human research has proved the role of conditioning in the mediation of the appetitive reflex modulation and motivation to eat (Drobes *et al.*, 2001). Classical conditioning models, derived from addiction research have been recently applied to binge eating (Overduin, Jansen and Eilkes, 1997; Overduin and Jansen, 1997).

'Cue reactivity' is defined as a range of physiological, cognitive and emotional responses elicited by cues (conditioned stimuli) that have been repeatedly paired with a specific behaviour such as alcohol, drug administration or binge eating (Staiger, Dawe and McCarthy, 2000). Over a hundred studies have found autonomic responding, such as increased heart rate, skin conductance and salivation, in addicts who were confronted with drug-related cues (Jansen, 1998). However, in contrast to addiction research, relatively few laboratory cue reactivity studies have been carried out in individuals with eating disorders (Overduin, Jansen and Eilkes, 1997; Staiger, Dawe and McCarthy, 2000) and the results are rather contradictory.

Conditioning models of binge eating assume that, through a learning process, cues predictive of binge eating (e.g. feelings of depression, anxiety, loneliness or boredom, or the sight and smell of food) acquire the power to elicit a variety of anticipatory physiological, behavioural, affective and cognitive responses (Bulik *et al.*, 1996; Vögele and Florin, 1996; Overduin, Jansen and Eilkes, 1997). These responses are subjectively experienced as craving, leading to increased food consumption, and play a significant role in dysfunctional eating behaviour (Jansen, 1998; Nederkoorn, Smulders and Jansen, 2000). The reduction of cue reactivity may be an essential part of treatment and continued elevated cue reactivity after treatment could predict relapse (Bulik *et al.*, 1996).

REACTIVITY TO FOOD CUES IN STATES OF NORMAL AND ABNORMAL PHYSIOLOGY OR PSYCHOPATHOLOGY

Now we will review some of the studies that have been carried out to examine the arousal elicited by food cues in individuals

with eating disorders and sub-clinical samples of restrained and binge eaters.

Restrained Eating

Homeostatic drives are thought to lead to periodic phases of disinhibited eating in people who chronically restrain their eating. Both bulimic and restrained subjects are characterized by failed attempts to restrict eating, overconcern with body shape and weight and an elevated frequency of binge eating (Overduin *et al.*, 1997). The conditioning model of binge eating predicts that cue reactivity should be larger in restrained eaters than in normal individuals. However, and contrary to these predictions, psychophysiological studies carried out in restrained and unrestrained eaters (Overduin, Jansen and Eilkes, 1997; Drobes *et al.*, 2001) have not found significant differences in the responses of both groups to food cues.

Binge Eating

Food cue reactivity has been investigated in sub-clinical samples of binge eaters and in individuals with a clear diagnosis of bulimia nervosa. For example, Vögele and Florin (1996) examined reactivity to exposure to favourite foods in non-binge eaters and to binge foods in binge eaters. All subjects responded with increased physiological arousal and feelings of hunger and desire to binge but significant differences between groups were also found. Binge eaters showed higher psychophysiological reactivity (increased blood pressure and electrodermal activity) throughout food exposure relative to controls. Interestingly, heart rate during food exposure predicted the later amount of food consumed in all subjects. This relationship was more marked in binge eaters than in the comparison group. In a later study, Drobes *et al.* (2001) also studied the emotional responding to food cues in binge eaters. Since binge eaters typically report high levels of dietary restraint, these authors predicted that they would show similar responses to food-deprived subjects given that these two groups might have an enhanced approach-oriented bias to food. Their hypothesis was confirmed as both groups showed enhanced startle reflexes while viewing food pictures. The same result was previously reported by Mauler *et al.* (1997) (cited in Drobes *et al.*, 2001) who found augmented probe startle responses to the presentation of food pictures in a bulimic group and in food-deprived normal eaters. Thus it appears that people with bulimia nervosa have arousal of the sympathetic nervous system in response to food cues.

Results from this research therefore suggest that binge eaters show an enhanced overall psychophysiological arousal to the presentation of food cues than do controls. However, the physiological response in terms of parasympathetic reactivity such as salivation to food cues in bulimia nervosa is less clear cut. Staiger and co-workers (2000) examined psychophysiological reactivity to individualized binge/favourite food exposure in bulimic subjects. They hypothesized that increasing cue salience would correlate with augmented reactivity and examined salivation and subjective responses to sensory properties of food (the sight and smell and the sight, smell, and taste of a binge/favourite food) and compared this to the responses elicited by a neutral food. Results confirmed the hypothesis that bulimic subjects would show greater urge to binge, stress and loss of control than the control group and this relationship would be more pronounced when exposed to the sight, smell and taste condition. However, there were no significant differences between groups in salivary reactivity.

In a longitudinal study, Bulik *et al.* (1996) examined salivary reactivity to individualized binge foods in a group of bulimic subjects. Before treatment (eight sessions of cognitive behavioural therapy), there was no evidence of an increase in salivation with the presentation of food. After treatment, however, food cues did lead to greater salivary reactivity. Thus it is possible that, before treatment, arousal of the sympathetic nervous system may have inhibited the parasympathetic response to food cues, namely salivation.

This result contrasts with those of other studies (LeGoff, Leichner and Spigelman, 1988) however, in which a greater increase in salivation was found in individuals with bulimia nervosa compared to controls. It is not easy to suggest a single conclusion for the contradictory results obtained in salivary reactivity in bulimic subjects. It has been hypothesized that variables such as body mass index, anxiety, depression, food restriction, dehydration, volume depletion or parotid gland hypertrophy could influence salivary reactivity and confound the results (Bulik *et al.*, 1996). In order to test this, Bulik *et al.* (1996) correlated all these variables with salivary reactivity but they failed to find any connection between them. Clearly, further research is needed to elucidate the possible variables affecting salivary reactivity in individuals with bulimia nervosa.

Another interesting topic of research in the addiction area has been to experimentally induce negative mood states and observe changes in the response to specific cues. In line with this, Laberg *et al.* (1991) assessed the physiological and subjective response of restrained eaters and patients with bulimia nervosa to slides before and after an experimental induction of negative mood. The slides consisted of pictures of the subject's actual body, food previously identified by the individual as typical binge food and control images of landscapes and houses. Heart rate was recorded continuously and self-reports of mood, craving and self-efficacy were obtained before and after the presentations of the slides. Patients with bulimia nervosa and restrained eaters did not differ in their responses prior to the negative mood induction. All participants had an initial deceleration in heart rate to food slides. After the induction of negative mood, patients with bulimia nervosa showed a greater deceleration in heart rate in response to food and body shape pictures than comparison women. Furthermore, bulimic patients reported an increased craving for food in the negative mood state. These findings suggest that negative affect focuses attention on food and increases craving in patients with bulimia nervosa and are congruent with clinical data suggesting that binge episodes are more likely in negative mood states.

Anorexia Nervosa

Very few studies of cue reactivity have included a group of individuals with anorexia nervosa. As noted above, one difficulty in interpreting the results of such studies is that starvation is associated with changes in autonomic nervous activity, and this may confound the response. One of the first reported studies of cue reactivity in anorexia nervosa was that by Salkind, Fincham and Silverstone (1980). These authors were interested in testing the conceptualization of anorexia nervosa as a fear-based disorder (i.e. fear of weight gain, weight phobia, morbid dread of fatness) and so also included as a control group patients with a specific phobia. The skin conductance changes in patients with anorexia and related feeding disturbances following exposure to food- and weight-related stimuli were either absent or small. This contrasted with the marked responses to the feared stimuli in subjects with specific phobias. Therefore, the hypothesis that anorexia might be considered a phobic disorder was not supported. However, 'fear of weight gain' remains one of the criteria for a diagnosis of anorexia nervosa in DSM-IV (APA, 1994).

Conclusion

Overall we can conclude that abnormal eating behaviours alter physiological and subjective reactivity to food cues. However, responses on some parameters are still uncertain. It is unclear

whether the difference in these results arises from differences in food cues or technology used from the studies or whether the negative results can be attributed to low power or to inappropriateness of some response parameters. Finally, it is not clear whether this reactivity to food cues confirms the conditioning model of binge eating or not. Future research should be aimed to clarify the veracity of this model and its accuracy to explain abnormal eating patterns.

REACTIVITY TO FOOD INTAKE IN STATES OF NORMAL AND ABNORMAL PHYSIOLOGY OR PSYCHOPATHOLOGY

Weight and appetite are regulated homeostatically. Food is more attractive and the drive to eat is increased in states of starvation (Keys *et al.*, 1950). Food intake is governed not only by states of depletion but also by the hedonic properties of food (Pinel, Assanand and Lehman, 2000). The sensory properties of foods influence food choice and intake (Shepherd and Farleigh, 1989). Therefore the physiological and psychological state of the individual as well as salience of the cue will impact on the psychophysiological response.

The anticipation of food or the exposure to the sensory properties of food (visual, olfactory and taste) as well as cognitive processes (such as the thought of food or eating) elicit cephalic phase responses which prepare the body to optimize the digestion, absorption and use of nutrients, and play a role in the amount of food that an individual can tolerate (Jansen, 1998; Nederkoorn, Smulders and Jansen, 2000). Therefore, it is hypothesized that they are also important in eating disorders in which the intake of a large amount of food is a characteristic feature (Nederkoorn, Smulders and Jansen, 2000). Specifically, the cue reactivity model predicts that the frequent large food intake of binge eaters and their chaotic eating pattern (alternating between dieting and binge eating) will induce stronger conditioned responses in binge eaters than in normal subjects, and that these anticipatory responses will be subjectively experienced as craving, making it more difficult for the subject to abstain from eating (Nederkoorn, Smulders and Jansen, 2000; Jansen, 1998). Although this relationship is particularly marked in individuals with eating disorders, food cues also increase feelings of hunger and craving in healthy individuals (Overduin, Jansen and Eilkes, 1997; Vögele and Florin, 1996; Nederkoorn, Smulders and Jansen, 2000).

Animal and human research has found that food exposure increases salivation, gastric activity, and insulin release. Woods and Strubbe (1994) found that the temperature of rats increased prior to a meal. Nederkoorn and co-workers (2000) found a similar increase in temperature of humans during food exposure. The response to eating differs from that of exposure alone. Skin conductance increased during food exposure and decreased during and after the meal. A parallel pattern of change was seen in the low frequency component of heart rate variability.

Léonard *et al.* (1998) also assessed the emotional response to food intake in patients with anorexia nervosa, bulimia nervosa and in a comparison group using three measures of psychophysiological response (skin conductance, heart rate and electroencephalogram). The results showed that meal intake did not lead to significant changes in skin conductance in controls or in people with bulimia nervosa. However, in people with anorexia nervosa there was a significant increase in skin conductance during eating. No changes were found on other physiological variables in any of the groups. It is possible that the negative finding in bulimia nervosa may be because the study was underpowered (there were only 10 women with bulimia) and also the cue may have been less salient for women with bulimia than for women with anorexia.

In an early study, Williamson *et al.* (1988) assessed sympathetic arousal following eating. Four groups were included in this study: bulimia nervosa, obese and a normal control group who all ate a test meal and another normal control group who did not eat the test meal. Results showed that both the bulimic and obese groups responded with increased heart rate after eating the test meal, differing from the normal control group who showed a deceleration in heart rate after eating. All groups had vasoconstriction immediately after eating but this rapidly reversed within 20 minutes in the bulimia nervosa group. All groups responded with decreased skin temperature. The bulimic group also differed from all other groups because subjects with bulimia nervosa responded to eating with an immediate augment in skin resistance followed by a subsequent decline. Contrasting to both normal groups, the obese group responded to food intake with decreased skin resistance. Finally, results for forearm EMG suggested that bulimic and obese groups responded to eating with enhanced EMG, whereas both normal groups showed a decrease in this response. Therefore, some of these physiological responses were congruent with a higher level of sympathetic arousal, whereas others showed opposite results, especially in bulimia nervosa. A possible explanation for these mixed results is that vomiting may produce reduced autonomic arousability and therefore it may be particularly hard to find a global sympathetic arousal response in individuals with bulimia nervosa.

Overall it is difficult to draw a general conclusion from these studies due to the different methodologies used in them. For example, the type of food and caloric content of the test meals, the amount of food the subjects were allowed to eat, sample characteristics and previous exposure of food cues were different for each study. Further research characterized by the use of a single methodology and similar groups of subjects is needed to clarify differences in psychophysiological responses to eating in individuals with and without an eating disorder.

STRESS AND BULIMIA NERVOSA

Stress, in particular interpersonal stress (Cattanach, Malley and Rodin, 1988; Polivy and Herman, 1993; Leal, Weise and Dodd, 1995; Koo-Loeb *et al.*, 2000), plays an important role in the aetiology and maintenance of eating disorders. Eating disorder patients subjectively report high levels of perceived stress as well as the presence of objectively determined severe life events and difficulties both concurrently (Soukup, Beiler and Terrell, 1990; Troop, Holbrey and Treasure, 1998) and at onset (Schmidt *et al.*, 1997; Welch, Doll and Fairburn, 1997). In addition, patients with eating disorders show poorer coping in terms of individual coping strategies (such as cognitive and behavioural avoidance of problems), lower confidence in coping and increased helplessness (e.g. Soukup, Beiler and Terrell, 1990; Neckowitz and Morrison, 1991; Troop *et al.*, 1994, 1998; Yager, Rorty and Rossotto, 1995). It is thought that the abnormal eating behaviours arise from a maladaptive response to stress and that individuals with eating disorders, therefore, will respond to stress with a greater psychophysiological reactivity relative to non-eating disordered controls.

In an early study that tested this hypothesis, Cattanach and co-workers (1988) assessed cardiovascular and affective responses to achievement challenge and interpersonal stress in women with bulimia nervosa. Strong cardiovascular responses and negative mood states were seen in both groups but, relative to the comparison group, bulimic individuals did not show significant differences in blood pressure, pulse rate or affective responses to the stressors. Subjective responses were significantly different between both groups. Individuals with bulimia reported a higher desire to binge than controls (particularly during interpersonal stress) and more

global stress, lower self-esteem, and lower mastery than the normal control group. Consistent with these results, Tuschen-Caffier and Vögele (1999) did not find significant differences in heart rate, blood pressure, respiration rate, and electrodermal activity between patients with bulimia nervosa, restrained eaters and controls during achievement challenge and interpersonal stress. However, subjective ratings did show substantial differences between groups since bulimic patients responded to the stressful tasks with enhanced desire to binge and hunger.

In contrast, however, Tuschen *et al.* (1995) found lower cardiovascular reactivity during achievement challenge in the bulimic group relative to the normal comparison group. Koo-Loeb, Pedersen and Girdler (1998) also found elongated PEP intervals, blunted blood pressure and heart rate in response to mental stressors in individuals with bulimia nervosa relative to a normal comparison group. Psychosocial responses of bulimic individuals also suggested that the bulimic group perceived more stress and were more anxious and depressed than the comparison group.

Overall, subjective ratings from these studies suggest that individuals with bulimia nervosa show higher levels of distress to mental stressors than control subjects and this is accompanied by an increased desire to binge. However, psychophysiological data are confusing. This dissociation between emotional responses and physiological reactivity could be due to a variety of factors. For example, the low power of the studies or the type and range of the stressors used through the different studies (see Table XXIII-5.1). Therefore, and as we suggested above, more studies using a similar methodology should be carried out to clarify physiological reactivity in response to stressors. It is also important to discern what kind of stressors may be important for stress-induced eating.

SUMMARY AND CONCLUSION

Research on psychophysiological factors and eating disorders has provided limited and confusing results (Laberg *et al.*, 1991). The psychophysiological reactivity to food cues remains unclear (Nederkoorn, Smulders and Jansen, 2000). As Nederkoon and coworkers (2000) suggest, deriving a global conclusion from these studies is problematic due to the diversity of groups (restrained eaters, bulimic patients, obese), cues (standardized food, individualized favourite food, pictures of food, real food) and psychophysiological measures (salivation, heart rate, skin conductance) used in these studies. Besides this, most of the studies present methodological problems, such as inadequate test stimuli, small sample size, inappropriate control groups and limited analysis of psychophysiological reactivity (Laberg *et al.*, 1991). Results from studies on psychophysiological responses to stress are similarly inconsistent. While some studies report no difference in psychophysiological responses between patients with bulimia nervosa and non-eating disordered women, others do find differences. More consistently, however, self-reports of urge to binge are greater in patients than in comparison women.

So what are we to make of the results reviewed? What are the implications for the theory and therapy of eating disorders? For the cue reactivity to food- and body-related stimuli, if not for responses to stress, the weight of evidence suggests there are differences in physiological arousal to food when people are in a state of deprivation. This is seen in normals who have missed a meal, in restrained eaters and in women with bulimia and anorexia nervosa.

It is uncertain as to whether the psychophysiological abnormalities that have been found represent aetiological risk factors for eating disorders or whether they are consequences of the illness or even factors that may maintain the illness. The psychophysiological differences found in the normal controls as a result of food deprivation suggest that it may be a maintaining factor.

Regardless of any possible role in the development of eating disorders, the use of psychophysiological measures in response to food-related stimuli may provide a useful marker of improvement and recovery. Measures of outcome tend to rely on objective behavioural indicators as these are relatively unequivocal in terms of making diagnoses (notwithstanding debates about the validity of particular criteria or diagnostic categories). However, there is considerable debate as to what constitutes 'recovery' rather than simply 'abstinence' from eating disordered behaviours. Perhaps it is here, potentially, that psychophysiological measures may be of greatest value in identifying differences between asymptomatic rather than recovered patients. One study has explored changes across treatment (Bulik *et al.*, 1996) but not specifically addressed whether 'normal' psychophysiological reactivity to food and food cues can be used as a marker of recovery. Future research on this possibility might be a useful line of enquiry.

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Neuropsychological Findings in Eating Disorders

Christoph J. Lauer

INTRODUCTION

Neuropsychological research in eating disorders initially was driven by the observation that anorexic patients with evidence for perinatal brain injury showed a poor treatment outcome; particularly, a smaller weight gain was found to be associated with more frequent complications in pregnancy and delivery. Such complications were suggested to increase the susceptibility to subtle cerebral damage, which then may lead to a poor clinical prognosis. Hereby, neuropsychological testing was thought to comprise the advantage of favouring identification of cortical dysfunctions caused by these subtle brain damages below anatomically or physiologically detectable levels. The general hypothesis formulated proposed that the pattern of neurocognitive deficits might be suggestive of dysfunction of relative specific brain systems (Braun and Chouinard, 1992). In following these assumptions, most of the early neuropsychological investigations applied formal test batteries such as the 'Luria-Nebraska Neuropsychological Battery' or the 'Wechsler Intelligence Scales' (WAIS). However, while such test batteries are useful in making general conclusions about the presence of impairments in individuals with a given brain injury, they are not flexible enough to allow the investigator to assess the variety of functions that may underlie a performance deficit on a complex cognitive task in patients with, for example, an eating disorder. Poor performance may be the result of a broad range of possibilities, including damage to one of several areas, the accumulative effect of mild deficits in multiple areas, or factors unrelated to specific brain dysfunctions (see: Keefe, 1995). Therefore, neuropsychology should provide more than a window into the everyday mental processes of psychiatric patients; it should provide an objective description of what areas of behaviour and cognition are likely to be a problem for the patient and — more important — what areas are not.

This paper is divided into five sections. First, a short overview of neurocognitive observations in non-eating disordered subjects (e.g. dieters) will be provided; thereafter, the findings obtained during the acute state of an eating disorders will be summarized, followed by the presentation of neurocognitive changes after treatment, the attempt to identify neuropsychological predictors of the treatment outcome and a short section aiming at the neurocognitive-based identification of patients dropping out of therapy. Finally, the significance of various factors mediating the neurocognitive deficits in eating disorder patients will be discussed.

Because little scientific effort has so far been undertaken to evaluate the neurocognitive function in overweight and obese subjects not suffering from an additional disease known to affect neuropsychological task performance (such as the Prader–Willy syndrome, the sleep apnoea syndrome, the Down syndrome), these types of eating disorders will not be considered in this paper.

NEUROPSYCHOLOGICAL FINDINGS IN FORMER PRISONERS OF WAR AND IN NON-EATING DISORDERED, NORMAL WEIGHT DIETERS

In the following the suggestion that severe starvation and weight loss may cause sustained impairments in brain morphology and cognitive functioning, Sutker and co-workers (1987, 1990) evaluated the neuropsychological task performances in former prisoners of war (POW) who had experienced severe weight loss during captivity due to biological and psychological stress. Compared to combat veterans not captured and interned, the high weight-loss POW (loss of more than 35% of preconfinement weight) were deficient on the 'Attention-Concentration' Factor of the WAIS-R and on measures of immediate and delayed memory as assessed by the Wechsler-Memory-Scale (WMS); on the other hand, no significant impairment was obvious on the WAIS-R subtests 'digit symbol' (sustained attention), 'block design' (visuospatial construction) and 'digit span' (span of attention, working memory). The authors concluded that their findings of deficits on measures of immediate recall combined with lowered 'Attention-Concentration' factor performances raises the possibility that memory problems cited frequently in POW self-reports may be more attributable to deficiencies in attention, concentration and perhaps organizing functions than to memory storage or retrieval processes.

In healthy normal weight non-dieting females, various degrees of 'acute' food deprivation (miss one meal, miss two meals and miss all food for 24 hours) did not result in obvious effects on neurocognitive functions such as sustained attention, attentional focus, simple reaction time or immediate memory, except for a significant slower psychomotor speed performance (finger tapping) after 24-hour of food deprivation (Green, Elliman and Rogers, 1995). However, the same group reported that normal weight female *dieters* displayed poorer vigilance performance, slower reaction times and poorer immediate recall of words when they were dieting compared to a period of normal food intake. Performance on psychomotor speed was not impaired (Green and Rogers, 1995). Because self-reported dietary restraint, but not anxiety and depression, was increased during dieting, the authors related their findings to an association between dieting behaviour and high levels of distractibility; they proposed that impaired cognitive performance is closely related to dieting or the perceived need to diet *per se*. An increased distractibility (reduced capacity to inhibit responding to task irrelevant information and to facilitate responses that are goal-directed; assessed by the Stroop Colour Naming Task) was also reported in restrained eaters after a 'high-caloric' preload (single Twix bar) compared to a 'low-caloric' preload (cream cracker) and to unrestrained eaters (Odgen and Greville, 1993); the restrained females took longer to colour name active state words, food words and body size words after the high-caloric preload than unrestrained eating females. Interestingly, not only do acute food deprivation and prolonged periods of dieting affect some facets of neurocognitive

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performance but also the micro-composition of the nourishment *per se*. Investigating the acute effects of isocaloric lunches differing in fat and carbohydrate content Lloyd and colleagues (1994) observed a slower reaction time following the fat- as well as the carbohydrate-rich lunches compared to the fat- and carbohydrate-balanced meals. However, because the subjects reported themselves as more drowsy and muddled after the fat- and carbohydrate-rich lunches, the reported limited attentional capacity might simply reflect the well-known phenomenon of a 'post lunch dip'.

Thus, the findings reported so far in non-eating disordered *dieters* demonstrate that various manipulations of individual eating habits result in a mild impairment of attention and concentration, as indicated by a slower reaction time, an increased distractibility and a slightly reduced capacity of the working memory. However, in non-eating disordered and non-dieting subjects such manipulations failed to provoke any changes in their neurocognitive task profile.

NEUROPSYCHOLOGICAL FINDINGS IN THE ACUTE STATE OF THE EATING DISORDER

One of the initial studies that investigated a broad range of neurocognitive test performances in patients with anorexia nervosa was conducted by Hamsher and colleagues about 20 years ago (1981). The authors followed the concept that at least some forms of anorexia nervosa may occur in the context of subtle brain dysfunction. Applying the cut-off scores that were known to differentiate well between patients with brain lesions and patients with other clinical disorders, the authors reported 45% of their anorexic patients to show impaired performances on two or more of the applied tasks; most frequently a retarded reaction time on a forced-choice task (35% of the patients) was observed, followed by an impairment on mental arithmetic tasks (15%; sustained attention), short-term visual memory (30%) and long-term information retrieval (20%; long-term memory). Other neurocognitive abilities assessed were more or less well preserved (percentage of affected patients $<15\%$; e.g. psychomotor speed, perceptual-analytic ability, spatial orientation and verbal fluency). The rather non-specific and sporadic nature of the test failures was described by the authors as being most characteristic of an attention-concentration deficit or cognitive inefficiency. The assumption of a reduced attentional capacity in eating disorder patients, along with other but sporadic reported difficulties, was underlined in most of the subsequent studies in anorexic and in bulimic patients (*vigilance and sustained attention*: Laessle *et al.*, 1989, 1990; Green *et al.*, 1996; Kingston *et al.*, 1996; *divided attention*: Lauer *et al.*, 1999; attentional capacity necessary for *response execution/inhibition*: Fairburn *et al.*, 1991; Cooper and Fairburn, 1992; Kingston *et al.*, 1996; *attentional flexibility*: Pendleton Jones *et al.*, 1991; Szmukler *et al.*, 1992; *speed of information processing*: Pendleton Jones *et al.*, 1991; Kingston *et al.*, 1996). Because psychomotor speed and reaction time are among the critical variables on almost all tasks assessing the various aspects of attention, one might simply attribute these findings to the general slowing of motor responses associated with starvation (Maxwell, Tucker and Townes, 1984; Green *et al.*, 1996). Alternatively, the poor performances on these tasks may be related to an increased susceptibility to interference and an increased distractibility (Ben-Tovim *et al.*, 1989; Fairburn *et al.*, 1991; Perpina *et al.*, 1993; Long, Hinton and Gillespie, 1994). In other words, most of the attentional deficits in patients with eating disorders seem to depend on an insufficient functioning of selective and flexible processing of more than one piece of relevant information.

In a number of studies, working (immediate) memory capacity, learning abilities, as well as short-term and long-term memory, were found to be impaired to a similar extent in both anorexia and bulimia nervosa (Touyz, Beumont and Johnstone, 1986; Pendleton Jones *et al.*, 1991; Green *et al.*, 1996; Kingston *et al.*, 1996; Mathias and Kent, 1998). In contrast to the rather 'unspecific' impairments of attention, these findings of mnemonic disabilities are suggestive of a 'specific' deficit and have been primarily discussed in terms of an impaired working memory that, according to Baddeley (1992), is formed by the phonological loop and the visuospatial sketch-pad in conjunction with a central executive component. However, other studies failed to demonstrate a significantly reduced capacity of either the phonological loop (e.g. Digit Span) or the visuospatial sketch pad (e.g. Block/Corsi Span) (see Hamsher, Halmi and Benton, 1981; Szmukler *et al.*, 1992; Lauer *et al.*, 1999), implying that the central executive component was dysfunctional; however, this component is particularly dependent on preserved attentional processes. Of interest here are also the findings of Beatty and colleagues (1990). These authors reported the process of learning to be deficient in their patients; however, the delayed recall (short- and long-termed) of that previously learned material was not. Thus, whereas the acquisition period of the new material was affected probably due to impaired attentional demands and information processing, the final encoding and the memory retrieval processes were undisturbed. Interestingly, Strupp and colleagues (1986) reported the effortful information processing (conscious learning) to be quite normal in their patient sample; however, the automatic information processing (learning without conscious intent) was found to be impaired. The authors discussed their finding in terms of heightened arousal (for example, anxiety and preoccupations concerning food and body weight) and attentional focusing. According to Bacon (1974), they suggested that arousal both narrows attentional focus and reduces overall cognitive capacity; therefore, an aroused subject might be expected to have sufficient cognitive capacity to perform well on the designated (effortful) aspect of the task, but to have insufficient capacity to automatically process additional information. Again, the suggestion of an insufficient attentional capacity allowed a satisfactory explanation without dealing with more complex neurocognitive functioning such as working memory or the ability to appropriately encode, store and retrieve information from the memory.

In only a few studies were higher order cognitive skills such as problem solving capacity, categorization and abstraction evaluated (Palazidou, Robinson and Lishman, 1990; Szmukler *et al.*, 1992; Lauer *et al.*, 1999). The performances of tasks measuring such skills depend on complex neurocognitive functioning (for example, to solve the Wisconsin Card Sorting Test, at least auditory attention, visual attention, motor skills, learning, abstraction, categorization, working memory, short-term memory and executive control are needed). No obvious impairments could be ascertained in two investigations applying the WCST (Palazidou, Robinson and Lishman, 1990; Lauer *et al.*, 1999), while Szmukler and co-workers (1992) reported a poor performance in the Austin Maze, which assesses complex serial learning, error utilization, flexibility, planning and adaptive behaviour abilities.

According to the cited findings, no definite conclusion can be drawn as to which neurocognitive functions are impaired in the acute state of the eating disorder. However, as already proposed by Hamsher and colleagues (1981) there is fairly good evidence that the most characteristic failure is a cognitive inefficiency in the form of attentional deficits irrespective of the type of eating disorder (restricting anorexics, bulimic anorexics, and bulimics). Although this conclusion sounds rather non-specific, most of the investigations revealed that especially the selective processing of information is affected in eating disorder patients. This results in a general slowing not limited to specific types of mental decision-making processes. However, this deficiency apparently seems not to diminish the final encoding, storage and retrieval of the information — as long as the information was subjected to conscious learning. In more popular terms, patients with an eating disorder need a bit more time than healthy subjects for sufficient

information encoding, because their overall attentional capacity is limited by additionally processing a certain amount of task irrelevant (disease related?) information. However, this conclusion does not refer to all eating disorder patients; as far as explicitly indicated in the respective reports, only about 38% of the patients presented with more or less obvious neurocognitive impairments (see: Lauer *et al.*, 1999).

RECTIFICATION OF THE NEUROPSYCHOLOGICAL MEASURES AFTER TREATMENT

A number of investigations followed the hypothesis that the neurocognitive deficits assessed at hospital admission might ameliorate in parallel with improvement of the primary and secondary eating disorder symptomatology (weight gain, decreased frequency of bingeing and purging episodes, rectification of the abnormalities in brain morphology and neuroendocrinology). A second, and probably more important point was to identify cognitive deficits predictive for a good but also for a poor clinical prognosis. Such identification would allow the development of additional and specific treatment strategies to increase the likelihood of a favourable outcome.

In addressing this question, two investigations applied a cross-sectional design. In the first study (Strupp *et al.*, 1986), which mainly focused on effortful versus automatic information processing, there were no obvious differences between acute underweight and weight-recovered anorexic patients. Pendleton Jones and colleagues (1991) found a poorer performance on tasks corresponding to attentional focusing, verbal memory and visuospatial reasoning in their underweight anorexics compared to weight-restored anorexics; however, these differences were subtle and nonsignificant.

In contrast, all the prospective studies (Hamsher, Halmi and Benton, 1981; Small *et al.*, 1983; Szmukler *et al.*, 1992; Kingston *et al.*, 1996; Lauer *et al.*, 1999) reported a rectification of the impaired neurocognitive skills, in particular of the attentional capacity, when the patients had successfully completed the respective therapeutic schedules (weight gain, reduction in the frequency of bingeing and purging). In one study, however, the mnemonic disabilities observed in the acute state of anorexia nervosa, continued to persist after a weight gain of at least 10% of the body mass index (Kingston *et al.*, 1996); the authors related their finding to an insufficient weight gain (incomplete nutritional rehabilitation) at the time of re-testing; but they also speculated that the mnemonic disability might have antedated the onset of the illness, although a relationship with the history of perinatal injury could be excluded.

Because one methodological problem is inherent to all neuropsychological prospective study designs — that is the repeated application of the same tasks at different time points — one might argue that the neurocognitive rectifications observed after clinical remission of the eating disorder simply are due to practice effects. However, in most of the studies, methodological attention had been paid to choose measures for which acceptable alternative forms were available, in order to minimize this effect. In addition, the time interval between pre- and post-treatment sessions lasted between 70 days (Szmukler *et al.*, 1992) and seven months (Lauer *et al.*, 1999), a timely length that further lowers the probability of significant practice effects. Therefore, it is certainly possible that the improved attentional abilities are, in part, related to a decrease in susceptibility to interferences and an increase of the flexibility/inhibition functions, as was reported by Kingston *et al.* (1996). This is further supported by the finding of Lauer and colleagues (1999) that the percentage of eating disorder patients who were able to resolve a Daily-Living Problem Task without any cues had increased from 73% at pretreatment to about 90% at post-treatment.

Thus, the neurocognitive deficits that had been present in eating disorder patients before the onset of a specific treatment schedule appear to vanish in parallel with the amelioration of the eating disorder symptomatology, irrespective of whether the patients suffered from an anorexic or a bulimic disorder. However, as will be outlined in more detail in the following section, this conclusion sounds too optimistic, because it refers to only 11% of the eating disorder patients (see below).

DO NEUROPSYCHOLOGICAL MEASURES PREDICT THE CLINICAL OUTCOME?

In four investigations, the authors attempted to identify neurocognitive predictors of the clinical outcome. However, the results reported were conflicting. Hamsher and colleagues (1981) found their anorexic patients who showed impaired performance on two or more neuropsychological tasks at *post*-treatment examination to be more likely to exhibit an unfavourable outcome (weight loss) after one year of follow-up than patients with very mild or no cognitive deficits at hospital discharge. Regarding the task performances at pretreatment examination, however, no such predictions could be confirmed. Small and colleagues (1983) reported the performance in the digit span task, which measures immediate (working) memory capacity, to be a powerful predictor of weight gain in their anorexic patients. In contrast, Szmukler and co-workers (1992) as well as Lauer and colleagues (1999) failed to identify any valuable predictors for the clinical outcome (either just prior to or eight weeks after hospital discharge), although they had applied a broad range of neuropsychological tasks.

NEUROPSYCHOLOGICAL FINDINGS IN PATIENTS DROPPING THE TREATMENT

In one study, the authors aimed at the neuropsychological identification of those patients who dropped out of the therapeutical schedule (Lauer *et al.*, 1999). However, the neurocognitive task performances as well as the personal and clinical characteristics of these patients were fairly good comparable to those patients who completed the programme. Again, the type of the eating disorder appeared not to be of importance.

Percentage of Patients with Neuropsychological Deficits

At present, one would suggest that certain cognitive deficits are evident in anorexic and bulimic patients, but disappear when the eating disorder patients recover. However, when looking at an *individual* level, this suggestion is not reasonable. Considering two or more impaired task performances as a cut-off, only 38% of patients are reported to be cognitively affected in the acute episode of the eating disorder (38 patients out of a total of 101 patients; Hamsher, Halmi and Benton, 1981; Szmukler *et al.*, 1992; Kingston *et al.*, 1996; Lauer *et al.*, 1999) and this figure drops to 27% at post-treatment. In other words, 62% of the patients with an acute eating disorder have well-preserved cognitive skills, while in the remaining patients displaying cognitive deficits (38%) a rectification can be observed in only 11%. Therefore, it appears not surprising that the reports on the cognitive abilities in eating disorders published so far do not provide a very consistent picture, especially as 'positive' findings depend not only on the severity of cognitive deficits but, more importantly, on the number of patients displaying such deficits. It is important to note also that the attempt to distinguish 'poor' and 'good' performing eating disorder patients on a personal (e.g. age, years of education) and a clinical level (e.g. Beck Depression Inventory, BDI; Eating Disorder Inventory, EDI) yielded completely negative results (Hamsher, Halmi and Benton, 1981; Lauer *et al.*, 1999).

FACTORS MODULATING NEUROCOGNITIVE PERFORMANCE IN EATING DISORDER PATIENTS

Eating Disorder Symptomatology

Interestingly, in only a few studies was the performance on neurocognitive tasks directly related to the degree of underweight, the frequency of bingeing and purging, and the severity of the psychopathology in the eating disordered patients (e.g. as measured by the EDI) and their ameliorations during treatment, respectively. The results reported, however, are somewhat disappointing. Correlations calculated between task performances and eating disorder symptomatology were all far from reaching statistical significance. In addition, group comparisons of 'good' performing and 'poor' performing patients revealed no obvious differences in the severity of the eating disorder. And finally, although in all prospective studies the rectification of the cognitive deficits had run in parallel with that of the eating disorder symptomatology, little evidence was found that the two processes are closely interrelated (Strupp *et al.*, 1986; Laessle *et al.*, 1990; Green *et al.*, 1996; Lauer *et al.*, 1999).

'Secondary' Psychopathology

It can also be speculated that the impaired cognitive functions in eating disorder patients are due to secondary psychopathological symptoms, in particular of depression. However, in almost all of the studies performed so far no evidence was found that the severity of depressive symptomatology had influenced the respective cognitive findings in these patients (Hamsher, Halmi and Benton, 1981; Beatty *et al.*, 1990; Pendleton Jones *et al.*, 1991; Szmukler *et al.*, 1992; Cooper and Fairburn, 1993; Green *et al.*, 1996; Kingston *et al.*, 1996; Lauer *et al.*, 1999). Occasionally, however, an association was reported between level of vigilance and severity of depression (Green *et al.*, 1996), a finding that closely matches that generally observed in patients with affective disorders (see further papers in this chapter). In addition, the actual level ('state') of anxiety at neuropsychological examination appeared not to affect task performances (Szmukler *et al.*, 1992; Kingston *et al.*, 1996; Green *et al.*, 1996; Mathias and Kent, 1998). However, the more enduring level of anxiety ('trait'; as measured by the MMPI) was closely associated with the neuropsychological task performances at pretreatment (Hamsher, Halmi and Benton, 1981) and accounted for nearly all of the neuropsychological deficits, though rather subtle ones, reported in the study by Pendleton Jones and colleagues (1991). Therefore, it could be argued that the neurocognitive deficits observed in eating disorder patients reflect simply the effect of more enduring anxiety (this chapter).

Unspecific Stress due to Hospital Admission

In 1981, Hamsher and colleagues had speculated that stress due to hospital admission might obscure task performance in eating disorder patients. A comparable effect also could be expected in those investigations evaluating the neurocognitive status of their patients two to three weeks after admission, that is, during the re-feeding/treatment period. To control for such a bias, Lauer *et al.* (1999) had investigated their patients four weeks *before* admission. However, the profile of cognitive deficits they observed was surprisingly similar to that usually found at or shortly *after* hospital admission. Therefore, it is rather unlikely that the level of performance in patients with eating disorders is affected by unspecific stress due to hospital admission.

Sleep Disorders

Recent observations in sleep research provide fairly good evidence for a relationship between learning and sleep states, particularly rapid eye movement (REM) sleep or dream sleep (Smith, 1996). Furthermore, patients suffering from severe insomnia were found to perform poorly on tasks measuring the capacity of attention and working memory (Pedrosi *et al.*, 1995; Hauri, 1997), observations that are comparable to the neurocognitive task profile in eating disorder patients. The initial electroencephalographic (EEG) sleep studies in patients with anorexia nervosa reported a decreased sleep maintenance caused by frequent awakenings associated with a prolonged wake time during the night, while the amount of REM sleep was unaffected (Crisp, Stonehill and Fenton, 1970); thus, the attentional deficits present in anorexia nervosa might be attributed to a disturbed nocturnal sleep profile. However, the sleep of bulimic patients, who display similarly impaired task performances, was quite undisturbed (Walsh *et al.*, 1985). Moreover, subsequent EEG sleep studies failed to replicate a definite disturbance of the sleep profile (increased numbers of arousals and wake time, decreased amounts of slow wave sleep or REM sleep) in both anorexic and bulimic patients (see: Lauer and Krieg, 2002). Therefore, it appears unlikely that disturbances in the regulation and the maintenance of nocturnal sleep account for the impaired neuropsychological performance in eating disorder patients.

Morphological and Functional Brain Alterations

As already mentioned, neuropsychological research in eating disorder patients initially was driven by the assumption that neurocognitive impairments might be due to subtle cerebral damage acquired by complications in pregnancy and delivery. However, Hamsher and co-workers (1981) were not able to confirm this initial hypothesis by using their patients' case records. One might also speculate that the cognitive deficits depend on morphological and functional brain alterations that are present in the acute episode of an eating disorder, but tend to normalize with clinical recovery (Krieg *et al.*, 1988, 1989; Herholz, 1996; Delvenne *et al.*, 1996; Kingston *et al.*, 1996). Palazidou and co-workers (1990) reported on a significant association between *morphological* brain alterations (size of the cortical sulci) and the performance on the digit symbol test; however, Laessle *et al.* (1989) and Kingston *et al.* (1996) failed to replicate such associations indicating that crude morphological measurements are too insensitive for matching with cognitive deficits. A more conclusive picture can be expected from studies in which neuropsychological assessments are combined with *functional* brain imaging. Several investigations have revealed a relative hypermetabolism in the caudate nuclei and the inferior frontal cortex associated with a relative hypometabolism in the parietal and superior frontal cortex in both anorexics and bulimics (Herholz *et al.*, 1987; Delvenne *et al.*, 1996, 1997; Naruo *et al.*, 2000; see also this chapter). After weight restoration, the parietal hypometabolism and the inferior frontal hypermetabolism tended to persist, whereas the remaining changes had normalized (Delvenne *et al.*, 1996). These findings appear to coincide with the assumption that the capacity of the working memory, which is functionally 'localized' in the frontal and parietal lobes (Shallice, 1982), is particularly affected in eating disorder patients (Green *et al.*, 1996), but also agree with the assumption that primarily the attentional capacity is deficient in these patients (Hamsher, Halmi and Benton, 1981; Pendleton Jones *et al.*, 1991; Lauer *et al.*, 1999).

Neurochemical Changes

Finally, one has to consider several neurochemical substances as the factors mediating the neurocognitive impairments. For example, steroid hormones are known to be specifically altered in the acute eating disorder but normalize with clinical remission (Pirke, Vandereycken and Ploog, 1988; Schweiger, 1991). In addition, these steroid hormones obviously influence human cognitive functions

(Wolkowitz *et al.*, 1990, 1993; Squire, 1992; Sapolsky, 1992). To date, two studies have demonstrated that patients with elevated cortisol concentrations performed poorer on tasks assessing vigilance, attentional demands and memory (Laessle *et al.*, 1992; Seed *et al.*, 2000). However, a direct correlation between the neurocognitive deficits and the cortisol measures could not be established. In a further investigation, Laessle and colleagues (1990) determined the association between β-hydroxybutyric acid (BHBA; elevated levels indicate metabolic adaptation to starvation) and vigilance performance in patients with bulimia nervosa. Interestingly, only the patients with pathologically high BHBA values performed worse on that vigilance task. The authors concluded that biological adaptation to (acute) starvation might be associated with alterations in CNS transmitter systems that regulate and allocate information processing capacity. Thus, although direct evidence is scarce at present, several neurochemical substances appear to be potential candidates that may serve as factors mediating between eating disorder symptomatology and neuropsychological deficits.

SUMMARY

The most robust neurocognitive finding in patients suffering from an eating disorder — and also but to a lesser extent in non-eating disordered dieters — can be characterized by a non-specific attention-concentration deficit. In particular, the selective information processing (enhanced distractibility) and the flexibility/inhibition functions (response selection) appear to be affected. These neurocognitive failures tend to vanish in parallel with the amelioration of the eating disorder. However, there is little evidence that any neuropsychological deficit assessed before the onset of a therapeutical regime might predict the clinical outcome. In addition, eating disorder patients with impaired task performances do not differ in their personal and clinical characteristics from patients presented with well-preserved neurocognitive functioning. Nevertheless, there is some evidence that elevated and enduring anxiety, neurochemical disturbances and, probably, metabolic brain alterations might mediate neuropsychological task performance. Finally, the fact that only fewer than 40% of eating disorder patients show obvious neurocognitive impairments should not limit our efforts to improve therapy by adding specific neuropsychological training programs that aim to ameliorate the attentional deficits. With this, a better clinical prognosis for *all* eating disorder patients might be achieved.

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XXIII-7

Neuroanatomical Bases of Eating Disorders

Rudolf Uher, Janet Treasure and Iain C. Campbell

INTRODUCTION

There are several lines of evidence that may eventually converge to provide an integrated model of brain dysfunction in eating disorders (ED). In this section we have combined current knowledge and have constructed a neuroanatomically based explanation of ED. The picture is far from clear; and consequently, much of what is written contains suggestions and hypotheses rather than established explanations.

Diagnostic categories of ED span a spectrum of heterogeneous conditions, ranging from anorexia to obesity, in which maladaptive eating habits constitute a major symptom and which are thought to arise from primarily psychological disturbance. The inclusion of simple obesity in ED is controversial (Bruch, 1973; Treasure and Collier, 2001). Restrictive anorexia nervosa (RAN), one of the extreme conditions at the end of the ED spectrum, constitutes probably the most clear-cut syndrome, and is therefore a useful model for research. Much more knowledge has been collected for anorexia nervosa (AN) than for other ED. Therefore, this chapter will be substantially based on AN research and bulimia nervosa (BN) will be discussed to a lesser extent.

In the second part of this paper (Symptoms and Physiology) four groups of ED symptoms and their neural correlates are examined (eating and hunger/satiety perception, body image, pain perception and cognitive functions). A third part (Direct evidence of neural disturbance in ED) summarizes evidence from lesions, electrophysiological and neuroimaging studies. Finally, the fourth part provides a conclusion.

SYMPTOMS AND PHYSIOLOGY

Given the heterogeneity of symptoms and syndromes within the spectrum of ED (Treasure and Collier, 2001), it is unlikely that a particular neuroanatomically defined disturbance will match on to DSM IV or ICD10 diagnostic categories, rather it may be helpful at this stage to examine brain function in relation to individual symptoms. However, a question may be raised: can these diverse symptoms be considered as separate disturbances or do they (all or some of them) represent several facets of a common psychopathological mechanism? For example, the apparent insensitivity of AN patients to hunger, pain and physical fatigue could be attributed to a general impairment or inhibition of signalling coming from the body. Moreover, as body image representation is dependent on interoceptive and exteroceptive sensory information, its distortion in ED may also be caused by primary or secondary sensory insensitivity (Smeets and Kosslyn, 2001). The concept of a 'proto-self' (Damasio, 1999) as a representation of bodily awareness that constitutes a basic level of consciousness can be considered as a model which links these various symptoms. The proposed neuroanatomical substrate for this representation of body physical status is comprised of brainstem nuclei, hypothalamus, basal forebrain, insular, secondary sensory and medial parietal cortices.

Four groups of ED symptoms and their neural correlates are examined in this section: eating and hunger/satiety, body image, pain perception and cognitive functions. Although not clinically apparent, the insensitivity to pain is dealt with in detail, because considerable research has been carried out on this topic and it may provide a useful link in the pathophysiology of ED. Other constituents of ED symptomatology: affective and emotional disturbance, impulsivity and obsessive–compulsive symptoms are shared with other psychiatric disorders (affective disorders, personality disorders, obsessive–compulsive disorder) and their description and neural mechanisms can be found in other sections of this book.

Eating, Hunger and Satiety

Deleterious eating behaviour, the most obvious manifestation of ED, is associated with an abnormal perception of hunger and satiety. People with AN seem to lack the feeling of hunger, whereas those with BN have diminished feelings of satiety and still feel hungry after having eaten a large meal (Owen *et al.*, 1985; Halmi, 1988; Halmi and Sunday, 1991; Hetherington and Rolls, 1991, 2001). The complementarity between hunger and satiety states seems to disappear in ED and people with AN of the bingeing–purging subtype (BPAN) may report feeling both full and hungry at the same time (Halmi and Sunday, 1991). In response to provocation by insulin or 2-deoxy-D-glucose the subjective rating of hunger by AN patients paradoxically decreases (Nakai and Koh, 2001). Despite this subjective absence of hunger, when AN patients were shown palatable food, they responded by increasing insulin secretion to an even greater extent than did healthy controls, but then chose not to eat the food (Broberg and Bernstein, 1989). This suggests that although their body is preparing for meal ingestion, people with AN do not follow this with appropriate behaviour (eating the food). In summary, hunger/satiety sensing is apparently disrupted in people with eating disorders and the subjective experience and behaviour are disconnected from the body's needs and autonomic functions. Several important questions remain unresolved: Do patients with AN not feel hunger or do they just not admit feeling it? Is this disturbance in hunger/satiety sensing primary (and possibly causative) or is it acquired during the course of the illness? Hetherington and Rolls (2001) in a recent review have argued in favour of a secondary, learned disturbance in ED.

Neural mechanisms involved in the response to food and its modulation by hunger and satiation have been extensively investigated with single neuron activity mapping in primates (Rolls, 1999). It has been established that taste, olfactory and visual pathways which process food-related stimuli converge to the amygdala and the caudal orbitofrontal cortex, where their

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reward value is appraised and a choice is made whether to engage in action aimed at ingesting the potential food. Whereas the amygdala provides a relatively slow and rigid mechanism of learned stimulus-reinforcement associations, the hierarchically superior orbitofrontal cortex adds the flexibility necessary to rapidly adapt the behaviour to a changing environment. Information on the organism's energy needs is conveyed from the hypothalamus and in both the caudal orbitofrontal cortex and in the lateral hypothalamus, the representation of food-related stimuli depends on whether the organism is hungry or satiated. The ventral striatum is implicated in the representation of reward value of food-related stimuli and also constitutes a link to the motor system. In the head of the caudate nucleus, which receives a direct input from the orbitofrontal cortex, an appropriate behavioural (motor) response is selected, once the decision is made. (For a detailed review of findings in primates, see Rolls, 1999.)

Human neuroimaging studies suggest that the neural circuitry involved in eating might be more widespread than suggested by primate studies. In the human amygdala, parahippocampal gyrus and anterior fusiform gyrus, neural activity in response to visual food-related cues was found to be hunger-dependent (LaBar *et al.*, 2001). Several loci in the orbitofrontal and medial prefrontal cortex are activated by food stimuli in a sensory specific manner — i.e. they are activated only to food that has not been eaten to satiety (O'Doherty *et al.*, 2000). A positron emission tomography (PET) study in healthy men identified hypothalamus, posterior orbitofrontal cortex, anterior cingulate cortex and insular cortex, but also hippocampus, precuneus, caudate nucleus, putamen and cerebellum as being activated in a hungry state (36 hours fasting) compared to the state of satiety (Tataranni *et al.*, 1999). Satiation on the other hand was associated with increased neural activity in the dorsolateral and anterior ventromedial prefrontal cortices and left inferior parietal lobule, whereas the activity in the hypothalamus, orbitofrontal cortex and insula decreased (Tataranni *et al.*, 1999). The influence of satiety on eating-related brain processing has been further investigated in a study, where chocolate was eaten to satiety and beyond, so that its reward value gradually diminished and even became negative. The activity (measured as regional cerebral blood flow — rCBF — with $H_2{}^{15}O$-PET) in response to eating another piece of chocolate gradually decreased in subcallosal and medial orbitofrontal cortex, insula, striatum and midbrain, whereas lateral orbitofrontal, prefrontal and parahippocampal regions became activated with increasing satiety (Small *et al.*, 2001). The human central appetite control system apparently is comprised of an orexigenic network and an inhibitory control (or anorexigenic) circuit and the balance between these two subsystems determines eating behaviour (Tataranni *et al.*, 1999). The orexigenic network (consisting of orbitofrontal and insular cortices, hypothalamus, parts of striatum and hippocampal formation) activates with fasting and it promotes feeding behaviour. The inhibitory anorexigenic circuit consists of anterior ventromedial and dorsolateral prefrontal cortices and acts to terminate eating, probably by direct inhibition of the orexigenic system (Karhunen *et al.*, 1997; Tataranni *et al.*, 1999; Gautier *et al.*, 2000; Small *et al.*, 2001), see Figure XXIII-7.1.

Little is known about neural correlates of the feeling of hunger. This subjective feeling is not separable from the biological state of acute negative energy balance (fasting) in the available studies on healthy volunteers but in AN the sensation of hunger and the energy balance status are dissociated. Furthermore, most but not all (Karhunen *et al.*, 1997; Gordon *et al.*, 2000), human studies have been performed on male volunteers, whereas the majority of ED patients are females. A PET study of food stimuli perception in females yielded results that were rather inconsistent with other studies; no increase of brain activity was recorded in the fasted state in this study and only parietal deactivation was seen (Gordon *et al.*, 2000). The question of gender differences in the neurophysiology of eating has however not yet been addressed.

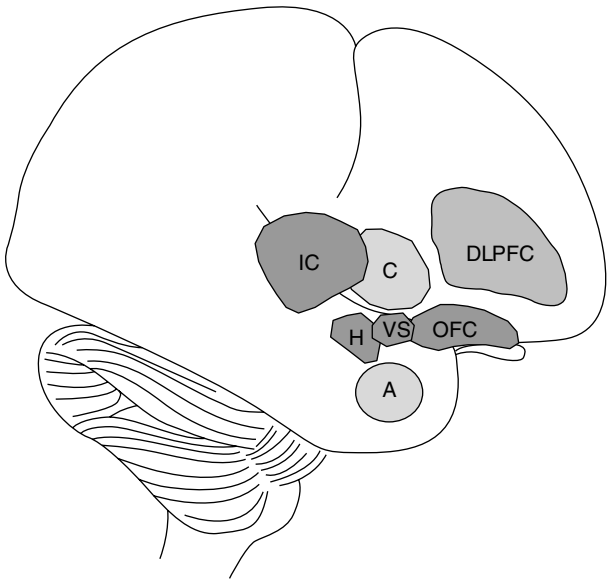

Figure XXIII-7.1 Brain circuits controlling eating behaviour: components of the **orexigenic circuit** are shown in dark shading (OFC — orbitofrontal cortex; VS — ventral striatum; H — hypothalamus; IC — insular cortex); other structures important for eating behaviour in light shading (A-amygdala, C — head of the caudate nucleus); and dorsolateral prefrontal cortex (DLPFC) is shown in medium shading as a structure inhibiting feeding and thus constituting the anorexigenic circuit

Body Image and Anosognosia

Patients with both AN and BN commonly believe their objectively thin bodies or body parts to be fat and large even though their perception of the bodies of other people is usually not distorted (Bruch, 1962; Slade, 1988). Furthermore, any subjective concern about emaciation and its consequences is conspicuously absent (Bruch, 1962), contrasting with their general concern for health and healthy eating in particular. The degree of abnormal body perception and/or conception in ED is akin to neurological disorders such as asomatognosia, microsomatognosia or neglect syndromes or indeed frank delusions or hallucinations. AN is not usually mentioned in writings on anosognosia, but the striking denial of being ill and the body image disturbance are in many ways reminiscent of neurological descriptions of anosognosic patients. As the disturbance in body image is an important constituent of ED psychopathology, we will review the concepts of body representation and its possible contribution to a neuroanatomically based theory of ED.

The body scheme (representing spatial aspects of size, shape and posture) and body image (including also the emotional and cognitive attitudes towards it) depend on multimodal sensory input including proprioception, visual, and haptic tactile sensations (Cumming, 1988; Damasio, 1999; Smeets and Kosslyn, 2001). Unlike early concepts localizing body representation to brain structures in a form of a homunculus, body image is currently viewed as a complex function of a widely distributed network spanning structures from the brainstem to associative cortices (Damasio, 1999). The importance of some of these structures has been highlighted by lesion studies. Considerable evidence implicates the associative parietal cortices, especially in the right hemisphere, and their connections to the thalamus, as lesions in

these areas often produce gross alteration of body perception such as asomatognosia and anosognosia (Cumming, 1988; McGlynn and Schacter, 1989). Anosognosia — unawareness of an obvious deficit is a common phenomenon in many neurological conditions: hemiplegia, cortical blindness (Anton's syndrome), hemianopia, visual agnosia, dementia, amnesia and aphasia, poriomania but also in personality disorder and personality change due to a head injury. In most cases, these disturbances are associated with extensive right parietal lesions (especially in somatic conditions like hemiplegia) or with prefrontal lesions (personality changes) (McGlynn and Schacter, 1989). It has been suggested that the right parietal cortex is the dominant locus for body scheme representation and the proximal prefrontal cortex is important for self-monitoring of the cognitive processes (McGlynn and Schacter, 1989). Thus, damage to these structures would prevent the somatic or cognitive deficits respectively from entering the consciousness. Furthermore, an association of temporal epilepsy with such experiences as micro- or macro-somatognosia (perceiving one's body as smaller or larger) (Trimble, 1988) provides evidence for the participation of temporal lobes in body image representation.

The laterality aspect of body image representation in AN has been examined in a recent study, where body images were morphed to various proportions (thicker and thinner than the real person) and projected separately to the left or right hemisphere in a divided visual field experiment. The left hemisphere tended to identify the subject's own (but not other's) body image with the falsely fatter morphed pictures more often and more quickly than the right hemisphere. The authors suggest that the memory of the subject's own proto-image in the left hemisphere is distorted, whereas the concrete examples represented in the right hemisphere are more accurate (Smeets and Kosslyn, 2001).

In summary, body image representation appears to be a widely distributed and complex function of the CNS with associative cortices of parietal, and possibly frontal and temporal lobes playing important roles; their functioning is nevertheless dependent on multimodal sensory information processing. A pathological disturbance of these structures may be suspected in ED but no particular evidence has been given.

Pain Perception

BN and AN patients have been reported to be less sensitive to pain but the mechanism of this insensitivity is not known (Lautenbacher *et al.*, 1990, 1991). In AN patients, the pain threshold correlates significantly negatively with the local skin temperature, which is suggestive of a primary thermoregulation deficit in AN. In BN patients, the pain threshold tends to correlate positively with body weight (Lautenbacher *et al.*, 1991). The decreased pain sensitivity in ED is not influenced by administration of naloxone and therefore an opiate mechanism seems unlikely to be an important factor. As the pain threshold correlated with the height of patients, it has been hypothesized that a subclinical neuropathy could be the cause (Lautenbacher *et al.*, 1990). The clinical observation that a large proportion of AN patients frequently complain of neuropathic symptoms such as paraesthesias and diminished position sense, also supports the notion of a peripheral neuropathy in AN. Nevertheless, a study aimed at identifying features of a peripheral neuropathy in AN found a selective deficit in pain perception with preserved warmth, cold and vibration recognition in both AN and BN. There was no difference between pain perception from proximal and distal loci, which is considered as an argument against a peripheral neuropathic cause, and therefore the authors concluded that a neuropathy is unlikely to account for elevated pain threshold in ED (Pauls *et al.*, 1991). An autonomic nervous system imbalance has also been suggested as a possible mechanism (Yamamotova and Papezova, 2000).

Decreased pain sensitivity appears to be state dependent in anorexia, because people recovered from AN have normal thresholds (Krieg *et al.*, 1993). Normal pain sensitivity was also found in healthy females after severe food restriction for three weeks (Lautenbacher *et al.*, 1991).

As both BN and AN patients often indulge in self-injurious behaviours, it is interesting that an elevated pain threshold (measured by the cold-pressor test) has also been found in people with borderline personality disorder who reported that they do not experience pain during self-harm (Russ *et al.*, 1992).

Neural mechanisms underlying decreased pain sensitivity in ED are unknown and no relay on the pain pathway can be pinpointed or excluded: receptors, afferent nerve fibres, spinal, medullar, thalamic or cortical mechanisms may be involved. Contrary to earlier views, cortical representation is also essential for pain perception. Neocortical areas surrounding the lateral sulcus (Sylvian fissure), notably the parietal operculum and anterior insula, have been identified as playing a crucial role (Treede *et al.*, 2000). Lesions of the anterior insular cortex produce asymbolia for pain (pain is recognized but not accompanied by negative emotions), which implicates this region in the affective underpinning of pain (Berthier, Starkstein and Leiguarda, 1988; Greenspan, Lee and Lenz, 1999). The anterior cingulate cortex performs the integration of a painful affect and behavioural response selection (Devinsky, Morrell and Vogt, 1995).

In summary, people with eating disorders have an elevated pain threshold, which may be a consequence of starvation as it is not present after weight recovery. Most authors consider primarily peripheral causes of this pain insensitivity, but a central mechanism cannot be excluded. While a peripheral mechanism would probably be restricted to pain perception, a central mechanism may be shared with other disturbances found in ED, such as cognitive impairment, hunger insensitivity and emotional dysregulation.

Cognitive Deficit

Neuropsychological examination of cognitive functioning is a valuable tool for detecting and localizing brain dysfunction and Lauer provides a comprehensive review of ED neuropsychology in Chapter XXIII-6 of this book. In this section, these findings will be summarized according to the neuroanatomical correlates of the cognitive functions impaired in ED.

Both deficits in attention and in visuospatial and tactile spatial processing have been consistently found across studies in AN patients (Fox, 1981; Hamsher, Halmi and Benton, 1981; Kingston *et al.*, 1996; Lauer *et al.*, 1999; Maxwell, Tucker and Townes, 1984; Rovet *et al.*, 1988; Szmukler *et al.*, 1992). Patients with anorexia nervosa are particularly impaired in tasks of spatial abilities which require them to copy a complex figure, complete missing parts of a picture or analyse spatial constructions. The right parietal associative cortex is considered to be central in visuospatial processing and spatial attention and several authors raised the suggestion of right parietal dysfunction in AN (Kinsbourne and Bemporad, 1984; Maxwell, Tucker and Townes, 1984; Braun and Chouinard, 1992; Neumarker *et al.*, 2000; Grunwald *et al.*, 2001). As further evidence for right-rather than left-hemispheric neural disturbance in AN, non-verbal and numeric processing have been reported to be more impaired in AN compared to relatively preserved verbal functions and often excellent language-based academic performance (Fox, 1981; Maxwell, Tucker and Townes, 1984; Strupp *et al.*, 1986; Neumarker *et al.*, 2000). Other studies do not however support this distinction (Jones *et al.*, 1991; Kingston *et al.*, 1996).

Deficits in executive functions, which are mainly attributable to the prefrontal cortices, have also been reported in AN. Problem solving (Szmukler *et al.*, 1992; Lauer *et al.*, 1999), working

memory (Green *et al.*, 1996; Kingston *et al.*, 1996), and cognitive and perceptual set shifting (Kingston *et al.*, 1996; Tchanturia *et al.*, 2001, 2002) have all been reported to be impaired in AN.

As cerebral atrophy is a common finding in AN, several studies combined neuropsychological tests and MRI (magnetic resonance imaging) structural measurements to address the question of whether there is a relationship between structural atrophy and cognitive deficits, but the correlations were generally insignificant (Kingston *et al.*, 1996; Neumarker *et al.*, 2000).

Neuropsychological studies in BN are scarce, but the available evidence indicates cognitive impairments similar to those in AN, with the addition of increased impulsivity (Ferraro, Wonderlich and Jocic, 1997; Lauer *et al.*, 1999, Tchanturia *et al.*, 2001).

In conclusion, there appears to be widespread cognitive impairment in AN. Deficits in attention, visuospatial and numeric processing, problem solving and set shifting are established (see Table XXIII-7.1). This pattern of cognitive impairment is suggestive of a predominantly parietal and prefrontal cortical dysfunction but this association has not been demonstrated in the available morphological neuroimaging studies. As the degree of cognitive disturbance is relatively mild, a rather subtle deficit can be expected, perhaps detectable by functional neuroimaging.

Table XXIII-7.1 Cognitive deficits in AN and their possible neuroanatomical correlates

Function	Evidence	Anatomical correlate
Attention, concentration	Hamsher, 1981 Szmukler, 1992 Laessle, 1989 Jones, 1991 Kingston, 1996 Lauer, 1999	Right frontal, Right parietal, Thalamus
Visuospatial	Fox, 1981; Hamsher, 1981 Szmukler, 1992 Jones, 1991 Kingston, 1996	Right parietal
(Haptic perception)	Grunwald, 2001	
Numeral processing	Fox, 1981 Witt, 1985 Small, 1983 Szmukler, 1992 Palazidou, 1990 Neumarker, 2000	Parietal
Automatic processing	Strupp, 1986 Kingston, 1996	Prefrontal/basal ganglia
Set shifting, flexibility	Tchanturia, 2001 Kingston, 1996	DLPF
Working memory	Green, 1996	DLPF, parietal
Problem solving	Szmukler, 1992 Lauer, 1999	Prefrontal
Psychomotor speed	Kingston, 1996 Lauer, 1999 Maxwell, 1984 Green, 1996	Brainstem + neocortex
Memory (immediate)	Jones, 1991 Kingston, 1996	Prefrontal, temporal, hippocampus
Verbal	Jones, 1991	LH
Non-verbal processing	Maxwell, 1984	RH

DLPF, dorsolateral prefrontal cortex; LH, left hemisphere; RH, right hemisphere.

DIRECT EVIDENCE OF NEURAL DISTURBANCE IN ED

In this section we provide an overview of the evidence from brain lesions and from electrophysiological and neuroimaging studies in ED patients.

Due to the ethical limitations of human studies, most of the knowledge on ED neuroanatomy *in vivo* derives from functional neuroimaging and surface electrophysiology studies. The fact that both these methods appear to be relatively more sensitive to the changes in cortical activity than to those occurring in deep subcortical structures, may have contributed to focus the attention on neocortical functions. The relative importance of some subcortical structures implicated in eating behaviour by the primate single neuron recording studies, e.g. hypothalamus and ventral striatum, cannot be adequately assessed by these methods. There is a relative dearth of the electrophysiological studies compared to the rapidly expanding neuroimaging research.

While functional neuroimaging simply implicates a region as active under certain conditions, the lesion studies provide valuable information on the indispensability of a structure for physiological eating behaviour. In human lesion studies it is however not possible to differentiate between cellular and fibre damage and between acute effects and subsequent adaptation.

Given these methodological differences and limitations, a careful comparison between results of various studies is necessary and only findings replicated by different methods should be taken into account, when constructing an integrated model of brain dysfunction in ED.

Disorders of Eating Associated with Brain Lesions

Although primarily genetic and psychological explanations for ED are prevalent in the recent literature, there is also evidence that relates disordered eating to specific brain lesions and neurological disease. Cases with an obvious somatic cause constitute a small minority of ED, but they are helpful in understanding neural mechanisms underlying eating and its disorders.

Lesions of prefrontal and temporal cortices, mesiotemporal structures and the hypothalamus have all been reported to produce the clinical manifestations of ED, notably AN (Chipkevitch, 1994; Griffith and Hochberg, 1988; Regard and Landis, 1997; Signer and Benson, 1990; Ward *et al.*, 2000). Lesion studies also provide evidence for a right-hemispheric dominance for eating physiology and pathology. Tumours causing anorexia were found to be predominantly located in the right anterior quadrant (Griffith and Hochberg, 1988) as were lesions causing the so-called 'gourmand syndrome', a benign preoccupation with fine eating (Regard and Landis, 1997). Both right temporal and right frontal lesions are associated with eating-related symptoms. Fisher (1994) reported a case where a sudden feeling of hunger was a first symptom of right anterior temporal haemorrhage and hunger is prominent among the symptoms of temporal lobe epilepsy (Gastaut, 1955). An association of AN with temporal lobe epilepsy has also been reported (Signer and Benson, 1990). Bilateral damage to the temporal lobes leads to the Klüver–Bucy syndrome which includes, among other symptoms, hyperphagia and hyperorality (Klüver and Bucy, 1939; Terzian and Ore, 1955). There are several case studies of anorexia associated with tumours affecting the hypothalamus (Heron and Johnston, 1976; Lewin, Mattingly and Millis, 1972; Weller and Weller, 1982) but only one of these tumours was limited to the hypothalamus alone (Lewin, Mattingly and Millis, 1972). An association of AN syndrome with right prefrontal lesions has been reported in several cases (Ward *et al.*, 2000).

In conclusion, lesion studies indicate the hypothalamus and frontal and temporal lobes as physiologically indispensable for eating. Predominance of the right-hemispheric cortical regions is supported by considerable evidence. Especially the right frontal

lesions seem to be causally associated with clear-cut AN syndrome in its whole complexity.

Electrophysiology

Sixty percent of adolescent AN patients have some disturbance of spontaneous electrical activity in their resting electroencephalogram (EEG) which, in most cases, does not improve with weight gain (Rothenberger, Blanz and Lehmkuhl, 1991). Poor modulation of brain responses to auditory stimuli of various intensity and uncoupling of cortical and subcortical systems has been suggested, based on an augmenting/reducing paradigm auditory evoked potential study; this partially normalizes with weight gain (Rothenberger, Blanz and Lehmkuhl, 1991). A study of event-related potentials (ERP) in eating disorders found a prolonged P300 latency (but normal P300 amplitude) in all three ED groups (BN, BPAN, RAN); this may reflect slowness of cognitive processing (Otagaki *et al.*, 1998). Finally, an extensive ERP study by Bradley and coworkers (1997) provides further support for a right hemisphere dysfunction in AN, demonstrating that AN patients fail to show the normal R > L asymmetry for non-verbal tasks seen in control subjects. They also found that the ERP's latency and amplitude abnormalities in AN were most pronounced in the central-parietal region.

Neuroimaging

Neuroimaging research provides direct information on brain dysfunction in eating disorders and Naruo gives a detailed review of both structural and functional neuroimaging studies in Chapter XXIII-8. Only findings most relevant to the neuroanatomy of eating disorders are summarized here.

Whereas most structural imaging findings point to an overall decrease in brain white and grey matter, functional imaging studies, performed either at rest or under a specific challenge, have reported dysfunction of specific brain regions. When AN patients are examined at rest, both global and regional changes in brain function have been reported in terms of increased or decreased brain perfusion or metabolism (Delvenne *et al.*, 1995, 1999; Takano *et al.*, 2001; Naruo *et al.*, 2001). The most frequently reported functional brain abnormalities include global cerebral hypofunction (Delvenne *et al.*, 1995, 1999), anterior cingulate and frontal hypofunction (Delvenne *et al.*, 1995; Nozoe *et al.*, 1995; Takano *et al.*, 2001; Naruo *et al.*, 2001), parietal hypofunction (Delvenne *et al.*, 1995, 1999), and basal ganglia hyperfunction (Delvenne *et al.*, 1999; Herholz *et al.*, 1987). Takano *et al.* (2001) also reported increased perfusion in thalamus and in the hippocampo–amygdalar complex, but these findings have not yet been replicated. Global brain hypometabolism most pronounced in the temporoparietal and frontal regions persists after recovery from AN (Rastam *et al.*, 2001).

In BN patients parietal hypometabolism (Delvenne *et al.*, 1999) and a loss of the normal right > left asymmetry of brain glucose metabolism (Wu *et al.*, 1990) (Andreason *et al.*, 1992) were demonstrated.

To further explore the neural basis of ED symptomatology, specific behavioural challenges (e.g. eating or viewing caloric food) have been used. In AN patients perfusion of the frontal lobes increased in reaction to eating a cake (Nozoe *et al.*, 1993), viewing colour photographs of food and drink activated the right orbitofrontal cortex (Uher *et al.*, 2001) and labelled high-caloric drinks elicited a response in amygdala, insula, anterior cingulate and prefrontal cortex (Ellison *et al.*, 1998). Interestingly, patients with anorexia and bulimia displayed inverse patterns of cerebral perfusion before and after eating a cake: in BN high frontotemporal perfusion at rest abated after eating a cake; in the AN group low frontotemporal perfusion at rest contrasted with an increase while eating (Nozoe *et al.*, 1995). Patients with binge-purging AN (but not restrictive AN) showed increases in glucose metabolism in the right frontal and parietal regions while visualizing a custard cake (Naruo *et al.*, 2000).

In summary, neuroimaging research provides most convincing evidence for a frontal lobe dysfunction in anorexia nervosa; this comprises a decreased resting activity as well as an overreaction of some frontal regions to specific stimuli. Some abnormalities (e.g. parietal hypometabolism) are common to AN and BN, whereas other findings differ between these diagnoses (e.g. frontal lobe reaction to eating).

CONCLUSION

As eating disorders are primarily psychiatric diseases, their genesis and symptomatology are presumed to result from alterations in brain neural networks. In this paper, evidence from physiology, neuropathology, neuropsychology and neuroimaging has been brought together to identify the underlying neural abnormalities. Data obtained with various research methods are summarized in Table XXIII-7.2 according to the brain structures they implicate.

Table XXIII-7.2 Brain structures possibly dysfunctional in ED

Structure	Indirect evidence	Direct evidence
Prefrontal cortex (including orbitofrontal cortex and anterior cingulate)	Eating behaviour Executive cognitive functions deficit (set shifting, problem solving) Personality characteristics	Brain lesions: Ward, 2000; Griffith and Hochberg, 1988; Regard and Landis, 1997 Neuroimaging: Nozoe, 1993, 1995; Delvenne, 1993, 1999; Takano, 2001; Naruo, 2000, 2001; Ellison, 1998; Uher, 2001
Parietal cortex	Visuospatial abilities Number processing Haptic perception Body image Awareness of bodily status Anosognosia Pain perception	Neuroimaging: Delvenne, 1995, 1999 Electrophysiology: Bradley *et al.*, 1997; Grunwald, 2001
Right hemisphere	Cognitive deficit predominantly in non-verbal and spatial skills Right-hemispheric dominance for eating behaviour	Brain lesions: Griffith and Hochberg, 1988; Regard and Landis, 1997; Ward, 2000 Neuroimaging: Nozoe, 1993, 1995; Naruo, 2000; Uher, 2001 Electrophysiology: Bradley *et al.*, 1997
Temporal cortex	Contributes to hunger (Fisher, 1994) and taste recognition (Small, 1997) Klüver–Bucy syndrome	Neuroimaging: Nozoe, 1993, 1995
Insular cortex	Taste, visceral sensing, pain, disgust, body scheme representation	Neuroimaging: Ellison, 1998
Striatum (basal ganglia)	Eating behaviour	Neuroimaging: Herholtz, 1987; Delvenne, 1999
Hypothalamus	Neurohumoral disturbances Eating behaviour	Tumours associated with anorexia: Lewin, 1972

This table reflects an apparent shift in emphasis from hypothalamic and endocrine mechanisms to the neocortical circuits based theories, which is characteristic of much of the current research. The structures in Table XXIII-7.2 are listed roughly in the order of their importance with the reservation that there is functional interdependency within cerebral networks. The available data consistently implicates the association cortices of frontal and parietal lobes as probable loci of dysfunction. Primary involvement of these high-order associative cortices with a multiplicity of functions may reflect the complexity of these diseases. In the face of this complexity and relative lack of consistency in some research results, substantial changes in our concept of ED are still likely to occur and a consensus on the aetiology and neural basis of eating disorders will remain a challenge for future research.

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XXIII-8

Brain Imaging

Tetsuro Naruo

INTRODUCTION

There is a general consensus that the aetiology of eating disorders is multidetermined, affected by biological, psychological and social factors that interact in a complex fashion (Garfinkel, Kennedy and Kaplan, 1995). And while it is clear that no one cause for eating disorders will be discovered, the role of the central nervous system (CNS) deserves special attention as the CNS must play a central role in mediating and maintaining eating behaviours. A fuller understanding of CNS control of eating may lead to improvements in the medical treatment of various disorders of eating. For these reasons, direct and non-invasive analytic methods of brain imaging are now widely applied in the study of eating disorders (Ellison and Foog, 1998; Krishnan and Gadde, 1998; Grady, 1999; Demaerel, 2000; Hendren, De Backer and Pandina, 2000).

This chapter will review the research findings from brain imaging techniques focusing on anorexia nervosa and bulimia nervosa, and categorizing the studies as either structural or functional. The literature on obesity will also be reviewed, as a substantial proportion of the obese in weight control programs have binge-eating behaviour (De Zwaan *et al.*, 1994) and are reported to have many characteristics in common with patients suffering from eating disorders (Stunkard, 1996).

Tables XXIII-8.1 and XXIII-8.2 present a brief summary of the studies reviewed in this chapter according to the brain imaging techniques used.

STRUCTURAL IMAGING FINDINGS

Computed Tomography

Anorexia Nervosa

Numerous important findings in patients with anorexia nervosa were detected by computed tomography (CT) in studies performed in the 1980s. These structural changes are characterized by an enlargement of the cortical and cerebellar sulci, the interhemispheric fissure and the cisterns, and a widening of internal cerebrospinal fluid (CSF) spaces (Datlof *et al.*, 1986; Lankenau *et al.*, 1985). According to these studies, as body weight increased, these changes, labelled 'pseudoatrophy' returned to normal (Kohkmeyer, Lemkuhl and Poutska, 1983; Artmann *et al.*, 1985).

However, there may be different patterns of recovery for the cortical sulci and ventricles over the course of weight restoration. In one of these studies, 15 anorexic patients out of 25 displayed enlarged ventricles and cortical sulci. After 3 month's treatment, the cortical sulci returned to normal while the ventricles remained enlarged (Dolan, Mitchell and Wakeling, 1988). The authors suggested that ventricular enlargement might require longer than 3 months to recover, or that with sufficient chronicity the observed changes in ventricular size might be irreversible. The observed reversal of sulcal widening following refeeding was interpreted as upholding a hypothesis that the observed changes are secondary to malnourishment.

Table XXIII-8.1 Brain imaging studies of eating disorders: structural brain imaging studies

Instruments	Reference	Subjects	Main findings
CT	Dolan *et al.* (1988)	25 AN	Decreased sulcal widening and persisting ventricular enlargement in recovered AN
CT	Krieg *et al.* (1989)	50 BN vs 50 AN vs 50 C	Enlarged CSF spaces in both AN and BN patients
MRI	Hoffman *et al.* (1989)	8 BN vs 8 C	Greater cortical atrophy in BN patients indicated by SCCR
MRI	Golden *et al.* (1996)	12 AN	Inverse correlation between BMI and total ventricular enlargement
MRI	Kingston *et al.* (1996)	46 AN vs 41 C	Association between lower BW and disturbed flexibility/inhibition and memory
MRI	Lamabe *et al.* (1997)	12 AN vs 18 C	Persisting cerebral grey-matter volume deficits after weight recovery

Table XXIII-8.2 Brain imaging studies of eating disorders: functional brain imaging studies

Instruments	Reference	Subjects	Main findings
SPECT	Gordon *et al.* (1997)	15 AN	Unilateral temporal lobe hypoperfusion
SPECT	Karhunen *et al.* (2000)	19 Obese vs 12 C	Correlation between hunger and the rCBF of the lt. frontal and temporal regions
SPECT	Naruo *et al.* (2000)	7 AN-R vs 7 AN-BPvs 7 C	Activation on the rCBF in rt. frontal cortical regions in AN-BP during imagining food
PET	Andreason *et al.* (1992)	11 BN vs 18 HC	Functional links between depressive symptoms and lt. prefrontal hypometabolism
PET	Delvenne *et al.* (1999)	10 AN vs 10 BN vs 10 C	Global hypometabolism in AN and a common parietal cortex dysfunction in ED
PET	Gautier *et al.* (2000)	11 Obese vs 11 C	Greater increases in rCBF in the prefrontal cortex in obese men to satiation
fMRI	Ellison *et al.* (1998)	6 AN vs 6 C	Activation in a limbic and paralimbic network in AN under food stimuli

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In another study, enlarged external CSF spaces were found in 43 (86%) of 50 anorectic patients (Krieg, Lauer and Pirke, 1989a). In a subgroup of 25 subjects examined both at admission and discharge the authors showed that while external CSF was still greater than normal controls, significant decreased had occurred compared to pre-treatment. Among several possible pathogenetic mechanisms thought to be responsible for these morphological brain alterations in patients with anorexia nervosa, hypercortisolemia was suggested as a possible mechanism as reversible changes on CT scan are found not to be unique to anorexia nervosa and have been reported in Cushing's disease, following therapeutic administration of corticosteroids, and in alcohol abuse and dependence.

Bulimia Nervosa

Morphological brain alterations have also been found in patients with bulimia nervosa via CT examinations. A study revealed that patients suffering from bulimia displayed enlarged external CSF spaces. The larger number of patients (13 of 28) than control subjects (2 of 18) displaying signs of cortical atrophy was statistically significant (Krieg, Backmund and Pirke, 1987). It is interesting to note that nine patients who had no history of anorexia nervosa also displayed cortical atrophy. The authors discussed various possible pathogenetic mechanisms, such as changes in the permeability of the blood vessels, diminished blood volume, protein loss with a subsequent movement of intracellular fluid into extracellular spaces or inhibition of brain protein biosynthesis, that have been thought to be responsible for the enlargement of the CSF spaces.

A comparison of 50 bulimia nervosa patients to 50 anorexia nervosa patients and 50 age-matched control subjects, showed increased rates and severity of sulcal widening and ventricular enlargement in both anorexia nervosa and bulimia nervosa subjects compared to controls though the degree and frequency of atrophic changes in bulimics were not so pronounced as in anorexics (Krieg *et al.*, 1989a, 1989b). In this study, the authors assumed that the morphological brain alterations might reflect the endocrine and metabolic reactions to starvation or abnormal eating behaviours as a low concentration of the plasma level of triiodothyronine was inversely correlated with ventricular size in the patients.

Magnetic Resonance Imaging

Anorexia Nervosa

Studies performed using magnetic resonance imaging (MRI) began to appear in the late 1980s, reporting similar findings to those using CT (Palazidou, Robinson and Lishman, 1990; Swayze *et al.*, 1996). MRI studies have expanded on the work done in earlier CT studies. For example, in a study of 12 subjects with anorexia nervosa after 11 months treatment, there was an inverse relationship between body mass index and total ventricular volume (Golden *et al.*, 1996).

Another study compared 12 weight recovered anorexic patients with 13 low-weight anorexic patients and 18 normal controls (Lambe *et al.*, 1997). The weight recovered patient's average BMI was 20.5 and time since weight recovery was more than 1 year. The low-weight patients' average BMI was 15.6. While weight recovered anorexia nervosa patients showed significantly larger grey-matter volume compared to ill subjects, it was still smaller than in the normal controls. The weight recovered group showed no significant difference from the control group on total white-matter volumes though remaining higher than the ill group.

Another study comparing anorexic patients before and after weight gain demonstrated correlations between structural brain abnormalities and neuropsychological function (Kingston *et al.*, 1996). Forty-six patients with anorexia nervosa were compared to 41 normal controls and found to be poor in performance on the attention, visuospatial and memory tasks. On tasks assessing flexibility and learning, no group differences were evident although an examination of deficits in individuals revealed that more anorexics were impaired on both types of tasks. Attention tasks improved, after the patients had gained 10% (BMI = 17.9) of their body weight. The enlarged ventricles and dilatated sulci were both reduced, but showed no correlation with the observed cognitive impairments. The authors also reported that lower weight, but not duration of illness, was associated with poorer performance on tasks assessing flexibility/inhibition and memory, and greater MRI ventricular size. The findings here should be interpreted with caution, but the study is important since it speculates on the relationship between cognitive function and structural findings.

A prospective cohort study using MRI was performed to examine the brains of female adolescents after weight recovery from AN (Katzman *et al.*, 1997). Of 13 patients who underwent an MRI study at low weight, six patients were re-scanned after weight recovery. Quantitative analysis showed that white matter and CSF volumes changed significantly on weight recovery but that there were significant grey-matter volume deficits and enlarged CSF spaces compared to aged-matched female controls. Both of these findings suggest that anorexia nervosa may exert different effects on grey-matter and white-matter volumes, with the former appearing to be more closely related to the degree of weight loss. These results suggest that the abnormalities found in grey matter, white matter, and CSF volumes may resolve to varying degrees and over time with weight restoration.

Since it is well established that glucocorticoids inhibit the proliferation of progenitor cells that occurs in the hippocampal dentate gyrus of adult mammals (Kuhn, Dickinson-Anson and Gage, 1996), hypercortisolemia and other abnormal factors induced by malnutrition may have a specific role in producing the distorted recovery processes of remyelination of grey- and white-matter volumes in patients with anorexia nervosa.

Bulimia Nervosa

Patients with bulimia nervosa have also been investigated with MRI scans. In one study, eight consecutive unmedicated bulimics without a history of anorexia nervosa or alcohol abuse were compared with eight sex and age-matched controls (Hoffman *et al.*, 1989). Using the sagittal cerebral/cranial ratio (SCCR): an easily obtainable and replicable method of quantifying relative cerebral size, the authors found cortical atrophy to be significantly greater in the bulimic group compared to controls. But they failed to find significant differences in ventricle/brain ration (VBR) between bulimics and controls. Methodologic differences in measuring or different metabolic patterns in various brain areas were thought to be responsible for the atrophy found in the two different areas because the correlation between VBR and SCCR was quite low. In discussion the authors described that hypercortisolisms, elevated beta-hydroxy-butyric acid and a loss of brain tissue water due to changes in vascular permeability might be possible causes of brain atrophy.

Anorexia Nervosa and Bulimia Nervosa

Another small study, comparing 18 eating disorder patients (eight anorexia nervosa, 10 bulimia nervosa patients) to 13 normal controls, examined the size and morphology of the pituitary gland using MRI (Doraiswamy *et al.*, 1990). Both anorexics and bulimics showed smaller pituitary gland areas compared with controls. Though the mean values were longer, none of the pituitary measurements in the bulimics differed significantly from those of the anorexics. The authors mentioned that the smaller pituitary sizes

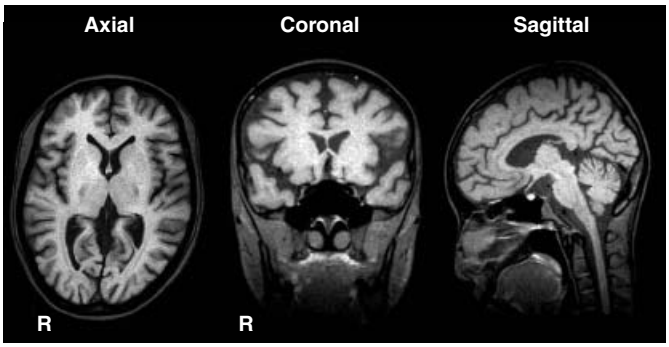

Figure XXIII-8.1 MRI displaying typical brain atrophy of a young female patient with severe anorexia nervosa. Three T1-weighted images show an enlargement of the cortical and cerebellar sulci, cisterns and ventricular dilatation

in the group of patients compared to controls may reflect pituitary atrophy secondary to nutritional or endocrine alterations.

Summary

In summary, early studies using CT and MRI indicated that the morphological alterations in patients with anorexia nervosa were due to weight loss. However, the occurrence of cortical atrophy in normal weight bulimics, as well as relative absence of ventricular enlargement in these patients, suggest more complicated possible mechanisms inducing cerebral atrophy in eating disorders.

Figure XXIII-8.1 shows an example of pseudo cerebral atrophy in anorexia nervosa revealed with MRI. There are obvious structural abnormalities such as an enlargement of the cortical and cerebellar sulci and cisterns, and ventricular dilatation.

FUNCTIONAL IMAGE FINDINGS

Single Photon Emission Computed Tomography

Anorexia Nervosa

Anorexia nervosa has been studied by single photon emission computed tomography (SPECT) since 1989. These studies have used radioactive tracers, allowing the measurement of brain blood flow. In the earlier studies regional cerebral blood flow (rCBF) was measured by the xenon-133 inhalation method.

One early study compared 12 anorexic patients to 12 normal controls at rest using CT and SPECT with xenon-133. This study revealed that the anorexic patients showed no significant reduction in rCBF compared to controls. However, there was a significant inverse relationship between VBR measured by CT and rCBF (Krieg *et al.*, 1989b).

Since the early 1990s ^{99m}Tc-hexamethylpropylene amineoxime (HM-PAO) has become available for the investigation of rCBF. HM-PAO behaves like a chemical microsphere quasipermanently trapped in the brain and allows imagings of regional cerebral perfusion (Inugami, 1988).

SPECT study using HM-PAO observed changes in rCBF before and after weight gain. Subjects were 15 patients with anorexia nervosa, three of whom were re-examined after weight gain. Eight of 15 subjects showed unilateral left-sided temporal lobe hypoperfusion and five of 15 right temporal lobe hypoperfusion. The three subjects re-scanned after weight gain showed a further 10% reduction in perfusion (Gordon *et al.*, 1997). Another study comparing rCBF of anorexic patients before and after 3 months of treatment, reported that the low blood flow in frontal, parietal and frontotemporal areas normalized after weight gain (Kuruoglu *et al.*, 1998).

HM-PAO also allows for activation studies. One preliminary study using a food intake stimulus in five normal controls and seven anorexic patients analysed ratios of the radioactive counts in a region of interest to the cerebellum in various cortical regions before and after eating, and before and after weight gain (Nozoe *et al.*, 1993). Patients showed a significant increase of the percentage of rCBF in the left inferior frontal region but had no significant difference of the ratios before treatment. After weight gain, the patients failed to show significant differences in the percentage of rCBF though they significantly increased the ratios in both sides of the temporal cortex in the rest period.

Another study using a food sight stimulus revealed different response patterns of the percentage of rCBF in anorexic patients with binge/purge behaviour compared to restricting anorexics (Naruo *et al.*, 2000). The results of this study indicate that anorexic patients with habitual binge/purge behaviour display increases in regional cerebral blood flow in the right cerebral hemisphere, especially in the inferior frontal and parietal region with a food-related visual stimulus.

There has recently been progress in converting the techniques of PET imaging into SPECT. Two recent studies using statistical parametric mapping (SPM) 96 analysis reported hypoperfusion in the anterior frontal areas in untreated restricting anorexia nervosa at baseline. The first study using HM-PAO found hypoperfusion in the frontal area, mainly bilaterally in the anterior cingulate cortex (ACC) in restricting anorexia nervosa patients compared to anorexia nervosa patients with binge/purge behaviour and healthy volunteers (Naruo *et al.*, 2001). The other SPECT study using Iodin-123-iodoamphetamine (IMP) and SPM analysis also found hypoperfusion in the ACC and medial prefrontal area. Furthermore, a significant hyperperfusion was found in the thalamus and the amygdala–hippocampus complex when compared to the normal subjects (Takano *et al.*, 2001). Since the frontal regions including the ACC have often been reported to be very important in controlling a wide range of higher brain functions, the identical finding concerning the ACC in these two studies suggested that dysfunction of the ACC may possibly be associated with the causing or maintaining of abnormal eating behaviour in anorexia nervosa patients.

Anorexia Nervosa and Bulimia Nervosa

A study using the same experimental design and radioactive tracer examined eight untreated patients with anorexia nervosa, five patients with bulimia nervosa, and nine normal controls (Nozoe *et al.*, 1995). Comparisons across the three groups using a food intake stimulus showed an increase in ratios of the radioactive counts in a region of interest to the cerebellum in the bilateral inferior frontal and left temporal regions in the bulimic patients at baseline. In the anorexic patients, an overall decrease of the normalized radioactivity before stimulus and a significant decrease in the left temporal region were observed at baseline. After exposure to food the intake stimulus only the anorexic patients showed an increased blood flow in a wide region of the cortex, more specifically bilaterally in the inferior frontal, parietal and occipital regions. The authors hypothesized that the increases in rCBF after a food intake stimulus in both studies could reflect changes necessary for patients with anorexia nervosa to control their eating behaviour. On the other hand, the small or negative changes in rCBF in the superior and inferior frontal regions in the bulimia nervosa patients could be correlated with their eating behaviour.

Bulimia Nervosa

Using iodine-labelled [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane ([^{123}I] β-CIT) and SPECT, the first exploratory investigation of brain serotonin and dopamine transporter availability in bulimia nervosa has recently been reported (Tauscher *et al.*, 2001). These authors found that 10 bulimic patients showed a 17% reduction in brain serotonin transporter availability in the hypothalamus and thalamus and a 15% reduction in striatal dopamine transporter availability as compared to controls. In particular, the reduction of [^{123}I] β-CIT binding in the hypothalamus and thalamus was reported to be in line with a previous study demonstrating decreased serotonergic transmission in bulimia nervosa. The authors note that it remains unclear whether the reduced serotonin transporter availability is an aetiologic defect, an adaptive mechanism, or an unrelated epiphenomenona of some other process.

Positron Emission Tomography

Anorexia Nervosa

The first preliminary study on anorexia nervosa using (18-F)-fluordeoxyglucose (FDG) and positron emission tomography (PET) was reported in 1987. It was a comparison study of five female patients with anorexia nervosa, before and after treatment, and 15 young male controls (median 29 years) (Herholz *et al.*, 1987). The results were that the anorexic patients before treatment displayed significant bilateral hypermetabolism in the caudate nuclei and temporal cortex compared to post-treatment. Whole brain metabolism was also reported to be higher in the anorexic patients before treatment compared to post-treatment and controls. The authors mentioned that since cortical pseudoatrophy would be associated with hypometabolism in the atrophic structures the observed hypermetabolism cannot be explained by partial volume effects.

Another study examined cerebral glucose metabolism using FDG in 20 patients with anorexia nervosa and 10 healthy controls. Compared to controls, the anorexic group showed global hypometabolism and an absolute as well as relative hypometabolism of glucose in cortical regions, with the most significant differences found in the frontal and the parietal cortices (Delvenne *et al.*, 1995). Within the anorexic and the normal volunteer groups, no correlations were found between absolute or relative cerebral glucose metabolism (rCMRglu) and BMI, anxiety, or Hamilton Depression Rating Scale scores. Different factors may explain the reduced glucose metabolism in anorexia nervosa. It might be the consequence of neurophysiological or morphological aspects of anorexia nervosa and/or the result of some associated symptoms such as anxiety or depression. Supported by cognitive studies, the authors also hypothesized a primary corticocerebral dysfunctioning in anorexia nervosa.

Bulimia Nervosa

Several studies in bulimia nervosa using PET and FDG or [^{15}O]water have been reported as follows.

A preliminary study of eight female bulimia nervosa patients, eight depressed women and eight normal controls revealed that the normal controls and the depressed patients had high metabolic rates in the right cortical regions, whereas the bulimic group instead had high rCMRglu in the basal ganglia (Hagman *et al.*, 1990). From these findings the authors concluded that although women with bulimia nervosa frequently present with symptoms of depression, the pathophysiologic changes associated with bulima differ from depression.

A study of 11 bulimia nervosa patients and 18 normal controls investigated the relationship between rCMRglu and the symptoms of depression, obsessive–compulsive disorder, and bulimia nervosa (Andreason *et al.*, 1992). It was demonstrated that in the patients with bulimia nervosa a lower left anterolateral prefrontal rCMRglu correlated with a greater amount of depressive symptoms, while left temporal lobe hypermetabolism and asymmetries observed in the bulimic patients appeared to be independent of the mood state. The authors also reported that they failed to observe a pattern characteristic of obsessive–compulsive disorder in the patients with bulimia nervosa.

In a study comparing 10 patients with bulimia nervosa of normal body weight, 10 patients with anorexia nervosa and 10 normal controls, the absolute rCMRglu was reduced only in the anorexic patients (Delvenne *et al.*, 1999). This was hypothesized to be related to the low body weight. On the other hand, both anorexic and bulimic patients displayed a decreased in rCMRglu in the parietal cortex. The authors hypothesized that this region might be particularly sensitive to nutritional factors, leading to characteristic changes in this region.

Recently a PET study using [^{15}O]water investigated rCBF in recovered bulimia nervosa patients (Frank *et al.*, 2000). Comparing nine recovered patients to 13 normal controls, a significant inverse relationship between rCFB in several cortical regions and the thalamus and length of recovery was found. The authors suggest that previously reported alterations in rCBF in bulimia nervosa patients during the ill state is a state-related phenomenon that remits with recovery.

Functional Magnetic Resonance Imaging

Anorexia Nervosa

Functional magnetic resonance imaging (fMRI) is a good tool for investigating regional brain abnormalities associated with specific cognitive processes. Because it can map fine changes in cerebral blood flow without using radioactivity, it is expected to be a useful way to investigate brain function in eating disorders.

Up to now, only one fMRI study has been published. The study compared with six patients with anorexia nervosa and six normal controls using videotaped pictures of drinks labelled high (e.g. chocolate milkshake) and low (e.g. still mineral water) calories, respectively (Ellison *et al.*, 1998). The anorexic group showed a higher level of anxiety along with an increased blood flow in response to the contrasting stimuli, especially in the left insula, anterior, cingulate gyrus and left amygdala-hippocampal region.

The authors suggested that the findings provided evidence that the fear associated with high caloric food in people who have anorexia nervosa is associated with activation in a limbic and paralimbic network. The abnormal activity in this area of the brain was suggested to be a linked with an abnormal preoccupation with food-related items.

Magnetic Resonance Spectroscopy

Anorexia Nervosa

Analysis of the chemical composition of the brain in anorexia nervosa using magnetic resonance spectroscopy (MRS) has been the subject of a few reports.

One study of four anorexic patients suggested that the main cause of the brain structure changes such as brain atrophy, was an abnormality in membrane phospholipid metabolism due to the starving state of anorexia nervosa patients (Kato *et al.*, 1997). Another study, comparing 12 anorexic patients with seven normal controls revealed that the ratio of choline containing compounds relative to total creatine in the grey matter was significantly higher in anorexia nervosa subjects compared to controls, but

that anorexia nervosa subjects showed significantly lower ratios of *N*-acetyl-aspartate relative to choline containing compounds compared to controls (Schlemmer *et al.*, 1998).

Anorexia Nervosa and Bulimia Nervosa

A study comparing a group of anorexia nervosa patients, bulimia nervosa patients, and normal controls using MRI and MRS in three regions of the brain, found that reductions in the myo-inositol and lipid compounds (containing lipids, proteins and lactate) within the frontal white matter correlated with reductions in body weight (Roser *et al.*, 1999). Furthermore, reduced lipid signals were found in the occipital grey matter, but on the other hand, in the cerebellum, the concentration of all metabolites except lipid was increased. The authors suggested that decrease in myo-inositol was related to hyponatoremia or hypercortisolemia in patients with eating disorders. They also suggested that both altered glucose oxidation and fatty acid metabolism in anorexic patients could explain the reduced spectral intensity of these compounds probably caused by a reduced concentration of free lipoproteins in brain parenchyma.

Summary

Progress in functional imaging techniques have made it possible to identify specific areas of abnormal brain functions regardless of structural changes. Interestingly, the recent studies have reported that untreated anorexia nervosa patients showed decreases in regional blood perfusion and metabolism of the anterior frontal areas. However, we should be careful in understanding the localized abnormal areas of brain functions because it is still very difficult to specify the neuronal projections that account for the observed changes in regional activity.

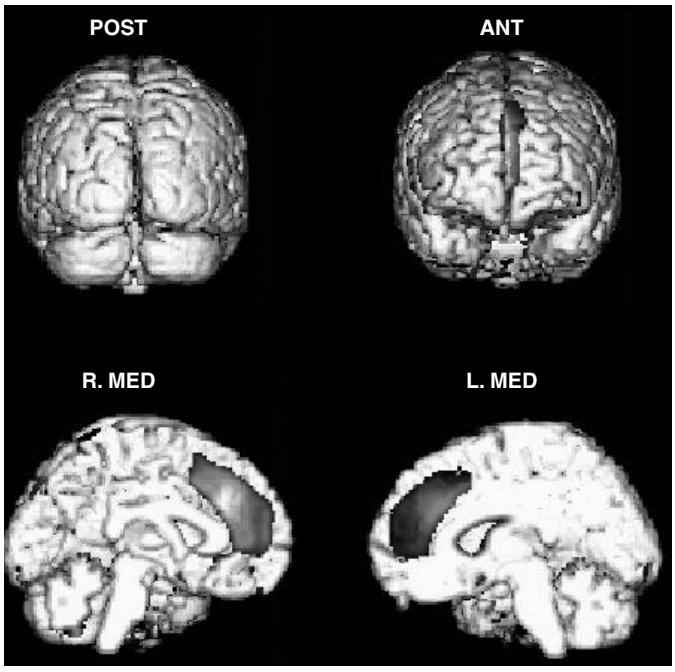

Figure XXIII-8.2 The 3D rendering image displaying area of hypoperfusion. The SPM 96 analysis of SPECT data shows significantly decreased of rCBF in the anorexia nervosa restricters in the bilateral ACC and parts of frontal regions when compared to controls. Height threshold = 3.09, $p = 0.001$, extent threshold = 550

Figure XXIII-8.2 shows the rendering images of an SPM analysis of SPECT data with HM-PAO displaying hypoperfusion both bilaterally in the medial prefrontal regions and in the ACC in restricting anorexia nervosa patients.

RESEARCH ON OBESITY

Although the aetiology of obesity is still unclear, genetic, metabolic, and social factors are all believed to play a role in its development and progression (Stunkard, 1996). Recently, behaviourally distinct subsets of obese persons have been considered to display particular patterns of disordered eating and elevated rates of psychopathology (Devlin, Yanovski and Wilson, 2000). Brain imaging investigations in obese patients may shed light on the mechanisms of the onset and maintenance of obesity. A literature review of this area revealed no studies of structure using CT or MRI, but did locate some functional brain imaging studies.

One study using ^{99m}Tc–ethyl-cysteine-dimer (ECD) and SPECT compared 11 obese women without binge-eating behaviour to 12 normal control females using a food-exposure stimulus. The obese women show higher rCB in right parietal and temporal cortices compared to controls (Karhunen *et al.*, 1997). In addition, higher activity in the right parietal cortex was found to be associated with an enhanced feeling of hunger when looking at food. The authors concluded that the increase in the rCBF of the right cortex after exposure to food could be associated with difficulties in the control of eating since the right hemisphere plays a role in the recognition and control of emotional expressions and related behaviours.

In another study using the same radiotracer in eight obese binge-eating patients and 12 normal controls, regional cerebral blood flow was mapped by SPECT, while the subjects were looking at a picture of a landscape or at a portion of food (Karhunen *et al.*, 2000). When exposed to food the obese patients showed different changes in the cerebral blood flow of the right and left hemisphere, especially in the frontal and prefrontal lobe compared to controls. The increase in blood flow in obese patients was positively correlate with the intensity of hunger. The authors concluded that the left frontal and prefrontal regions could play a role in binge-eating behaviour in humans.

Another study used PET to investigate the functional anatomy of satiation, comparing 11 obese and 11 lean men. It was demonstrated that refeeding after 36 hours of fasting resulted in increases in rCBF in the ventromedial and dorsolateral prefrontal cortex, and decreases in limbic/paralimbic areas and striatum in both groups (Gautier *et al.*, 2000). However, the obese group displayed a significant increase in blood flow in the prefrontal cortex, but a significant decrease in blood flow in the limbic/paralimbic areas, temporal and occipital cortex and cerebellum. The authors suggested that obese individuals have a greater activation of the prefrontal cortex and a greater deactivation of the limbic/paralimbic areas in response to satiation. They also speculated that differences in postprandial amino acid metabolism between lean and obese men might underlie the observed differences in neural activity.

CONCLUSION

The majority of structural studies have examined subjects with anorexia nervosa; few studies have reported on structural changes in bulimia nervosa or obesity. It is now generally agreed upon that alterations in structures such as cerebral atrophy and enlargement of the ventricles are secondary to low body weight (Addolorato *et al.*, 1998). The more recent use of volumetric methods assessing grey- and white-matter volumes has made it possible to assess these changes. While these structural abnormalities typically revert

to normal with weight restoration, the process of this recovery is poorly understood. The precise aetiology of these changes is controversial, hypercortisolemia being an attractive explanation, as associations have been demonstrated between hypercortisolemia and the magnitude of structural brain abnormalities (Katzman *et al.*, 2001).

The use of functional brain imaging techniques has led to significant improvements in our understanding of the characteristic brain functional changes in eating disorders and obesity. However, the limitations of these techniques must be acknowledged because of the following reasons. Changes in regional brain activity are considered to reflect the activity of terminal neuronal fields which include those from local interneurons and afferent projections arising in other sites (Tataranni *et al.*, 1999). Comparisons of studies across countries are complicated by varying diagnostic criteria and differences in establishing appropriate comparison groups. Spatial resolution, contrast resolution of individual subtraction images, and accuracy of the image deformation algorithm make it difficult to specify in greater detail the structures that are responsible for the observed increases in regional brain activity (Woods, 1996).

Furthermore, significant challenges remain for functional imaging. These include the effects of prolonged starvation, the administration of medication, and the effect of comorbidity such as depression and obsessive–compulsive disorders. It is also not clear as to what constitutes optimal experimental designs to compare scans at rest or after specific stimuli or tasks. Technical problems in accounting for individual variations in the shape and volume of the brain have yet to be overcome. It is not yet clear whether the findings to date are only state effects, secondary to starvation, or whether any might be pre-existing trait phenomena. Future studies should try to address these various problems.

Finally, brain imaging techniques are believed to serve as the bridge between the molecular clinical domains of this field (Mazzoitta, 2000). Therefore, using the rapidly developing brain imaging techniques in the study of eating disorders is likely to provide us with important information regarding the understanding of the disease and its possible treatment.

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XXIII-9

The Genetics of Eating Disorders

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INTRODUCTION

Anorexia nervosa (AN) and bulimia nervosa (BN) are disorders characterized by abnormal patterns of eating behaviour and disturbances in attitudes and perceptions toward weight and shape. In AN, there is an extreme fear of weight gain despite increasing emaciation. BN usually emerges after a period of dieting (Bulik *et al.*, 1997; Mussell *et al.*, 1997) and is characterized by alternating patterns of binge eating and compensatory behaviour. Binge eating, which is the consumption of a large amount of food in an uncontrollable manner, is typically followed by either self-induced vomiting, excessive exercise, fasting, and/or the misuse of laxatives, diuretics or enemas. Although abnormally low body weight excludes a BN diagnosis, 25% to 30% of patients with BN have a prior history of AN (Eckert *et al.*, 1995; Bulik *et al.*, 1997; Strober, Freeman and Morrell, 1997; Garfinkel, Moldofsky and Garner, 1980). Common to individuals with AN and BN are pathological concern with weight and shape, depression, and anxiety (Mitchell *et al.*, 1986; Keck *et al.*, 1990; Fornari *et al.*, 1992; Bulik *et al.*, in press).

The aetiology of these disorders is presumed to be multiply influenced by developmental, social, and biological processes (Garner, 1993; Treasure and Campbell, 1994). However, the exact nature of these interactive processes remains incompletely understood. Cultural attitudes towards thinness have relevance to the psychopathology of eating disorders, but they are unlikely to be sufficient to account for the pathogenesis of these disorders. Notably, dieting behaviour is quite common in industrialized countries throughout the world, yet AN and BN affect only an estimated 0.3% to 0.7%, and 1.7% to 2.5%, respectively, of females in the general population (APA, 1994). Moreover, numerous descriptions of AN date from the middle of the 19th century suggesting that factors other than modern culture play an aetiologic role. In addition, both syndromes have a relatively homogeneous clinical presentation, sex distribution, and age-of-onset, supporting the possibility of some biological susceptibility. This is not to discount the role of culture, as the introduction of Western ideals of thinness may serve to release a biological propensity toward eating disorders (Becker, 1999) possibly by increasing behaviours such as dieting that may trigger the spiral of disordered eating.

Recent findings from behaviour genetic studies suggest that this biological vulnerability might be genetic in nature. In this paper, we will highlight these emerging findings and suggest areas for future research.

HERITABILITY

Family Studies

Family studies provide initial data regarding genetic influence on a disorder by establishing whether it clusters amongst biologically-related individuals. Controlled family studies have generally found increased rates of eating disorders in relatives of women with AN and BN compared to relatives of controls (Biederman *et al.*, 1985; Lilenfeld *et al.*, 1998; Strober *et al.*, 1990, 2000). Findings from the largest and most systematic studies (Lilenfeld *et al.*, 1998; Strober *et al.*, 2000) suggest a 7–12-fold increase in the prevalence of AN and BN in relatives of eating disordered probands. This clustering of eating disorders in families of AN and BN individuals provides strong support for familial transmission of both disorders. However, given that first-degree relatives share both genes and environments, these studies cannot differentiate genetic versus environmental causes for the observed familiality. Systematic studies of twins are the means by which to disentangle the relative aetiological influence of genes and environment.

Twin Studies

Twin studies differentiate genetic from environmental effects by comparing similarity for a trait/disorder between identical (monozygotic (MZ)) and fraternal twins (dizygotic (DZ)). This comparison is based on the fact that MZ twins share all of their genes identical by descent, whereas DZ twins share, on average, half of their genes identical by descent. Consequently, MZ twin correlations that are $\simeq$ two times greater than DZ twin correlations suggest genetic effects. In general, greater MZ relative to DZ twin similarity for AN and BN has generally been found (Holland *et al.*, 1984, 1988; Fichter and Noegel, 1990; Treasure and Holland, 1990). Estimates indicate that roughly 58–76% of the variance in the liability to AN (Klump *et al.*, 2001; Wade *et al.*, 2000), and 54–83% of the variance in the liability to BN (Bulik, Sullivan and Kendler, 1998; Kendler *et al.*, 1991) can be accounted for by genetic factors. Although the confidence intervals on these estimates are wide, consistent findings across studies support moderate heritability of these traits (Bulik *et al.*, 2000). For both AN and BN, the remaining variance in liability appears to be due to unique environmental factors (i.e., factors that are unique to siblings in the same family) rather than shared or common environmental factors (i.e., factors that are shared by siblings in the same family).

Eating disorder symptoms themselves also appear to be moderately heritable. Twin studies of binge eating, self-induced vomiting, and dietary restraint suggest that these behaviours are roughly 46–72% heritable (Sullivan, Bulik and Kendler, 1998; Klump *et al.*, 2000). Likewise, pathological attitudes such as body dissatisfaction, eating and weight concerns, and weight preoccupation show heritabilities of roughly 32–72% (Klump *et al.*, 2000; Rutherford *et al.*, 1993; Wade *et al.*, 1998, 1999). Taken together, findings suggest a significant genetic component to AN and BN as well as the attitudes and behaviours that contribute to, and correlate with, clinical eating pathology.

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Developmental Differences

A caveat to the above conclusions is that there appears to be developmental differences in genetic effects across adolescence. Two recent twin studies (Klump *et al.*, 2000, in press) from the Minnesota Twin Family Study (MTFS) have examined this issue by comparing genetic influences on eating attitudes and behaviours in population-based samples of 680 11-year-old twins and 602 17-year-old twins. In the first of these studies, essentially no genetic influence was found for weight preoccupation scores and overall eating pathology in 11 year-old twins, whereas 52–57% of the variance in these attitudes and behaviours could be accounted for by genetic factors in the older cohort (Klump *et al.*, 2000). Increased genetic influence across age was also found for body dissatisfaction scores, although effects were much less dramatic. The authors speculated that these findings may reflect an activation of aetiologic genes during puberty.

In a follow-up study (Klump, McGue and Iacono, in press), the 11-year-old cohort was divided into a pre- and post-pubertal group in order to directly examine the effect of puberty on the heritability of these traits and behaviours. Findings revealed a pattern of results similar to those reported in the initial study (Klump *et al.*, 2000). Genetic factors accounted for 0% of the variance in overall eating pathology scores in pre-pubertal twins, but accounted for 53% of the variance in post-pubertal twins. Although sample sizes were small in the post-pubertal group ($n = 39$ pairs), the similar pattern of twin correlations in the two studies suggested that puberty may account for the dramatic age differences observed earlier. Increased heritability in post-pubertal relative to pre-pubertal twins *who were the same age* provided strong evidence of potential pubertal activation of the heritability of eating pathology that may be mediated by ovarian hormones. These findings are important for highlighting not only developmental differences in genetic effects, but also the potential role of ovarian steroids in the heritability of these disorders.

COMORBIDITY

Individuals with AN and BN commonly present with comorbid psychopathology — most notably affective and anxiety disorders (Bulik *et al.*, 2000). Family and twin studies have been effective in illuminating the causes of comorbidity (Neale and Kendler, 1995) and addressing to what extent comorbidity among these disorders might arise as a function of a shared genetic effect.

Psychopathology

Substance Use Disorders

Family studies investigating substance use disorders suggest relatively low prevalence among relatives of restricting AN probands (Holderness, Brooks-Gunn and Warren, 1994; Lilenfeld *et al.*, 1998). In contrast, rates are elevated in relatives of probands with BN. However, results from three studies (Kaye *et al.*, 1996; Schuckit *et al.*, 1996; Miller *et al.*, 1998) indicate that there is no evidence of a cross-transmission of BN and substance use disorder in families, and twin data (Kendler *et al.*, 1995) have shown that the genes influencing susceptibility to alcoholism were independent of those underlying risk to BN.

Major Depression

Several family and twin studies have examined the covariation between eating disorders and major depression. Studies of AN probands have yielded relative risk estimates for depression in the range of 2.1 to 3.4 (Strober *et al.*, 1990; Lilenfeld *et al.*, 1998). Likewise, studies of BN probands indicate that their first-degree relatives are significantly more likely to develop major depression than relatives of controls (Lilenfeld *et al.*, 1998). However, most studies considering the effects of proband comorbidity on familial risk have shown that affective illness is more likely to be transmitted by probands with this same diagnostic comorbidity (Strober *et al.*, 1990; Lilenfeld *et al.*, 1998). These later studies suggest that although eating disorders and depression may share some aetiologic factors, there are also unique factors specific to each. Two recent twin studies support this conclusion, as both found evidence for shared as well as unique genetic influences on major depression and both AN and BN (Walters *et al.*, 1992; Wade *et al.*, 2000).

Anxiety Disorders

Several different anxiety disorders have been examined for their genetic relationships with eating pathology. In general, evidence supports shared genetic transmission between these disorders and both AN and BN. For example, although obsessive–compulsive disorder (OCD) appears to segregate independently from AN and BN in families (Lilenfeld *et al.*, 1998), shared familial transmission has been found between obsessive-personality disorder (OCPD) and AN and BN (Lilenfeld *et al.*, 1998). In addition, shared familial transmission has been found between broadly defined AN and BN and separation anxiety and overanxious disorder (Keel *et al.*, submitted), and between BN and both simple phobia and panic disorder (Kendler *et al.*, 1995). Taken together, these findings suggest the existence of a broad, genetically influenced obsessive phenotype with core features of rigid perfectionism, anxiety, and a propensity towards behavioural constraint.

Personality and Physical Characteristics

Personality Traits

Individuals with AN and BN exhibit characteristic personality traits including high levels of stress reactivity, negative emotionality, and harm avoidance (Brewerton, Hand and Bishop, 1983; Casper, 1990; Kleifield *et al.*, 1994a, 1994b; Bulik *et al.*, 1995, 2000; O'Dwyer, Lucey and Russell, 1996; Klump *et al.*, 1999, 2000). These characteristics persist after recovery (Casper, 1990; O'Dwyer, Lucey and Russell, 1996; Klump *et al.*, 1999) from the disorder and are independent of body weight (Klump *et al.*, 2000), suggesting that they may be trait disturbances contributing to the disorders' development. The moderately heritable nature of these traits (Tellegen *et al.*, 1988) suggests that relationships may be genetic in nature.

Four studies have examined familial relationships between personality traits and eating disorders, with many suggesting familial co-transmission. Klump *et al.* (1999) found increased levels of negative emotionality and stress reactivity, and decreased levels of well-being, in family members of restricting AN probands compared to control relatives. Likewise, Lilenfeld *et al.* (2000) found increased perfectionism and stress reactivity scores in non-eating disordered relatives of bulimic probands compared to control relatives. Carney, Yates and Cizadlo (1990) failed to find shared transmission between DSM-III-R personality disorder characteristics and BN, although their use of lower prevalence personality disorder symptoms rather than more normative personality characteristics may have prohibited detection of significant effects.

Recent twin study results suggest that familial relationships between personality and eating pathology may be genetic in nature (Klump, McGue and Iacono, in press), as genetic rather than environmental influences have been found to underlie phenotypic

and familial relationships between these characteristics. However, genetic influences that are independent of those operating in personality also appear to contribute to eating pathology (Klump, McGue and Iacono, in press).

Body Mass Index

Vulnerability to obesity has been found to be a risk factor for bulimia nervosa (Fairburn *et al.*, 1997). Body weight is highly heritable (Stunkard *et al.*, 1990) leading to questions of shared genetic transmission between body weight and eating pathology. One study directly examined this question by investigating shared genetic transmission of body mass index (BMI) and disordered eating including body dissatisfaction, weight preoccupation, overall eating pathology, and the use of compensatory behaviours such as self-induced vomiting and laxative abuse (Klump *et al.*, 2000). Findings suggested some shared genetic transmission, although again, the majority of genetic influence on these disordered eating variables was independent of the genes influencing BMI.

Shared Transmission between AN and BN

Cumulating evidence suggests that AN and BN likely share some aetiologic features. Clinically, approximately 50% of women with AN develop BN during the course of their illness and ~30% of women with BN report a history of AN (Bulik *et al.*, 1997; Eckert *et al.*, 1995; Garfinkel, Moldofsky and Garner, 1980; Strober, Freeman and Morrell, 1997).

Family and twin studies indicate an increased risk of both AN and BN in relatives of AN and BN probands (Walters and Kendler, 1995; Lilenfeld *et al.*, 1998; Strober *et al.*, 2000), suggesting a shared familial component between the two disorders. In addition, subthreshold forms of eating disorders appear to lie on a continuum of liability with full eating disorders (Kendler *et al.*, 1991). These findings suggest the existence of a broad eating disorder phenotype with possible shared genetic predispositions.

Summary

Research reviewed above suggests that AN and BN may share genetic transmission with each other and with body weight, personality, anxiety, and possibly major depression. However, findings also suggest that there are genetic influences on eating pathology that are independent of those influencing the traits/disorders mentioned above. This complexity is the norm rather than the exception in psychiatric genetics and highlights the need for additional research to further characterize the genetic variance of these disorders.

MOLECULAR GENETIC STUDIES

A detailed comparison of the molecular genetic designs that can be brought to bear upon complex traits is beyond the scope of this chapter (Lander and Schork, 1994; Risch and Merikangas, 1996; Martin, Boomsma and Machin, 1997). Briefly, there are two general strategies in humans: linkage and association studies (Sham, 1998).

Linkage studies can be used in gene discovery: with a sufficiently large number of multiplex pedigrees or extreme sibling pairs (Allison *et al.*, 1998), anonymous genetic markers scattered across the genome can be used to identify the chromosomal regions that may contain genes that contribute to a disorder such as AN or BN. This appealing strength is tempered by the low power (Risch and Zhang, 1996) and resolution (Roberts *et al.*, 1999) likely for linkage studies of complex traits.

Association studies are conceptually equivalent to the familiar case-control design. This design is particularly useful and powerful when prior knowledge of the pathophysiology of a trait suggests a number of candidate genes. However, the use of this design is controversial because of the risk of false positive findings when studying a sample that contains individuals of evolutionary diverse ancestry (Kidd, 1993). Obtaining genotypes on other family members can reduce this risk but at the cost of reduced statistical power.

Association Studies

Evidence linking AN and BN to monoamine functioning (Gorwood *et al.*, 1998) have led researchers to target serotonin and dopamine-related genes in association studies. Several groups have reported an increase in the −1438/A allele of the 5-HT_{2A} receptor gene in AN women compared to controls (Collier *et al.*, 1997; Enoch *et al.*, 1998; Sorbi *et al.*, 1998; Nacmias *et al.*, 1999). However, additional studies of this and other serotonin-related genes (5-$HT_{1D\beta}$, 5-HTT, 5-HT7, tryptophan hydroxylase receptor (TPH)) have failed to find significant associations in AN (Hinney *et al.*, 1997a, 1997b, 1999a; Campbell *et al.*, 1998, Han *et al.*, 1999; Ziegler *et al.*, 1999) or BN (Burnet *et al.*, 1999; Nacmias *et al.*, 1999) individuals. Studies have also failed to find increased allele frequencies of the dopamine D_3 (Bruins-Slot *et al.*, 1998) and D_4 (Hinney *et al.*, 1999b) receptor genes in AN relative to controls. These genes have not yet been examined in individuals with BN.

The primary role of weight control, feeding, and energy expenditure in the pathology of AN and BN has lead researchers to examine genes related to these processes. Results thus far have been mixed, as tests for association between AN and neuropeptide Y5 and Y1 (Rosenkranz *et al.*, 1998), the β_3 adrenergic receptor gene (Hinney *et al.*, 1997c), the melanocortin-4 receptor gene (Hinney *et al.*, 1999c), and the leptin gene (Hinney *et al.*, 1998) were all negative. However, studies have found an increase in the D11S911 allele located near the UCP-2/UCP-3 gene in AN subjects relative to controls (Campbell *et al.*, 1999), as well as an increase in the oestrogen receptor β 1082/G allele in AN relative to obese and overweight subjects (Rosenkranz *et al.*, 1998). Once again, associations between most of these genes and BN have not been investigated.

Findings suggest possible associations between the 5-HT_{2A} receptor gene, the UCP-2/UCP-3 gene, and the oestrogen receptor β gene with AN. However, additional research is necessary to clarify conflicting findings and replicate initial results. Moreover, association studies of BN are needed, as this disorder has been much less studied than AN and findings thus far have been generally negative.

Linkage Studies

We and a number of collaborators (Kaye *et al.*, 2000) have recently completed the first study to date (Grice *et al.*, submitted) using genome-wide linkage analyses in AN or BN. This multicentre study is funded by the Price Foundation of Switzerland and uses allele-sharing linkage analyses to identify genes contributing to eating disorders in 196 families with two or more family members with AN, BN, or eating disorder not otherwise specified (EDNOS). Initial analyses of this dataset show only modest evidence for linkage, with peaks observed on chromosomes 4, 11, 13, and 15 with NPL scores >1. The highest peak was a NPL score on chromosome 4 (Grice *et al.*, in preparation). These modest results are likely due to decreased power to detect linkage as a result of large number of loci influencing the phenotype as well as considerable sample heterogeneity (i.e., inclusion of AN, BN, and EDNOS). These possibilities suggest that additional studies using more homogeneous phenotypes and larger numbers of subjects would increase power to identify genetic effects.

We have recently completed a larger, genome-wide linkage study of approximately 400 families with two or more family members with AN, BN, or EDNOS. This larger study will provide the necessary power to detect linkage and may prove to be the first to identify susceptibility loci for these disorders. In addition, we are currently in the process of collecting genetic data on approximately 700 AN individuals and their parents. This homogeneous sample will be used to conduct association analyses such as those described above and will provide additional power to detect genes of modest to large effect.

CONCLUSIONS

Data described above are clear in establishing a role for genes in the development of eating pathology. Estimates from the most rigorous studies suggest that >50% of the variance in liability to eating disorders and disordered eating behaviours can be accounted for by additive genetic effects. The remaining variance appears to be due to unique rather than common environmental effects. These high estimates indicate a need for studies identifying the specific genes contributing to this large proportion of variance. Twin and family studies suggest that a number of heritable characteristics that are frequently comorbid with AN and BN may share genetic transmission with these disorders, including anxiety disorders/traits, body weight, and possibly major depression. Developmental twin research is beginning to shed light on why eating disorders tend to develop within a relatively narrow developmental window. Additional work is required to enhance our understanding of how puberty (and which aspects of puberty) influences the apparent activation of genetic effects on disordered eating.

Molecular genetic research of these disorders is in its infancy. However, promising areas for future research have already been identified (e.g., 5-HT2A receptor gene, UCP-2/UCP-3 gene, oestrogen receptor β gene), and several large-scale linkage and association studies are currently underway. These studies are likely to provide invaluable information regarding both the appropriate phenotypes to be included in genetic studies as well as the genes with the most influence on the development of these disorders.

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XXIII-10

The Therapeutic Armamentarium in Eating Disorders

James E. Mitchell, Scott Crow, Tricia Cook Myers and Steve Wonderlich

INTRODUCTION

In this paper we will briefly summarize the available literature on the empirically tested treatments for patients suffering from eating disorders. We will address the traditional eating disorders of anorexia nervosa and bulimia nervosa, and will also include binge-eating disorder, a condition included as an example of 'eating disorders — not otherwise specified' in the DSM-IV as a disorder for further study. We focus on treatments that have been shown to be effective in randomized trials, but include other clinical information when it appears relevant and necessary to the reader's understanding of that particular area.

In perusing this text, many readers will notice several trends that typify this literature. First and of greatest clinical concern, the reader will notice the relative paucity of literature on the treatment of patients with anorexia nervosa, despite the fact that anorexia nervosa was the first identified eating disorder and clearly is the most severe, with a significant well-documented risk for morbidity and mortality. There are a number of factors that have contributed to the relative lack of research in this area, including the following: (1) anorexia nervosa is a relatively rare condition and it is difficult for individual treatment centres to acquire the necessary number of subjects to complete randomized trials; (2) patients with anorexia nervosa, by virtue of their illness, many times are not particularly motivated to be cooperative with treatment and therefore it is difficult for them to be compliant with research treatment protocols; (3) when initially seen many patients with anorexia nervosa are critically ill, and require a multiplicity of interventions (e.g., medical stabilization, occasionally hospitalization, family involvement, individual counselling, medication management) which markedly complicates the ability of researchers to design clinical trials which adequately control for all of these variables; (4) anorexia nervosa patients are often quite difficult to treat, again given the nature of their illness, and this undoubtedly dissuades many potential investigators from pursuing work in this area. All of these factors have contributed to what currently is an apparent and quite worrisome lack of knowledge regarding the best treatments for patients with this disorder.

Second, readers of this text will probably also notice that although the treatment literature on bulimia nervosa is better developed, this entire literature has been published in the last 20 years. This is attributable to the fact that bulimia nervosa was only first identified as a discrete diagnostic entity in 1979 (Russell, 1979) and randomized clinical trials were first implemented several years after that.

Third, readers may notice that the literature on binge-eating disorder is also quite limited. This again is attributable to the fact that binge-eating disorder was only described in its current form in the DSM-IV, which was published in 1994. At that time there were little data regarding this group of patients, and our current understanding of this disorder has developed since then.

Fourth, an observation that may strike some readers as surprising is the finding that psychotherapy plays a clearly important and, in some cases, central role in the treatment of patients with eating disorders. The reader is therefore reminded that psychological interventions that result in psychological changes often result in corollary changes in the underlying biology, and therefore discussions of such therapies in a text on biological psychiatry is quite appropriate.

We turn now to the treatment literature on these three conditions.

Pharmacotherapy of Bulimia Nervosa

The pharmacotherapy of bulimia nervosa can be conveniently divided into three areas: (1) antidepressant trials, which have employed a variety of types of agents using various experimental designs ranging from acute treatment studies to relapse prevention studies; (2) studies examining the utility of non-antidepressant pharmacological approaches — none of which have been definitive, but several of which are interesting and show promise; and (3) a handful of studies regarding the relative efficacy of pharmacotherapy and psychotherapy for patients with bulimia nervosa.

Antidepressant Therapy of Bulimia Nervosa

Early in the course of our understanding of bulimia nervosa, research groups studying this condition noted that many of the patients were depressed (Russell, 1979). Based on this observation, it was hypothesized that patients might be better able to control their bulimic symptoms if their depression were treated. Some researchers even went so far as to hypothesize that bulimia nervosa might be a variant of affective disorders, although few would endorse such a model currently (Pope *et al.*, 1983).

Given this background, a number of antidepressants were tried. Following the initial observation that tricyclic and MAO inhibitors both seemed beneficial for these patients, a series of randomized treatment trials were undertaken. These studies are summarized in Table XXIII-10.1, and as can be seen the list has grown rather lengthy. In examining these studies, several issues emerge. First, the number of compounds studied and the classes of compounds studied (e.g., MAOIs, tricyclics, SSRIs) is large but not exhaustive. Second, the sample size in most of the studies has been modest, the exceptions being the large multicentre studies involving fluoxetine that were funded by Eli Lilly, who subsequently sought and received FDA approval to market fluoxetine for bulimia nervosa in the US. Third, although not illustrated, there is a great deal of variability in response rates to placebo. This probably speaks to a number of issues, one of which is the lack of standardization of the protocols for administering agents in this population, a problem we have discussed previously in the literature (Mitchell *et al.*, 2000). Fourth, the reductions in the frequencies of target eating behaviours

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Table XXIII-10.1 Placebo-controlled antidepressant trials for bulimia nervosa

Reference	*N*	Duration (weeks)	Treatment	Outcome ↓ BE (%)	Outcome AB (%)
Pope *et al.*, 1983	36	8	Imipramine		0
Sabine *et al.*, 1983	19	8	Mianserin		
Mitchell and Groat, 1984	32	8	Amitriptyline	72	19
Hughes *et al.*, 1986	22	6	Desipramine	91	68
Agras *et al.*, 1987	22	16	Imipramine	72	30
Horne *et al.*, 1988	81	8	Bupropion	67	30
Barlow *et al.*, 1988	24	6	Desipramine	4	
Blouin *et al.*, 1988	10	6	Desipramine	45*	
Kennedy *et al.*, 1988	18	13	Isocarboxazid		33
Walsh *et al.*, 1988	50	12	Phelelzine	64	35
Pope *et al.*, 1989	42	4	Trazadone	31	10
Fichter *et al.*, 1991	40	35 (days)	Fluoxetine	**	
Kennedy *et al.*, 1993	36	8	Brofaromine	62	
FBNC, 1992	387	12	Fluoxetine	67	
Goldstein *et al.*, 1995	398	16	Fluoxetine	50	
Romano, 1999	150	52	Fluoxetine	**	

*Vomiting frequency;
**Relapse prevention trials.

are generally large and consistently superior to those seen with placebo. Fifth, despite this finding, the majority of subjects treated with these agents are not free of symptoms at the end of treatment, a particularly worrisome finding given the fact that research suggests that subjects who are not abstinent at the end of treatment may subsequently relapse to a more severe syndrome.

A few other studies have addressed relapse prevention (Walsh *et al.*, 1991; Fichter *et al.*, 1996; Romano, 1999), and indeed the data suggest that there is clearly an effect for drug therapy, although findings in these studies are also discouraging in that some patients relapse on medication, and a high dropout rate occurs at long-term follow-up, suggesting that the maintenance of patients on drug therapy, even if their initial response is favourable, may be difficult to accomplish.

Given these limitations, the agent that has been studied in the largest number of clinical trials is fluoxetine hydrochloride, and of particular importance clinically, the first multicentre trial demonstrated superiority for 60 mg over 20 mg in the treatment of bulimia nervosa (FBNC, 1992). The second multicentre treatment study (Goldstein *et al.*, 1995) and the relapse prevention trial (Romano, 1999) both employed 60 mg as an initial dosage. This suggests that subjects can either be started at this dosage or should have the dosage fairly rapidly escalated to this dosage to achieve optimal results.

Although such data cannot be easily summarized, studies using antidepressants have also demonstrated significant improvement on other variables such as mood, anxiety, and measures of core psychopathology as measured by various instruments. Therefore, the drugs do not appear just to suppress the core symptoms of binge eating and vomiting, but appear to have more global effects on the syndrome.

Accompanying this growing literature has been a growing acceptance of pharmacotherapy on the part of non-medical mental health practitioners and on the part of patients. While there was initial resistance on the part of health care professionals in the early 1980s to refer patients for pharmacotherapy, and while many patients were very resistant to the idea of drug treatment, most therapists now appreciate the worth of this approach, and not uncommonly patients seek treatment with pharmacotherapy.

Other Pharmacotherapies for Bulimia Nervosa

A variety of other agents have been tried, some for intriguing theoretical reasons. Phenytoin was tried for bulimia nervosa following the observation that a group of patients with binge-eating behaviour had abnormal electroencephalograms (Green and Rau, 1974), although subsequent studies have failed to find an exaggerated rate of EEG abnormalities in patients with bulimia nervosa (Mitchell, Hosfield and Pyle, 1983). The results of this study were ambiguous, yet surprisingly no attempt has been made to further examine the utility of this agent (Wermuth *et al.*, 1977).

Narcotic antagonists were used following the observation that the endogenous opioid system is involved in the modulation of feeding, particularly stress-induced and hedonically driven feeding in various animal models (de Zwaan and Mitchell, 1992). While some early work using short acting narcotic antagonists suggested positive effects (Jonas and Gold, 1986, 1987), randomized treatment trials at usually applied dosages have failed to find evidence of efficacy (Ingoin-Apfelbaum and Apfelbaum, 1987; Mitchell *et al.*, 1989), while open-label trials employing higher doses, which can only be used if one is willing to accept the risk of possible hepatotoxicity, suggested positive effects (Jonas and Gold, 1988). Given the current state of knowledge, until non-hepatotoxic agents are available, narcotic antagonists cannot be recommended as treatment, although their utility as adjunctive agents in other situations at usual doses could reasonably be explored.

d-Fenfluramine was not found to be particularly useful (Russell *et al.*, 1988; Fahy, Eisler and Russell, 1993). However, this compound has been shown subsequently to cause valvular heart disease (Cannistra and Gaasch, 1999). This is a moot point now, given the removal of both fenfluramine and d-fenfluramine from the market secondary to drug-induced valvular dysfunction.

A report demonstrating the efficacy of ondansetron, marketed as an anti-nauseant, over placebo in a randomized clinical trial recently appeared (Hartman *et al.*, 1997). The results are interesting theoretically but the magnitude of the improvement was not in excess of those achieved in trials finding modest benefits with antidepressants.

Psychotherapy of Bulimia Nervosa

Since the early 1980s numerous studies have investigated the efficacy of psychotherapy for bulimia nervosa. Cognitive-behavioural therapy (CBT) has been shown to be more beneficial than placebo antidepressant medication (Whittal, Agras and Gould, 1999) in the treatment of bulimia nervosa. Randomized trials indicate that CBT is superior to both support groups and wait-list controls (Lacey, 1983; Lee and Rush, 1986; Leitenberg *et al.*, 1988; Agras *et al.*, 1989). CBT has also been shown to be as effective or more effective than non-directive (Kirkley *et al.*, 1985), short-term focal (Fairburn *et al.*, 1986), supportive–expressive (Garner *et al.*, 1993), and supportive non-directive therapies (Walsh *et al.*, 1997). In fact most controlled treatment research to date has supported CBT as the psychotherapy of choice in the treatment of bulimia nervosa.

Recently, however, there has been some evidence that a therapy that does not specifically address the bulimic behaviours may be as effective as CBT in decreasing bulimic symptoms. Specifically,

interpersonal therapy (IPT), originally developed as a treatment for depression (Klerman *et al.*, 1984), may also be efficacious for the treatment of bulimia nervosa. Fairburn and colleagues (1991) compared CBT, behavioural therapy and a therapy that contained many elements of IPT and concluded that although CBT was most helpful in reducing bulimic symptoms at the end of treatment, at one- and six-year follow-up IPT and CBT were found to be equally effective. More recently, a multicentre study replicated this finding using IPT in its originally developed form (Agras *et al.*, 2000).

CBT and IPT differ in some significant ways. CBT addresses eating-related thoughts and behaviours and can be divided into three stages. The first stage focuses on education about the cognitive model of bulimia nervosa, which proposes that bulimic behaviours are maintained by weight and shape-related thoughts and attitudes, and specific behavioural techniques such as self-monitoring, regular eating, alternative behaviours and weekly weighings. The second stage continues to address these behavioural techniques; however, the main emphasis is on restructuring faulty cognitions and increasing problem solving skills. Additionally, this stage of treatment may also address low self-esteem, if applicable. Relapse prevention is the focus of the final stage of treatment.

CBT appears to be effectively delivered in both individual and group formats with a typical treatment length of 20 sessions. There appears to be considerable benefit to asking the patient to attend sessions twice per week during the first month of treatment, after which sessions are held on a weekly basis. CBT can also include, or be augmented with some form of dietary counseling. Lastly, there is evidence that CBT can be effectively delivered in the form of self-help manuals when guided by a non-specialist (Cooper, 1994; Waller *et al.*, 1996).

IPT, on the other hand, concentrates on current interpersonal functioning and tends to eschew issues related to eating problems and weight. During the initial phase of IPT treatment, interpersonal problems associated with the eating disorder are identified with the remainder of the therapy sessions geared toward resolving these interpersonal deficits.

One drawback of all treatments for bulimia nervosa, including antidepressant medication, is the low rate of abstinence from bulimic behaviour at the end of treatment. Another problem is the unavailability of empirically supported psychotherapies for those with bulimia nervosa due to lack of psychotherapist training (Mussell *et al.*, 2000). In addition, there is evidence that if a patient hasn't responded to CBT by the sixth session, she is unlikely to improve with additional sessions of CBT (Agras *et al.*, 2000).

These concerns have prompted researchers to begin to examine alternative approaches, such as stepped care models of treatment. Although controlled trials have supported the use of CBT and, in two studies, IPT, in the treatment of bulimia nervosa, the majority of psychotherapists are not trained to deliver such treatments. Stepped care models have arisen due to this growing disparity between research and practice. Given that supervised CBT self-help manuals and antidepressant medication are more readily available than full CBT, they seem to be appropriate first line interventions. Full CBT could then be offered to those individuals who fail to respond to these initial interventions. A study examining this approach is currently underway.

Pharmacotherapy vs Psychotherapy in the Treatment of Bulimia Nervosa

Given the growing literature on the efficacy of antidepressant treatments for bulimia nervosa, as well as the growing literature on the efficacy of various forms of psychotherapy, one logical step was to compare the relative efficacy of each approach versus the efficacy of the combination of these approaches. Five such studies have been published. In an initial trial comparing imipramine to intensive outpatient cognitive-behavioural group psychotherapy to the combination, clear superiority was found for CBT, with evidence of greater improvement on some variables (e.g., depression and anxiety) when active medication was added (Mitchell *et al.*, 1990). A second trial utilizing desipramine and individual CBT found similar results with added benefit on some variables (e.g., dietary restraint) with the combination (Agras *et al.*, 1992). Two studies are difficult to interpret because of a high dropout rate during treatment and a high dropout rate to follow-up (Leitenberg *et al.*, 1994; Goldbloom, Olmsted and Davis, 1996). The fifth study employed the most sophisticated design. Published by Walsh *et al.* (1997), the study involved randomization of outpatient bulimia nervosa subjects to one of five treatment cells: (a) medication management, which involved treatment with desipramine followed by treatment with fluoxetine if abstinence was not achieved; (b) and (c) treatment with individual cognitive-behavioural therapy with medication management or placebo; and (d) and (e) treatment with a control psychotherapy condition — supportive psychotherapy — with medication management or placebo.

The results showed that CBT was superior to supportive psychotherapy, and medication management was superior to placebo. In a *post-hoc* analysis, the best results overall were achieved using both medication management and CBT.

In summary, the literature on psychotherapy versus drug therapy versus combined treatment suggests that CBT is clearly superior to drug therapy alone, and arguments can be made for using the combination, particularly among patients who are depressed at baseline.

Pharmacotherapy of Binge-Eating Disorder

There are both similarities and differences in the state of our knowledge concerning the pharmacologic treatment of bulimia nervosa and binge-eating disorder. On the one hand, many of the trials conducted using subjects with binge-eating disorder thus far have been informed by previous work in bulimia nervosa. Often the same agents have been employed (for example, SSRIs and desipramine). Similarly, the major focus of treatment has been on the diminishment of binge-eating frequency as the major outcome variable. Much as with bulimia nervosa, the attendant symptoms have received less focus. One point of departure from the treatment of bulimia nervosa involves the issue of weight. The majority of individuals with binge-eating disorder have body mass indices that would currently be classified as overweight or obese, and presumably are at increased risk for the medical morbidity and mortality typically associated with obesity. Previous work suggests that binge eating may or may not be a predictor of poor outcome in weight loss therapy and of weight regain after treatment (Ho *et al.*, 1995; Hsu, Betancourt and Sullivan, 1996; McGuire *et al.*, 1999; Sherwood, Jeffery and Wing, 1999). However, most overweight or obese individuals seeking treatment for binge-eating disorder are very interested in losing weight, so treatments which result in control of binge eating but not weight loss are relatively disappointing to them. Therefore, it appears that successful treatment of those with binge-eating disorder would include the successful treatment of their weight problem as well.

Most of the drug trials completed to date have involved antidepressants, with the SSRIs being used most commonly (Marcus *et al.*, 1990; McCann and Agras, 1990; Alger *et al.*, 1991; de Zwaan, Nutinger and Schonbeck, 1992; Stunkard *et al.*, 1996; Hudson *et al.*, 1998; McElroy *et al.*, 2000). These studies have reported outcomes in terms of both binge-eating frequency and weight loss, but have targeted the issue of weight loss to varying degrees. Trials involving desipramine, fenfluramine, fluvoxamine, and sertraline have all found greater decreases in binge eating and greater rates of abstinence at the end of treatment in drug-treated patients compared to those receiving placebo. On the other hand, trials of

fluoxetine and imipramine have been negative in terms of binge-eating outcome.

Only three studies, Marcus *et al.* (1990), using fluoxetine, Hudson *et al.* (1998), using fluvoxamine, and McElroy *et al.* (2000), using sertraline, have shown active drug to produce more weight loss than placebo. In the Marcus *et al.* fluoxetine trial, which was lengthy (approximately one year), there was substantially more weight loss in the active drug group (13.9 kg vs 0.6 kg). Most of the weight loss occurred early in the trial, and subjects appeared to reach a plateau. Additionally, after the end of treatment, fluoxetine patients experienced fairly prompt regain of lost weight. In the fluvoxamine trial, although there was more weight loss in individuals receiving active drug, the difference between drug and placebo-treated patients was quite small (1.3 kg). In the sertraline trial, a weight loss of 5.6 kg occurred with active drug vs 2.4 kg with placebo.

Of historical note, there is one report of a trial of d-Fenfluramine in the treatment of binge-eating disorder (Stunkard *et al.*, 1996). Interestingly, d-Fenfluramine was superior to placebo in terms of binge-eating outcome, but there was no evidence for difference in weight loss over only eight weeks. However, d-Fenfluramine has been taken off the market in the United States.

Psychotherapy of Binge-Eating Disorder

A number of treatment studies targeting weight loss have examined the outcome for binge eating versus non-binge-eating obese subjects. The available studies are ambiguous as to whether or not obese binge eaters lose comparable amounts of weight as non-binge eaters in obesity treatment (Marcus *et al.*, 1988, 1990; Wadden, Foster and Letizia, 1992; de Zwaan, Mitchell and Mussell, 1992) but do suggest that abstinence from binge eating can be promoted by instituting a regimented diet (Yanovski, Gormally and Lese, 1994). In fact, these studies suggest that behavioural therapy in conjunction with dietary interventions for weight loss may be adequate to treat binge-eating disorder, at least in the short term. Because the presence of binge eating does not seem to increase dropout rates, it seems reasonable to include individuals with binge-eating disorder in weight management programs (Ho *et al.*, 1995).

Comorbid psychopathology is also common in individuals with binge-eating disorder. Therefore, another subset of studies has targeted binge eating as well as comorbid depression and anxiety. Initially, treatment manuals for bulimia nervosa were modified to address binge-eating disorder (e.g., Fairburn, 1995). Similar to treatment for bulimia nervosa, CBT for binge-eating disorder includes self-monitoring, stimulus control, alternative behaviours, and a pattern of regular eating, as well as cognitive restructuring, problem solving, and relapse prevention. These treatments successfully reduce the frequency of binge eating; however, weight reduction, a primary goal for many of these patients, has been modest at best with the cognitive-behavioural interventions (Wilfley *et al.*, 1993). As a result, many BED treatment approaches have been expanded to include a weight loss component. This subset of studies has incorporated sequential or concurrent treatment for obesity, binge eating and comorbid psychopathology.

In a series of studies (Agras *et al.*, 1995, 1997; Eldredge *et al.*, 1997), overweight participants were initially treated with 12 weeks of CBT. Participants who stopped binge eating then received weight loss therapy while those who continued to binge eat received IPT or additional sessions of CBT. The results showed that participants lost weight when treated with subsequent weight loss therapy and were still able to maintain abstinence from binge eating. Although there was no added benefit for treatment with subsequent IPT, a substantial number of participants who initially failed to reduce binge-eating frequency were successful with an extended course of CBT. At one-year follow-up those treated with CBT and weight loss therapy showed a 64% reduction in binge eating and a 33% abstinence rate. Those who were abstinent from binge eating were able to maintain a 4 kg weight loss while participants who were still binge eating gained an average of 3.6 kg.

Another study investigated the addition of CBT to the refeeding phase of a combined treatment of behavioural weight loss and VLCD for binge-eating obese (de Zwaan, Mitchell and Mussell, 1996). These authors found that although the acute treatment package was effective in reducing binge eating and in inducing weight loss, the addition of CBT did not prevent weight regain compared to the rate of regain in those not receiving CBT.

Cognitive-behavioural self-help manuals can also be beneficial for individuals with binge-eating disorder. When guided by a non-specialist, this type of therapy is cost-effective, timely, and may be less stigmatizing than traditional CBT. This method of treatment seems to be effective when guidance is provided via videotape and written instructions (Peterson *et al.*, 1998), or by telephone (Wells *et al.*, 1997), and in a face-to-face format (Carter and Fairburn, 1998).

In conclusion, the available evidence seems to support an integrated treatment of CBT and dietary intervention for optimum treatment of binge-eating disorder. Binge eating, weight loss and comorbid psychopathology should all be addressed. In addition, it may be appropriate to recommend guided self-help prior to implementing more formalized treatments, although this question needs further study.

Pharmacotherapy of Anorexia Nervosa

As noted above, relatively little information exists about effective pharmacologic strategies for the treatment of anorexia nervosa. We will review the limited number of controlled trials that have been conducted. In looking at this literature a few generalizations should be considered. First, a wide variety of different classes of agents have been tried. This undoubtedly reflects the difficult challenge of treating individuals with this illness, and the fact that most trials to date have been negative. Second, the outcome measures used to decide whether treatments are beneficial usually have been either weight gain or the maintenance of a relatively healthy body weight. As described elsewhere in this volume, fear of becoming fat, body image disturbance and perfectionism and obsessionality are critical core features of anorexia nervosa; addressing these problems in pharmacologic treatment seems a desirable and probably a necessary goal. Unfortunately, to date, these symptoms have received little attention in the controlled treatment literature. Third, anorexia nervosa is uniformly viewed as an illness needing long-term treatment, yet most of our knowledge to date is limited to short-term trials.

The placebo-controlled drug trials for anorexia nervosa that have been conducted are listed in Table XXIII-10.2. In contrast to bulimia nervosa, for which antidepressants are the most commonly tried agents, only a minority of trials have employed antidepressants. Typically, these trials have been conducted in individuals in intensive outpatient or inpatient treatment setting, with a goal of increasing body weight. To date, one study has shown support for the use of medication to aid short-term weight gain in subjects at low weight. That study (Halmi *et al.*, 1986) compared cyproheptadine in a dose of 32 mg per day, amitriptyline in a dose of 175 mg per day, and placebo over a short period. In that trial, the rates of weight gain with both active drugs exceeded that seen with placebo. Unfortunately, other trials of cyproheptadine (Vigersky and Loriaux, 1977; Goldberg *et al.*, 1979) and amitriptyline (Biederman *et al.*, 1985) have been negative. Similarly, in a small trial the antidepressant clomipramine was not more effective than placebo (Lacey and Crisp, 1980). Given the high rate of comorbidity between anorexia nervosa and obsessive–compulsive

Table XXIII-10.2 Placebo-controlled drug trials for anorexia nervosa

Reference	*N*	Duration (weeks)	Treatment	Outcome (weight gain vs placebo)
Virgersky and Loriaux, 1977	24	8	Cyproheptadine	No difference
Goldberg *et al.*, 1979	81	Variable	Cyproheptadine	No difference
Halmi *et al.*, 1986	72	4	Cyproheptadine	Cyproheptadine > placebo in rate of weight regain
Lacey and Crisp, 1980	16	Variable	Clomipramine	No difference
Gross *et al.*, 1981	16	4	Lithium	No difference
Biederman *et al.*, 1985	25	5	Amitriptyline	No difference
Halmi *et al.*, 1986	72	4	Amitriptyline	Amitriptyline > placebo in rate of weight regain
Vandereycken and Pierloot, 1982	18	6	Pimozide	No difference
Vandereycken, 1984	18	6	Sulpiride	No difference
Gross *et al.*, 1983	11	4	Tetrahydrocannabinol	No difference
Casper *et al.*, 1987	4	8	Clonidine	No difference
Stacher *et al.*, 1993	12	12	Cisapride	No difference

disorder, clomipramine would seem a logical choice; however, the dose (50 mg) was relatively low and might well have been subtherapeutic.

A wide range of other agents have been tried. These include lithium (Gross *et al.*, 1981), the antipsychotics pimozide (Vandereycken, and Pierloot, 1982) and sulpiride (Vandereycken, 1984), and drugs noted to increase appetite, tetrahydrocannabinol (Gross *et al.*, 1983) and clonidine (Casper, Schlemner and Javaid, 1987). In each case, active drug and placebo did not differ in efficacy. Finally, of historical note, cisapride was used in one trial (Stacher *et al.*, 1993). This agent was theoretically appealing given the symptoms of impaired intestinal motility among anorectic individuals, but this drug, too, was no more effective than placebo, and it has been taken off the market in the United States due to concerns about cardiovascular toxicity.

Another area of interest in the pharmacotherapy of anorexia nervosa involves the use of medications in individuals restored to a normal or near normal body weight, in an attempt to prevent relapse. One controlled trial has been reported thus far (Kaye, 1996). In this relapse prevention trial, subjects receiving active drug were less likely to relapse than those receiving placebo. The results of this trial are interesting in light of other work using fluoxetine in acutely ill, low weight anorectic individuals. In one double-blind, placebo-controlled protocol, fluoxetine in a dose of up to 60 mg per day did not differ from placebo in terms of weight gain or other measures in a series of inpatients (Attia *et al.*, 1998). Similarly, in a separate study, neither the short-term in-hospital course nor the long-term follow-up course of the group of anorectic patients was improved by the use of fluoxetine (Strober *et al.*, 1999). One potential explanation for these seemingly contradictory results could be that the efficacy of fluoxetine in anorexia nervosa may be highly dependent on the phase of the illness in which it is being used. The results of these trials seem to concur with the prevailing clinical opinion that when individuals are at very low weight, pharmacotherapy is unlikely to be effective. On the other hand, pharmacotherapy both for anorexia nervosa and perhaps for comorbid problems as well, appears to be more effective once weight has been restored.

Based on the results of these studies, pharmacotherapy for anorexia nervosa at this time is typically confined to one of two situations. The first is the use of medications, typically fluoxetine, in an attempt to maintain weight in weight-recovered individuals. Second, a variety of psychopharmacologic agents are used to provide the appropriate treatment for comorbid psychopathology.

Psychotherapy of Anorexia Nervosa

Despite the fact that anorexia nervosa has been studied for a long period of time, the number of empirical trials in patients in this population has been quite limited. Part of this reflects the difficulty in conducting trials in this area, as detailed in the introduction to this chapter. However, this situation is beginning to be remedied, in that the number of randomized trials which have recently appeared or which are now underway has increased dramatically, and one can only hope that the result of these studies will better inform our treatment of this group of patients, many of whom remain chronically ill, and some of whom die of the disorder.

One group of investigators that has been quite interested in examining various treatment methodologies for patients with anorexia nervosa includes Arthur Crisp and his colleagues at St. George's Hospital in London. In a series of publications, treatment techniques for anorexia nervosa have been examined (Hall and Crisp, 1987; Deeble *et al.*, 1990; Crisp *et al.*, 1999; Gowers *et al.*, 1994). This series of studies has documented that 'dietary advice' can be useful in inducing weight gain, compared to psychotherapy alone. Perhaps in the most controversial of these studies, 90 subjects with anorexia nervosa were randomly allocated to one of four treatment cells: inpatient treatment, assessment interview only, and one of two outpatient treatment cells. All three of the active treatment regimens resulted in improvement, and there appeared to be no significant advantage for the inpatient treatment program, despite the fact that the outpatient treatment programs offered were of relatively brief duration.

In another series of studies, Russell, Dare, Isler, and colleagues (for summaries please see Russell *et al.*, 1987; Eisler *et al.*, 1997) have sequentially examined different models of outpatient treatment for weight restored anorectic patients. In an initial study, these investigators found that family therapy was more effective for younger patients who had been ill for a briefer period of time, while the older more chronic patients did better with individual therapy. The second study, which included adult patients with anorexia nervosa who had been weight restored, found that family therapy was superior to individual psychoanalytic or individual supportive therapy. A third study found that a conjoint therapy approach, wherein patients were seen with their families, was less effective in inducing weight gain, but more effective in changing various psychological parameters, than family therapy where the patients and the family were each seen alone.

Another group of investigators has examined family therapy versus individual therapy with adolescents, employing either a behavioural family systems therapy approach or an ego-oriented

individual therapy approach (Robin *et al.*, 1994, 1995, 1999). The results of these studies suggest that the behavioural family systems therapy produced greater overall weight gain and was more likely to be associated with resumption of menses; however, both treatments were effective in producing improvement in eating attitudes, depression and eating-related family conflict.

Also of note, a pilot study by Treasure *et al.* (1995) found that a group of adult anorexia nervosa patients reported subjectively greater improvement when treated with cognitive analytical treatment as opposed to educational behavioural treatment, but there were no differences in other outcome parameters. Most recently Geist *et al.* (2000) contrasted family therapy versus family psychoeducational treatment in 25 adolescents requiring hospitalization for eating disorders, and found no significant group differences, suggesting that both interventions appeared to be effective, in a study with a relatively small sample size.

Taken together, these studies indicate quite clearly that structured forms of psychotherapy appear to benefit patients with anorexia nervosa. The findings regarding inpatient versus outpatient treatment in the protocol by Crisp and colleagues are of concern, and seems to be in conflict with the clinical impression of many researchers in the field, and clearly needs to be replicated. Other work suggests that many patients, particularly younger patients, may benefit from family involvement.

DISCUSSION

Both pharmacotherapy and psychotherapy play a role in the treatment of all three eating disorders. Bulimia nervosa appears to respond optimally to treatment with CBT, with the possible addition of fluoxetine in those comorbidly depressed. Pharmacotherapy plays a more limited role in the treatment of anorexia nervosa, but is probably useful in preventing relapse is limited among those also receiving counselling. Our knowledge of the treatment of binge-eating disorder, although results to date suggest that interventions that target both weight loss and binge eating are most useful.

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XXIV

Sleep Disorders

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Animal Models of Sleep Disturbances: Intrinsic and Environmental Determinants

Peter Meerlo, Bernard M. Bergmann and Fred W. Turek

INTRODUCTION

Although each mammalian species has its own sleep characteristics in terms of the total amount and distribution of sleep over the day (Campbell and Tobler, 1984; Zepelin, 2000), most of them share the fundamental regulatory principles with human beings (Borbely, 1982, Mistlberger *et al.*, 1983; Tobler, 2000). First, most species display a daily rhythm in sleep and wakefulness that is under the control of an endogenous biological clock in the brain. Second, within sleep, there is a cyclic alternation of two different states, non-rapid-eye-movement sleep (NREM) and rapid-eye-movement sleep (REM). And third, there is a homeostatic drive for each of the two sleep states that accumulates when the sleep state is absent. The longer an organism is awake, the higher its need for sleep. Remarkably, the exact functions of NREM and REM sleep are still unknown, but both states apparently serve vital functions. Studies in rats have shown that prolonged deprivation of either or both sleep states ultimately results in death (Rechtschaffen *et al.*, 1983; 1989). Studies of biological rhythms and sleep states in animals have greatly extended our knowledge of the regulatory mechanisms of sleep and provided clues to its functions. In addition, because of the similarities in sleep between human beings and other mammalian species, laboratory animals also provide a useful tool to gain insight into human sleep disorders.

Sleep disturbances can have a variety of underlying causes and contributing factors, and often are the outcome of a complex interplay between intrinsic factors and environmental influences. This chapter will summarize how sleep in animals can be affected by intrinsic factors (e.g., the physiological profile of an individual as determined by its genes, development, and age) as well as external factors (such as stressors and other environmental influences). Animal research allows detailed studies of each of these possible components and, as such, animal models can be a relevant source of information on the aetiology and mechanisms of sleep disorders.

We have chosen to categorize and present the animal literature on sleep disturbances roughly according to their intrinsic or extrinsic cause since that is what many studies use as a starting point. Although there are a number of animal models that mimic a specific sleep disorder (e.g., narcolepsy), a more common approach is to examine the influence of a certain intrinsic factor or environmental condition on sleep rather than modelling a sleep disorder *per se* (e.g., the influence of ageing or stress). The results of studies using the latter approach do not always allow a symptom-based classification as generally is used for human sleep disorders. For example, since insomnia in humans is often ascribed to stress, studying the influence of stressors on sleep in animals and its possible physiological pathways is highly relevant to the understanding of human sleep disorders. However, studies in mice and rats show that various stressors induce dynamic and complex changes in sleep that cannot simply be listed as insomnia. Therefore, we choose to present these models under the heading of stress, rather than insomnia.

The first sections hereafter describe how sleep disorders may be related to genes and development. The genetic models include successful models of narcolepsy as well as selected lines of rats with changes in particularly REM sleep. The developmental models show how influences during early stages of life can affect adult sleep and potential sensitivity to sleep disorders. These models also are characterized in particular by changes in adult REM sleep regulation. The following section discusses animal models of sleep in ageing, and the paragraphs thereafter deal with alterations in sleep due to disturbances of the circadian clock. Finally, a number of sections summarize the changes and disturbances in sleep that may occur in response to environmental factors, such as light and temperature, and, probably the most commonly cited cause of disturbed sleep, stress.

GENETIC MODELS

A variety of genetic tools and approaches are used to study gene effects on sleep in animals, including comparisons of different strains of rats or mice, knockout mice, or mice strains with other mutations. Whereas many of these studies are undertaken to unravel basic physiological mechanisms underlying sleep regulation, some of the models provide relevant information on genetic factors in sleep disturbances or genetic influences on the sensitivity to sleep disorders.

Narcoleptic Dogs and Mice

Probably the first true animal model of a sleep disorder was the finding of narcoleptic behaviour in dogs (Mitler *et al.*, 1974). Narcolepsy is a seriously disabling neurological disease that is characterized by disturbed night-time sleep and excessive daytime sleepiness, as well as pathological manifestations of REM sleep and cataplexy, often triggered by emotional expressions (Bassetti and Aldrich, 1996; Guilleminault and Anagnos, 2000). Early studies identified a narcoleptic phenotype in several breeds of dogs, including Doberman pinchers, which have played an important role in unravelling the genetic basis of the disease and also contributed to the development of treatment strategies (Baker *et al.*, 1982; Nishino and Mignot, 1997). More recently, mice were discovered with narcoleptic phenomena similar to those seen in dogs

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(Chemelli *et al.*, 1999). Taken together, the different animal models point to a link between narcolepsy and a dysfunction of the hypocretin/orexin neuropeptide system in the brain (for review, see Siegel, 1999). Hypocretin/orexin neurons are located in the hypothalamus and have widespread projections to various brain areas, including the brainstem, the limbic system, and the cerebral cortex (Peyron *et al.*, 1998). Originally, the hypocretin and orexin peptides were discovered independently by different research groups, and the function of these peptides appeared to be related to the regulation of food intake (De Lecea *et al.*, 1998; Sakurai *et al.*, 1998). In subsequent studies the orexins and hypocretins not only turned out to be identical, but also were found to have a major effect on sleep. The narcoleptic dogs, which are now known to have a mutation in one of the hypocretin receptors, have disturbed sleep and cataplexy attacks much like those in human patients (Lin *et al.*, 1999). Moreover, mice whose gene coding for the hypocretin/orexin peptide has been inactivated exhibit clear, narcolepsy-like symptoms, including an increase in sleep time during their normal activity phase, sleep-onset REM sleep episodes, and sudden behavioural arrests (Chemelli *et al.*, 1999). Pharmacological studies in rats have confirmed a role for hypocretins/orexin in sleep-wake regulation, and administration of the peptide increases wakefulness and suppresses REM sleep (Hagan *et al.*, 1999; Piper *et al.*, 2000).

Taken together, the results suggest that hypocretins/orexins serve as an arousal factor and suppress REM sleep, and a lack of hypocretin/orexin signalling might be the cause for much of the narcoleptic symptomatology (Siegel, 1999). Based on these results from animal research, subsequent studies found that human narcolepsy is indeed associated with a specific loss of hypocretin/orexin neurons and undetectable levels of the peptide in the brain and cerebrospinal fluid, supporting a role for hypocretin peptides in sleep disorders such as narcolepsy, and opening new avenues for drug development and treatment procedures (Nishino *et al.*, 2000; Peyron *et al.*, 2000; Thannickal *et al.*, 2000).

Rat Strains with REM Sleep Disorders

A number of studies on selected lines of rats have explored alterations in REM sleep regulation and its possible relevance to human disorders such as depression. Human depression is characterized by changes in REM sleep regulation, especially an increase in REM sleep early in the night, and it has been suggested that a dysregulation of REM sleep may be linked to the development of the mood disturbance (Berger and Riemann, 1993; Vogel *et al.*, 1980). Two rat lines in particular have been studied in this context, the Flinders Sensitive Line rats (Shiromani *et al.*, 1988) and the Wistar Kyoto rats (Dugovic *et al.*, 2000). The Flinders Sensitive Line was selectively bred for hypersensitivity of the cholinergic system (Overstreet, 1993) whereas the Wistar Kyoto rat was originally bred as a normotensive control for spontaneously hypertensive rats (Louis and Howes, 1990). Both of these rat strains show behavioural abnormalities that have been suggested as a model of human depression (Overstreet, 1993; Pare and Redei, 1993). Subsequent analysis of sleep in the Flinders Sensitive rats indeed revealed an increase in the amount of REM sleep, without major changes in NREM sleep (Shiromani *et al.*, 1988; Benca *et al.*, 1996). The Wistar Kyoto rats were found to have more fragmented sleep and an overall increase in the total amount of REM sleep (Dugovic *et al.*, 2000). These models thus support the hypothesis of a link between alterations of REM sleep regulation and mood disturbances such as depression. Although originally these selection lines of rats were not developed as models of sleep disorders, they hold great potential to study whether altered REM sleep regulation has detrimental effects and perhaps is a causal factor in the development of depression-like behavioural disturbances.

DEVELOPMENTAL FACTORS

In addition to genetic models of sleep disturbances, there is evidence from animal research that adult sleep characteristics and perhaps sensitivity to sleep disorders may depend on developmental factors as well. Manipulations and environmental stimuli around the time of birth can lead to profound changes in physiological function and behaviour later in life. Maternal stress, malnutrition, and alcohol ingestion are among several factors that induce developmental changes in the fetus and cause alterations in the offspring's sleep regulation that last into adulthood.

Maternal Stress

One example of developmental influences on sleep regulation is the effect of so-called maternal or prenatal stress. In this model, pregnant female rats are repeatedly subjected to stress, which often comprises immobilization several times a day during the last 1–2 weeks of gestation (Barbazanges *et al.*, 1996). Some of the effects of prenatal stress on the development of the young appear to be mediated by an increase in maternal corticosterone (Barbazanges *et al.*, 1996). After birth, the young are raised in a standard way until adulthood, when physiological function and behaviour are studied. In adulthood, the offspring of mothers subjected to such stress have increased sleep fragmentation and increased amount of REM sleep (Dugovic *et al.*, 1999). Maternal immobilization stress also induces certain alterations in HPA axis function and behaviour that are reminiscent of human depression (Weinstock, 1997). It has therefore been proposed that prenatal stress, similar to the genetic models with increased REM sleep, may be a valuable model to study the relationship between altered sleep and mood disorders (Dugovic *et al.*, 1999). Important in this context is the finding that experimentally depriving adult rats of REM sleep results in hyperactivity, fearlessness, and aggression (Hicks and Moore, 1979; Hicks *et al.*, 1979). Thus, whereas too much REM sleep may be associated with passive and depression-like behaviour, too little REM sleep may result in hyperemotionality and mania.

Maternal Food and Alcohol Intake

There is considerable evidence from studies in female rodents that the caloric content and composition of food, as well as alcohol and drugs during pregnancy, strongly affect the development of the foetuses. Especially maternal malnutrition and alcohol ingestion can result in serious morphological, physiological, and behavioural abnormalities in the offspring, including alterations in sleep regulation. A study on maternal protein malnutrition in rats showed that during adulthood the offspring had more NREM sleep but their REM sleep was less than half that of control animals (Datta *et al.*, 2000). In rat pups that were exposed to alcohol in utero, quiet sleep was reduced and more frequently interrupted by waking episodes (Hilakivi, 1986). In adulthood, the offspring showed a strong reduction in the amount of REM sleep (Stone *et al.*, 1996), an effect that, for unknown reasons, may be particularly severe in the female offspring (Sylvester *et al.*, 2000). Although the changes in sleep after maternal malnutrition and alcohol intake were not hypothesized to reflect a specific sleep disorder, it has been suggested that such sleep changes, particularly the decrease in REM sleep, may have detrimental effects on cognitive functions. Importantly, in rats that are exposed to alcohol in utero, the deficits in REM sleep during adulthood seem to predict certain cognitive deficits during ageing (Stone *et al.*, 1996).

It is noteworthy that the reduction in REM sleep in offspring after maternal malnutrition and alcohol intake is opposite to the increase in REM sleep seen after maternal stress. Apparently, developmental influences on adult sleep-wake patterns strongly depend on the nature of the stimulus. Moreover, these studies on maternal malnutrition and alcohol intake support the hypothesis that not only too much, but also too little REM sleep may be associated with specific malfunctions and disease-like manifestations. As already mentioned above, REM sleep deprivation appears to result in hyperemotionality. In addition, although the evidence is contradictory, other studies have linked REM sleep loss to a decrease in cognitive performance and disturbances in learning and memory (for review, see Graves *et al.*, 2001; Siegel, 2001; Smith, 1996).

AGEING

Many reports suggest that the prevalence of certain sleep disorders and insomnia increase during human ageing (for review, see Bliwise, 2000). However, the study of sleep and human ageing is severely complicated by factors such as disease and medication, and several reports suggest that poor sleep in the elderly is in many cases secondary to medical illness rather than chronological age (Foley *et al.*, 1995; Gislason and Almqvist, 1987). Controlled studies in animals can shed light on such issues, and measurements in a variety of mammalian species have explored the changes in sleep that occur with ageing. The most commonly used animal model for studies on ageing and sleep is the rat. In old rats of about 20–24 months, ageing-related changes in sleep include a dampening of the sleep-wake rhythm amplitude, more fragmentation of sleep, a decrease in deep NREM sleep, and, sometimes, slight overall decreases in the amount of sleep, especially REM sleep (Mendelson and Bergmann, 1999; Rosenberg *et al.*, 1979; Van Gool and Mirmiran, 1983). However, the changes can be fairly subtle, and not all studies have found clear changes in basal sleep patterns in old rats (see Mendelson and Bergmann, 1999; Zepelin *et al.*, 1972). The age-related changes in sleep may vary considerably between individual animals as well as between strains of rats (Li and Satinoff, 1995; Shiromani *et al.*, 2000), suggesting that the consequences of ageing may strongly depend on genetically determined individual characteristics.

Importantly, although age-related changes in *baseline* sleep may not always be consistent, there seems to be a more robust change in the compensatory sleep *rebound* after a period of sleep deprivation (Mendelson and Bergmann, 2000; Shiromani *et al.*, 2000). Young rats respond to extended wakefulness with an increase in the time spent in NREM sleep and an increase in NREM sleep intensity, as reflected in an increase in EEG slow waves, as well as an increase in REM sleep (Borbely and Neuhaus, 1979; Tobler and Borbely, 1986). In old animals, however, the compensatory NREM sleep rebound is diminished (Mendelson and Bergman, 2000; Shiromani *et al.*, 2000). The REM sleep rebound after sleep deprivation was also found to be attenuated, but only after a very long period of sleep deprivation of 48 h (Mendelson and Bergmann, 2000), and not after milder 12-h sleep deprivation (Shiromani *et al.*, 2000). Moreover, whereas in young animals extended wakefulness is generally followed by an immediate recovery rebound, in aged rats the rebound is not only attenuated but also sometimes delayed (Mendelson and Bergmann, 2000). Taken together, the results seem to point to a decline in the homeostatic response to sleep loss in ageing rats.

Age-related changes in sleep have been reported for several other mammalian species, including mice (Welsh *et al.*, 1986), hamsters (Naylor *et al.*, 1998), and cats (Bowersox *et al.*, 1984). Many of the sleep changes that occur during ageing in these species are comparable with those seen in the rat. A notable difference is that, in both hamsters and cats, ageing is associated with a slight increase in the total daily amount of NREM sleep. The reason for this difference is not fully understood, but it has been explained as an attempt to compensate for lower sleep efficiency as reflected in a decrease in electroencephalographic (EEG) slow waves (Naylor *et al.*, 1998).

Some of the ageing-related changes in sleep in the various animal models, such as a higher fragmentation of sleep and a decrease in sleep-wake rhythm amplitude, are thought to be due to alterations in the biological clock that is driving the sleep-wake rhythm (see next section). In addition, the decline in the compensatory response to sleep deprivation in the rat suggests that also the homeostatic mechanisms underlying NREM sleep regulation are affected by age. Interestingly, part of the age-related sleep changes in rats can be prevented by housing them in an enriched environment (Van Gool and Mirmiran, 1986), an observation which suggests that an increase in behavioural activity and sensory input may alleviate the consequences of ageing. A recent study in humans indeed confirmed that structured physical and social activity can improve night-time sleep in the elderly (Naylor *et al.*, 2000). These latter findings illustrate the potential value of animal models in finding treatment strategies and therapies for sleep disturbances in ageing.

CLOCK DISTURBANCES

In most mammalian species, the alternation of sleep and wakefulness occurs in a rhythmic fashion, and sleep is preferentially consolidated to certain periods of the day. The regulation of such daily rhythmicity, which is not restricted to sleep but is manifest in most biological functions, is under the control of an endogenous oscillator, or 'clock', located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972; for review, see Klein *et al.*, 1991). Since the periodicity of endogenous circadian rhythms often slightly deviates from 24 h, synchronization to the environment occurs through daily adjustment of the clock by light (Pittendrigh, 1981). For this purpose, the clock in the SCN receives photic information from the environment via a direct neuronal input from the retina (Moore and Lenn, 1972).

Several studies have shown that destruction of the clock by lesioning the SCN results in a fragmented sleep pattern without a clear daily rhythm in mice (Ibuka *et al.*, 1980), rats (Eastman *et al.*, 1984; Mistlberger *et al.*, 1983; Mouret *et al.*, 1978; Tobler *et al.*, 1983), and squirrel monkeys (Edgar *et al.*, 1993). In the mice and rat studies, destruction of the SCN abolished the normal expression of the circadian sleep-wake cycle, but it did not change the total amount of sleep. In the squirrel monkeys, however, lesioning the central oscillator resulted not only in an arrhythmic sleep-wake pattern but also in a pronounced increase in total sleep time. This finding may imply that, at least in some species, alterations in clock function may affect not only the timing and consolidation of sleep but also the homeostatic regulation of sleep duration.

The *Clock* Mutation

An alternative approach to study the effects of changes in clock function on sleep is to examine animals that have a genetically altered clock system. One such model is the *Clock* mouse, which has a mutation in one of the genes that is involved in the regulation of circadian rhythmicity (Vitaterna *et al.*, 1994). Under a normal light-dark cycle, homozygote *Clock* mice still maintain fairly normal activity patterns. However, under conditions of constant darkness, when the endogenous clock is no longer synchronized

to the environment, the *Clock* mice have unstable activity rhythms with an abnormally long, 28-h period. Eventually, the animals even become arrhythmic (Vitaterna *et al.*, 1994). Under both conditions of synchronization to a light-dark cycle and constant darkness, the *Clock* mice have a dampened amplitude of the sleep-wake rhythm and spend less time in NREM sleep than their wild-type counterparts (Naylor *et al.*, 2000). This study confirms that changes in circadian clock function are a potential pathway to disturbance of sleep.

The Ageing Clock

As discussed in a previous section, ageing is often associated with increased sleep fragmentation and reduced sleep-wake rhythm amplitude, part of which may be due to an age-related decline in the functional activity of the endogenous pacemaker in the SCN (for review, see Turek *et al.*, 2001; Weinert, 2000). In line with this, aged rodents sometimes exhibit decreased numbers of SCN cells with vasopressin, one of the primary neurotransmitters in the clock (Van der Zee *et al.*, 1999). Moreover, peak neuronal discharge in the SCN is reduced and more irregular in old animals (Satinoff *et al.*, 1993). In addition, advanced age has been associated with a decrease in responsiveness of the circadian clock to the phase-shifting effects of light and other synchronizers (Penev *et al.*, 1995; Van Reeth *et al.*, 1993; Zee *et al.*, 1992). Taken together, the data from animal studies indicate that in the aged the endogenous oscillator in the SCN is less sensitive to input stimuli that are normally involved in synchronizing the clock to the environment, and that the SCN has an altered output to the rest of the brain and body. However, although a great deal is known about the molecular machinery of the biological clock in the SCN and studies in rodents have revealed several pathways by which the SCN might affect the sleep-wake rhythm (Aston-Jones *et al.*, 2001), it is still unclear which of these pathways might deteriorate during ageing (Shiromani *et al.*, 2000).

Dissociations Between Clock and Environment

In addition to alterations in the central circadian clock itself, disturbed sleep may also arise from a misalignment between the endogenous clock and environmental determinants of the sleep-wake rhythm. In humans, this can occur, for instance, after a long-distance flight when the internal clock is temporarily out of synchrony with the environmental light-dark cycle at the place of arrival, a condition that is referred to as jet lag. In animals, the effects of long-distance flights can easily be mimicked by shifting the light-dark cycle of the housing facility. In one study, rats were subjected to an 8-h advance of the light-dark cycle, comparable to a transatlantic flight from the USA to Europe (Sei *et al.*, 1992). Sleep patterns were studied for 3 days preceding, and 5–7 days following the shift. Especially the rhythm of NREM sleep was severely disturbed and had a strongly dampened amplitude. The daily REM sleep rhythm, however, was less affected. Perhaps the REM sleep rhythm was strongly determined by the clock whereas the dampening of the NREM sleep rhythm may have been the result of interactive effects of the endogenous clock and direct effects of light (see next section). Overall, the amount of both NREM and REM sleep was somewhat increased during the simulated jet lag. Eventually, light input to the clock will lead to resynchronization of the endogenous clock to the new light-dark cycle, but in this particular study recordings did not extend beyond 5–7 days, when alterations in the sleep-wake rhythm were far from normalized. This in itself suggests that long-distance flight may have quite persistent effects. Animal models for jet lag hold great potential for studying concomitant sleep disturbances and treatment strategies.

Taken together, studies on the circadian regulation of sleep and wakefulness imply that alterations in the biological clock or in the light input to the clock can be a major cause of disrupted sleep. The progress that has been made in identifying the molecular and neurobiological substrates of circadian rhythms in animals has broadened our understanding of clock function and the way rhythm disturbances may contribute to disrupted sleep in our society.

ENVIRONMENTAL INFLUENCES

There are a variety of environmental factors, such as light and ambient temperature, that can directly or indirectly affect sleep quality. In human beings, the possibility of self-regulating light exposure and temperature conditions has turned these environmental influences into lifestyle factors that can have positive effects on sleep but may also contribute to the occurrence of disrupted sleep. Although most of the animal studies on light and ambient temperature were undertaken from a fundamental interest and were not presented as models of sleep disorders, some of them provide information that is relevant in the context of disrupted sleep due to inadequate sleep hygiene.

Light Effects

Light is a factor that may have both positive and negative effects on sleep. As mentioned in a previous section, light is important for the regulation of normal circadian rhythmicity, and well-timed exposure to light keeps the sleep-wake rhythm in synchrony with the outside world. However, light at the wrong time of day for prolonged periods of time may shift the clock and lead to disrupted sleep. In addition to direct input to the SCN, there is light input to various other brain regions, including the serotonergic raphe nuclei in the brainstem (Shen and Sembra, 1994), via which route light may modulate sleep and arousal as well (Jouvet, 1999; Portas *et al.*, 2000).

Rodents such as rats are primarily nocturnal animals. Maintained under a 12-h light:12-h dark schedule in the laboratory, both NREM and REM sleep in the rat predominantly occur during the light phase. However, under natural conditions, rats would probably avoid light and spend most of the daytime hours in the darkness of their burrows (Terman *et al.*, 1991). Thus, as in human beings, sleep would most likely occur mainly in darkness. Several studies in rats have shown that light can have potent effects on sleep architecture and sleep intensity. A number of studies that compared sleep under a light-dark cycle with sleep under constant darkness showed that *removal* of the light influence significantly enhances sleep continuity and increases NREM sleep EEG amplitude, a measure of sleep intensity (Tobler *et al.*, 1994; Trachsel *et al.*, 1986). Under these conditions, the overall amount of NREM sleep is not strongly affected by light, but the amount of REM sleep is sometimes suppressed (Trachsel *et al.*, 1986). Another approach that has been used to investigate the effects of light on sleep involves exposing rats to ultra-short light-dark cycles — for instance, cycles with consecutive periods of 1-h light and 1-h darkness (for review, see Borbely, 1978). Also under these conditions, the amount of REM sleep is lower in the light than in the dark. The amount of NREM sleep, however, is higher in the short light phases than in the short dark phases (Alfoldi *et al.*, 1991; Borbely, 1976; Lelkes *et al.*, 1990). Clearly, the effects of light on the amount of NREM sleep are complex, but the data suggest that exposure to light during the normal sleeping phase may disrupt sleep by at least suppressing NREM sleep intensity and REM sleep duration. Animal studies on the effects of light on sleep and the mechanism by which it acts become increasingly important in our rapidly developing 24-h society where we are frequently exposed to artificial light at unusual times, as in shift work.

Ambient Temperature

Another environmental variable that can have both positive and disruptive effects on sleep is the ambient temperature. The effects of ambient temperature on sleep have been studied in various mammalian species, including mice (Roussel *et al.*, 1984), rats (Schmidek *et al.*, 1972), hamsters (Sichieri and Schmidek, 1984), and cats (Parmeggiani and Rabini, 1970). A number of studies suggest that moderate and short-lasting thermal loads may promote NREM sleep (Moriarty *et al.*, 1993; Obal *et al.*, 1995), a finding that is in agreement with increases in NREM sleep in human beings after heating in a warm bath or a sauna (Horne and Reid, 1985; Putkonen *et al.*, 1973). However, exposure to more extreme ambient temperatures for prolonged periods of time may seriously disrupt sleep and reduce NREM sleep time. The amount of REM sleep is generally maximal around thermoneutrality, that is, the ambient temperature at which the metabolic rate is minimal. Both higher and lower ambient temperatures cause a decrease in REM sleep time (Parmeggiani and Rabini, 1970; Rosenthal and Vogel, 1993; Schmidek *et al.*, 1972; Szymusiak and Satinoff, 1981). Studies of rats under very low ambient temperatures around or below 0 °C have shown severe reductions and even complete loss of REM sleep (Amici *et al.*, 1994; 1998). During recovery from cold exposure, REM sleep levels are markedly increased above baseline levels, indicating that during cold exposure the animals are deprived of REM sleep. Clearly, these data indicate that extreme ambient temperatures should be avoided in order to prevent sleep from being disturbed. However, the sleep-stimulating effect of a mild temperature load preceding sleep could perhaps be used as a basis for developing treatments to alleviate sleep complaints.

STRESS

Probably the most frequently cited cause of disrupted sleep and insomnia in humans is 'stress' (Roehrs *et al.*, 2000). Accordingly, there is a great interest in using stress as a starting point for animal models of sleep disturbances. However, animal research shows that the relationship between stress and sleep is rather complex. Stress-induced changes in sleep show a dynamic temporal pattern that depends on the nature and duration of the stressor.

Acute Stress

Stress is usually defined as a non-specific physiological response to any kind of demand that an organism is facing (Selye, 1936). Traditionally, the autonomic sympatho-adrenal axis and the hypothalamic-pituitary-adrenal (HPA) axis are considered to be the main neuroendocrine systems involved in the integrated stress response (Axelrod and Reisine, 1984; Johnson *et al.*, 1992). The increased catecholaminergic activity and HPA axis activity, in a complex interplay with various other neuroendocrine systems, orchestrate an adequate response to the challenge an animal or human being is dealing with. Obviously, coping with environmental challenges requires alertness, and since stress is a state of physiological activation and arousal, by definition, it inhibits sleep. Indeed, exposing animals to stressors is invariably associated with at least a short-lasting increase in wakefulness. Moreover, many of the classical neuropeptides and hormones involved in the stress response are known to promote wakefulness, including catecholamines (Jones *et al.*, 1977; Cespuglio *et al.*, 1982; Lin *et al.*, 1992), corticotropin-releasing factor (CRF) (Ehlers *et al.*, 1986; Opp, 1995), and adrenocorticotropic hormone (ACTH) (Chastrette *et al.*, 1990; Gillin *et al.*, 1974). These data support the notion that acute stress and the concomitant physiological arousal may be an important cause of sleep disruption. The acute stress-induced arousal in animals can be used as a model to study and test the effects of potential hypnotic compounds (James and Piper, 1978).

Interestingly, studies in rats and mice have shown that upon removal of a stressor the arousing effect is rapidly overcome, and that stress may actually promote and increase sleep during the subsequent recovery period (Meerlo *et al.*, 1997; Meerlo and Turek, 2001; Meerlo *et al.*, 2001; Rampin *et al.*, 1991). In addition to this biphasic effect of stress on sleep, the picture is further complicated by the finding that different stressors may have different effects on NREM sleep and REM sleep. For example, whereas *immobilization* of rats and mice in a small tube results in an increase of REM sleep during subsequent recovery (Meerlo *et al.*, 2001; Rampin *et al.*, 1991), a *social conflict* with an aggressive conspecific was found to promote NREM sleep (Meerlo *et al.*, 1997; Meerlo and Turek, 2001). Importantly, although both social conflict and immobilization cause strong sympathetic and HPA axis activation, the respective increases in NREM sleep or REM sleep are apparently due to certain specific aspects of the stimuli. In this respect, one should perhaps not speak in general terms about the effects of stressors on sleep but, rather, refer to the effects of specific stimuli.

What are the specific elements of the different stressors that affect sleep? Clearly, the effects of a stressor may very well consist of multiple, interacting components, some non-specific, and some specific to a particular stimulus. The mechanism underlying an increase in NREM sleep as it is seen after a social conflict is still unknown, but for stressful stimuli that increase REM sleep, various factors have been proposed, including CRF (Gonzalez and Valatx, 1997), corticotropin-like intermediate lobe peptide (CLIP) (Bonnet *et al.*, 1997; Bonnet *et al.*, 2000), and prolactin (Bodosi *et al.*, 2000; Meerlo *et al.*, 2001). In particular, differences in prolactin release may explain some of the differential effects of stress on REM sleep. Prolactin is commonly referred to as a stress hormone, but the change in prolactin levels strongly depends on the nature of the stressor. In line with its effects on sleep, immobilization stress in rodents indeed appears to be characterized by an increase in prolactin that is much larger than what is observed after other stressors such as social conflict or cold stress (Lenox *et al.*, 1980; Meerlo *et al.*, 2001). Moreover, in a comparison between two different strains of mice, immobilization stress induced an increase in REM sleep during recovery only in one strain of mice, which had a large prolactin response (Meerlo *et al.*, 2001). The latter finding also illustrates how modulation of sleep by environmental factors depends not only on the nature of the stimulus but also on the genetic make-up of the individual.

Taken together, studies in rodents show that the final effect of an acute and short-lasting stress on sleep depends not only on the non-specific arousal common to all stressors but also on the specific aspects of a given stimulus. Whereas many of the classical *non-specific* stress response factors initially increase arousal and inhibit sleep, factors *specific* to a particular situation or stimulus may increase either NREM sleep or REM sleep during recovery sleep. Importantly, an increase in NREM sleep or REM sleep after exposure to a particular stressful stimulus is not necessarily a manifestation of dysregulation. Rather, it may be interpreted as a necessary homeostatic recovery process, although the exact nature of this recovery process is still unknown, in the case of both stress-induced sleep and sleep in general (Meerlo *et al.*, 1997).

Although exposure to an acute stressor in animals is generally associated with an increase in wakefulness for as long as the stimulus persists, it seems puzzling that this arousing and sleep-inhibiting effect of the stressor is so rapidly overcome. Moreover, especially the finding of an increase in sleep following certain stressful stimuli may seem to contrast with the general notion that stress is a major cause of sleep disturbances in human beings. However, there is very little information about the effects of acute stressors on human sleep, at least not in response to traumatic

experiences comparable to, for instance, social defeat in rodents. Thus, on the basis of the animal studies, one could hypothesize that, also in our own species, specific stressors may have a stimulatory effect on sleep systems shortly after the initial arousing effects. In fact, anecdotal information suggests that this may indeed be true (Oswald, 1962).

Studying the influence of acute and severe stressors may be a valid approach that mimics what could happen in real life, but, given the minimal and short-lasting wakefulness that occurs afterwards, these acute stress models do not appear to be extremely useful as models for the persistent insomnia so common in our society. For that purpose, one should perhaps use other or chronic stress models.

Repeated or Chronic Stress

In contrast to the acute and severe stress models discussed above, the 'stress' causing sleep disturbances in humans often seems to relate to a subtle cognitive phenomenon, that is, feelings of discomfort, not necessarily associated with an acute challenge (Roehrs *et al.*, 2000). Often, such feelings of discomfort may be based on memories of past events as well as worries and expectations about the future. In that respect, the human brain may be capable of turning a single acute stressor or life event that occurred in the past into a more persistent and chronic stress state. Although certain human cognitive processes may be difficult to model in animals, there are a number of studies that have examined the effects of chronic stress on sleep-wakefulness patterns in the rat.

In one model of chronic stress, sleep was measured in rats that were subjected to a paradigm of around-the-clock intermittent electrical shocks (Kant *et al.*, 1995). In this model of sustained stress, the animals display a wide variety of stress symptoms including elevated plasma levels of stress hormones such as corticosterone, a decrease in food intake and body weight, and disturbed temperature rhythm and activity pattern. The amount of sleep was remarkably reduced the first day of the treatment but normalized in the course of the days thereafter. However, the distribution of sleep over the 24-h cycle remained somewhat disturbed throughout the stress period. The amount of sleep was reduced in the light phase, the normal resting phase of rats, but this was compensated for by an increase in sleep during the dark phase. However, whereas rats under laboratory conditions have the possibility of such compensatory responses, human beings often do not have that possibility. These data show that, although a rat may rapidly overcome the arousal of a single stressful event, repetition of the stressor may add up and culminate in disrupted sleep.

In another study that applied a model of 'chronic mild stress', male rats were subjected to a mixture of noxious stimuli, once or twice a day, for various durations. The stimuli included cage tilt, soiled cage, deprivation of food and water, and housing in a mouse cage, which rats apparently dislike, but also exposure to continuous lighting and stroboscopic lighting (Cheeta *et al.*, 1997). The most significant finding from this study was a reduction in REM sleep latency during the protocol. The day after 3 weeks of a chronic mild stress treatment, the overall amount of sleep as well as the amount of REM sleep was somewhat increased, a finding which may in part reflect a rebound due to sleep that was lost during the actual stress exposure. Unfortunately, stimuli such as the lighting that was applied, although relevant in the context of environmental influences on sleep discussed earlier, may have effects on sleep that have little to do with stress *per se*.

Moreover, both models of chronic stress discussed here partly rely on direct stimulation of the animals, a fact which may explain some of the changes in sleep, whereas stress-related sleep disturbances in humans often appear to be of a more psychological nature. It may very well be that the physiological and neurobiological mechanisms resulting in disrupted sleep due to repeated electrical shocks and stroboscopic light are quite different from those involved in psychological stress in humans. These models are important first steps toward developing relevant models for stress-related sleep disturbances and insomnia, but, perhaps, research on the relationship between stress and sleep would gain by models that are based on, for instance, conditioned fear and arousal in which animals anticipate the occurrence of adverse events. Such an approach may have more resemblance to the psychological stress in humans, and with such a model one may be able to study the central mechanisms by which sleep is disrupted and how such disturbances could best be treated.

CONCLUDING REMARKS

Although the function of sleep is still an unresolved mystery, sleep is clearly necessary for optimal performance and well-being. The results from studies in a wide variety of animal models show that alterations and disruptions of sleep are often associated with changes in cognitive performance, emotionality, and disease. Sleep disturbances are a widespread and serious problem in our society, but animal research continues to push advances in the understanding of the causes and mechanisms of human sleep disorders, and to aid the improvement of treatment procedures and medicines.

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XXIV-2

Neurotransmitter Systems Regulating Sleep-Wake States

Barbara E. Jones

Three distinct physiological and cognitive states exist in mammals: waking, slow-wave sleep (SWS) and rapid-eye-movement sleep (REMS). Waking is a multifarious state but is generally characterized by fast waves on the electroencephalographic (EEG) record in association with behavioural activity and responsiveness; SWS is characterized by slow waves on the EEG record in association with behavioural quiescence and decreased responsiveness; however, REMS, or 'paradoxical sleep' (PS), is characterized by a unique dissociation of EEG, characterized by fast waves indicative of cortical arousal, and behaviour, characterized by quiescence and diminished responsiveness, indicative of sleep. Viewed in terms of metabolism and energy expenditure, waking requires variable yet relatively high levels of energy expenditure by the brain and body; SWS requires low levels and thus allows conservation and restoration of energy stores in both brain and body; however, REMS requires high levels of energy expenditure by the brain while maintaining minimal energy use by the body. These states occur within a circadian cycle and an ultradian cycle. Depending upon the species, the active, waking phase of the circadian cycle occurs either during the day and light, as in humans, or during the night and darkness, as in cats and rats. Sleep is concentrated to differing degrees in the opposite phase, when it occurs, according to an ultradian rhythm. Across the phylogenetic scale, the period of the ultradian sleep-wake rhythm is correlated with basal metabolic rate and body weight, and thus is apparently determined in part by energy expenditure, storage, conservation and restoration. In addition to their regulation by underlying rhythms, the sleep-wake states are also regulated by homeostatic processes, such that the deprivation of sleep results in an increased drive for sleep and increased occurrence of sleep upon recovery. The rhythmic and homeostatic nature of sleep-wake states indicates the existence of underlying, alternating and accumulating processes that may depend upon changes in chemical transmitters and their receptors in the brain.

Neurotransmitters include small molecules such as glutamate and GABA, acetylcholine and the monoamines, which may act directly upon ion channels or indirectly upon them through second messengers to modify membrane potentials and activity of neurons. They may also include peptides, which function as transmitters, modulators or hormones. Although, to date, no single transmitter/modulator molecule has been found that serves a specific or exclusive function in promoting or generating waking, SWS or REMS, specific neuronal systems containing particular neurotransmitters have been shown to be integral to the promotion of each of these states.

From early physiological studies, it has been known that no specific centres are present in the brain for the generation of any one state, but that redundant neuronal systems are distributed through the brainstem and forebrain, systems which are collectively important for the promotion and maintenance of individual states (Jones, 2000). For **waking** (Figure XXIV-2.1), neurons distributed through the brainstem reticular formation (RF) and concentrated within the oral pontine and mesencephalic fields comprise the ascending reticular activating system, which, by lesions in humans and animals, is known to be critical for the generation and maintenance of the EEG and behavioural components of waking (Moruzzi and Magoun, 1949). This system of neurons gives rise to ascending pathways projecting dorsally into the non-specific thalamo-cortical projection system, which in turn stimulates widespread activation of the cerebral cortex (Dempsey *et al.*, 1941). Other fibres ascend ventrally into and through the hypothalamus up to the level of the basal forebrain (substantia innominata [SI]), from where cortical activation is also relayed in a widespread manner (Starzl *et al.*, 1951). These systems collectively stimulate cortical activation, characterized by high-frequency (beta and gamma) EEG activity. Fibres also terminate in the posterior hypothalamus, where lesions are known in humans and animals to produce a comatose state (Ranson, 1939). As evident as well from electrical stimulation (Hess, 1957), the posterior hypothalamus is an important higher control centre for the sympathetic nervous system, thus coordinating peripheral autonomic responses (increased temperature, respiration, heart rate, blood pressure) with cortical activation. In addition, the descending projections from the brainstem reticular formation, including the caudal pontine and medullary reticular formation, serve to stimulate somatic motor activity, reactivity and postural muscle tonus (as evident in electromyographic [EMG] activity) through facilitatory reticulo-spinal influences (Magoun and Rhines, 1946).

For **SWS** (Figure XXIV-2.2), the medulla appears, to play a role in promoting sleep, particularly neurons in the region of the solitary and vagal, parasympathetic nuclei, which may inhibit the activating neurons located in the ponto-mesencephalic tegmentum (Batini *et al.*, 1959; Favale *et al.*, 1961). Within the forebrain, sleep marked by EEG slow waves may be elicited by low-frequency stimulation of the thalamo-cortical projection neurons (Akert *et al.*, 1952). Most potent there is the influence shown to emanate from the preoptic and anterior hypothalamic (POAH) region, where lesions have produced insomnia and electrical stimulation has promoted sleep, probably in part due to inhibition of the posterior hypothalamus and ponto-mesencephalic reticular formation (von Economo, 1931; Nauta, 1946; Hess, 1954). This region serves in an antagonistic manner to the posterior hypothalamus, inhibiting sympathetic activity and facilitating parasympathetic responses (decreased temperature, respiration, heart rate, blood pressure). SWS appears to emerge from the dampening of cortical activation, somatic motor activity (as evident by decreased EMG) and sympathetic nervous activity along with a shift to a predominance of parasympathetic activity.

For **REMS** (or PS, as it is often also called in animals) (Figure XXIV-2.3), the brainstem appears to contain the essential structures for the generation of the state (Jouvet, 1962). The oral pontine reticular formation (PnO) is necessary for the triggering of ascending and descending parameters of the state, including

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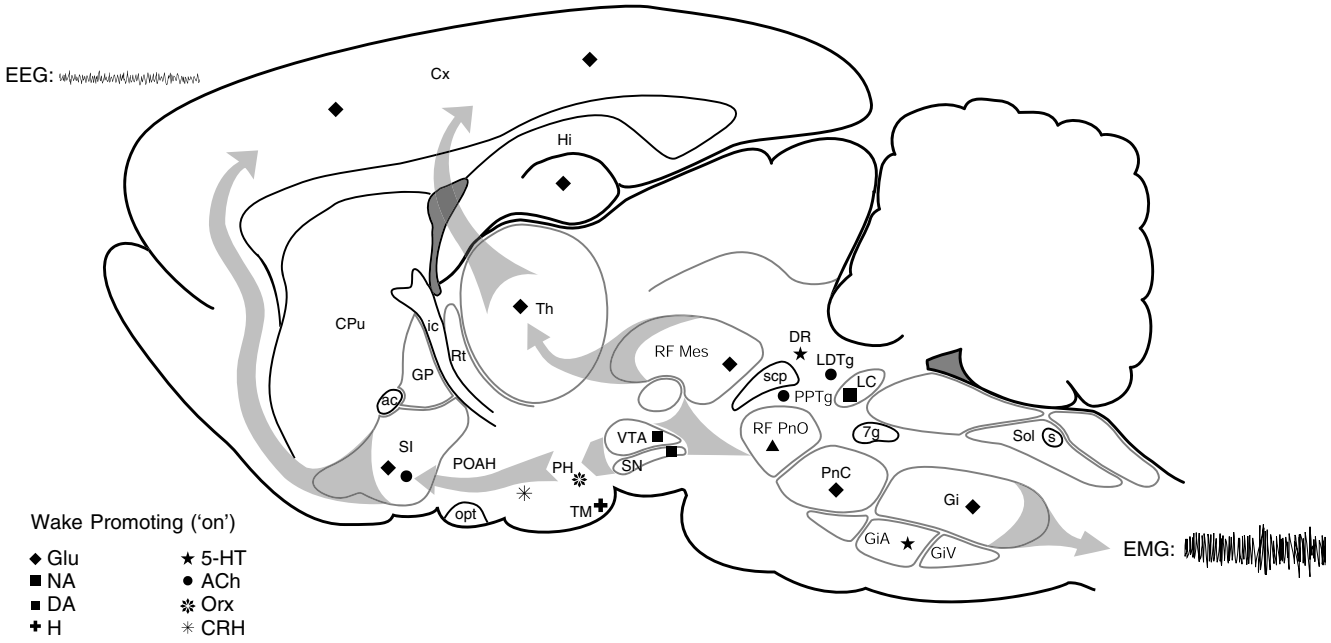

Figure XXIV-2.1 Wake-Promoting Systems. Schematic sagittal view of rat brain showing the major neuronal systems and their major excitatory pathways (arrows) involved in promoting the EEG fast activity (upper left) and EMG high muscle tone and activity (lower right) characteristic of the waking state. The major ascending pathways emerge from the brainstem reticular formation (RF, most densely from the mesencephalic, RF Mes, and oral pontine RF PnO, fields) to ascend along a) a dorsal trajectory into the thalamus (Th) where they terminate upon (midline, medial, and intralaminar) nuclei of the non-specific thalamo-cortical projection system, which projects in turn in a widespread manner to the cerebral cortex (Cx), and b) a ventral trajectory through the lateral hypothalamus up to the basal forebrain where they terminate upon neurons in the substantia innominata (SI) (and septum, not shown), which also project in turn in a widespread manner to the cerebral cortex (and hippocampus, Hi) (Jones, 1995). Descending projections collect from multiple levels of the reticular formation (though most densely from the caudal pontine, PnC, and medullary gigantocellular, Gi, fields) to form the reticulo-spinal pathways. The major transmitter systems that promote waking and discharge maximally ('on') during waking contribute to these ascending and descending systems and are represented by symbols where their cell bodies are located. Glutamatergic (Glu) neurons comprise the vast population of neurons of the reticular formation, the diffuse thalamo-cortical projection system and a contingent of the basalo-cortical projection system. Noradrenergic (NA) neurons of the locus coeruleus (LC) send axons along the major ascending and descending pathways to project in a diffuse manner to the cortex, the subcortical relay stations, brainstem and spinal cord. Dopaminergic (DA) neurons of the substantia nigra (SN) and ventral tegmental area (VTA) project along the ventral pathway in the nigro-striatal system and meso-limbo-cortical system, respectively. Histaminergic (H) neurons of the tuberomammillary nucleus (TM) project in a diffuse manner to the forebrain and cortex. Serotonergic neurons containing 5-hydroxytryptamine (5-HT) of the midbrain (including the dorsal raphe, DR) project to the forebrain, including the cerebral cortex (and hippocampus), as well as the subcortical relay stations, and those of the medulla (in raphe pallidus and obscurus, not shown, as well as pars alpha of the gigantocellular field [GiA]) project to the spinal cord. Cholinergic neurons, containing acetylcholine (ACh), are located in the laterodorsal and pedunculopontine tegmental (LDTg and PPTg) nuclei in the brainstem, from where they project along with other reticular neurons dorsally to the thalamus and ventrally to the posterior hypothalamus and basal forebrain, as well as to the brainstem reticular formation. They are also located in the substantia innominata (SI, and septum, not shown), from where they project to the cortex (and hippocampus). Orexinergic (Orx) neurons in the (perifornical and lateral) mid- and posterior hypothalamus (PH) project diffusely through the forebrain, brainstem and spinal cord to exert an excitatory influence at multiple levels. Corticotropin-releasing hormone (CRH) is contained in neurons within the paraventricular nucleus of the hypothalamus which project to the pituitary, as well as in other scattered neurons (not shown) projecting to other forebrain and brainstem areas that collectively stimulate the hypothalamo-pituitary-adrenal axis and central arousal systems. Some state-specific GABAergic neurons (triangle) may be on during waking to prevent activity of REM-promoting neurons in the oral pontine reticular formation (PnO) (Figure XXIV-2.3) (See Colour Plate XXIV-2.1)

cortical activation, phasic REM along with motor twitches, and tonic postural muscle atonia (Carli and Zanchetti, 1965). The ascending pathways stimulating cortical activation travel along the same routes as those utilized in waking, although the ventral extrathalamic pathway through the basal forebrain and into the limbic cortex appears to be most important (Jouvet, 1962). The descending motor inhibition (as evident from the flat EMG) appears to emerge from the oral pontine reticular formation and to relay through the ventral medullary reticular formation (Magoun and Rhines, 1946). Both visceral and somatic sensory-motor circuits and reflexes are inhibited by this system.

The representation from the classical physiological studies of the distribution of neuronal systems most critically involved in generating waking, SWS and REMS remains valid today. Within those regions and neuronal systems, particular transmitters/modulators have been localized and shown through specific lesions, unit recording, and pharmacological or more recently genetic modulation to play, if not specific, nonetheless differentiated, important roles in promoting waking, SWS or REMS.

WAKE-PROMOTING TRANSMITTER SYSTEMS

Glutamate (Glu) is the most common excitatory neurotransmitter in the brain and is critical for the waking state (Figure XXIV-2.1). It is utilized as the primary transmitter of the neurons within the brainstem reticular formation (RF) and thus is the transmitter of the ascending reticular activating system (Jones, 1995). It is also the transmitter of the non-specific thalamo-cortical projection system (Ottersen *et al.*, 1983) and of a contingent of the basalo-cortical

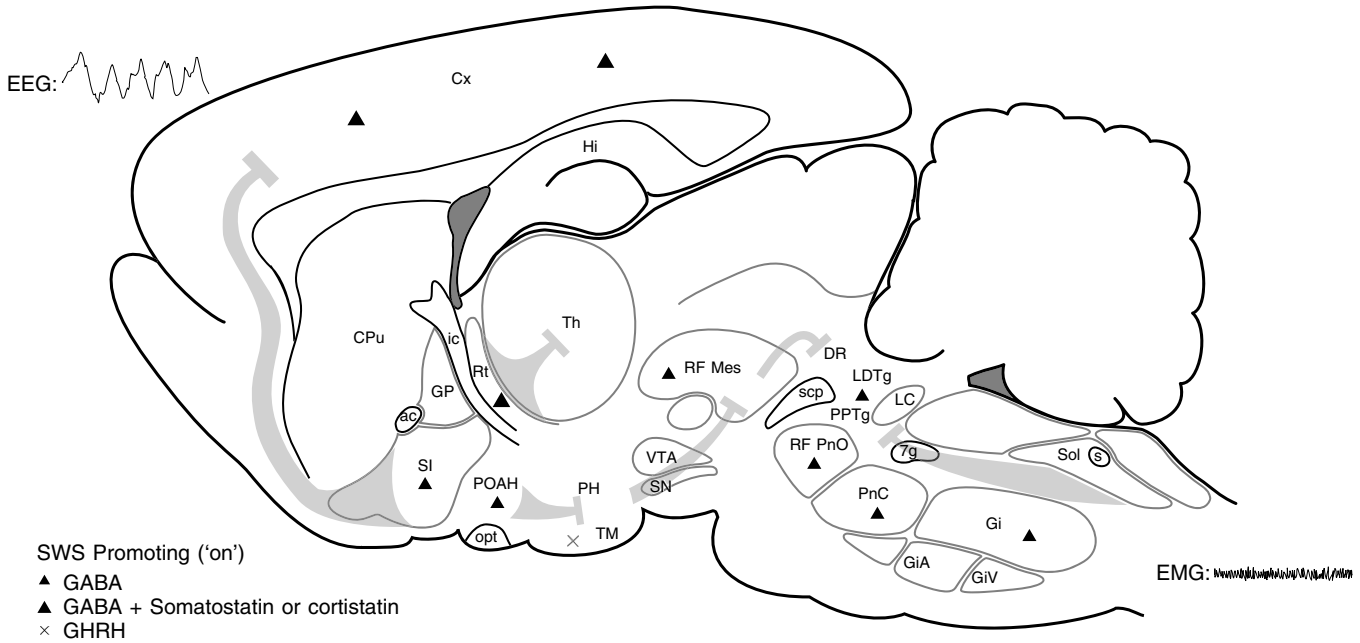

Figure XXIV-2.2 SWS-promoting systems. Schematic sagittal view of rat brain showing the major neuronal systems and their major inhibitory pathways (ending as blocks) involved in promoting the EEG slow-wave activity (upper left) and EMG reduced muscle tone (lower right) characteristic of the slow-wave sleep (SWS) state. Neurons in the region of the solitary tract nucleus (Sol) may exert an inhibitory influence on neurons in the ponto-mesencephalic tegmentum. A dampening influence on the brainstem activating system and the posterior hypothalamus (PH) also emerges from the basal forebrain and preoptic-anterior hypothalamic areas (POAH). From the basal forebrain (substantia innominata [SI]), an inhibitory influence is also exerted upon the cortex (Cx). The major transmitter systems that promote SWS and discharge maximally ('on') during SWS are represented by symbols where their cell bodies are located. These comprise largely GABAergic neurons that, by unit recording and/or c-Fos studies, have been shown to be active during SWS. Particular cortically projecting GABAergic neurons in the SI may have the capacity to dampen cortical activation directly during SWS. Locally projecting GABAergic neurons in the SI may inhibit the cholinergic and glutamatergic cortically projecting neurons. Other GABAergic neurons in the SI and POAH project caudally to the posterior hypothalamus (including the tuberomammillary nucleus [TM]) and brainstem (including RF Mes, DR, LDTg and LC), where they may inhibit multiple wake-active neurons of the activating systems. GABAergic neurons in the reticularis (Rt) nucleus, which surround and innervate the thalamic (Th) nuclei, discharge in bursts to generate spindles while inhibiting the thalamo-cortical projection neurons during SWS. These and local GABAergic neurons in the cortex (Cx) also contain somatostatin or the related peptide corticostatin, which may serve to prolong the inhibition in promoting the slow-wave activity of this state. GABAergic neurons in the brainstem may inhibit local neurons of the ascending reticular activating system as well as those of the descending reticulo-spinal system. Neurons containing growth hormone-releasing hormone (GHRH), primarily located in the arcuate nucleus and projecting to the median eminence, are actively involved in stimulating growth hormone and also promoting slow-wave activity during SWS (See Colour Plate XXIV-2.2)

projection system (Manns *et al.*, 2001). Cortical activation thus depends upon glutamatergic transmission. Glutamate release from the cerebral cortex is highest in association with cortical activation (Jasper *et al.*, 1965). The reticulo-spinal neurons important for increasing muscle tonus, enhancing behavioural responsiveness and stimulating locomotion are also glutamatergic (Grillner *et al.*, 1995).

Drugs that block glutamate transmission by pre- and/or postsynaptic mechanisms (especially the NMDA receptor) comprise some of the anaesthetics, including ketamine (Yamamura *et al.*, 1990) and halothane (MacIver *et al.*, 1996), that produce a loss of consciousness and sensory-motor responsiveness.

Noradrenaline (NA) is a wake- and arousal-promoting transmitter/modulator that is contained in the diffuse projecting locus coeruleus (LC)/subcoeruleus neurons of the pons (Figure XXIV-2.1) and in other hypothalamically projecting and spinally projecting pontine and medullary cell groups. Although lesions of the locus coeruleus nucleus do not result in long-term deficits in cortical activation or waking (Jones *et al.*, 1977), stimulation of the locus coeruleus by electrical or chemical means elicits cortical activation and a vigilant, waking state (Berridge and Foote, 1991). Locus coeruleus neurons discharge at their highest rates during waking, decrease their rate during SWS and cease firing during REMS (Hobson *et al.*, 1975; Aston-Jones and Bloom, 1981b). During waking, they discharge maximally in association with arousal, including attentive and active behaviours, orientation to sensory stimuli, response to painful stimuli and reaction to stressful conditions (Foote *et al.*, 1980; Aston-Jones and Bloom, 1981a; Rasmussen *et al.*, 1986; Abercrombie and Jacobs, 1987a, b). Release of noradrenaline is greatest in association with cortical activation and behavioural arousal, including stress (Nisenbaum *et al.*, 1991; Shouse *et al.*, 2000). In situations of stress, the increased activity of these neurons occurs in parallel with activation of the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis. Through their diffuse projection system that includes the entire forebrain and spinal cord, the locus coeruleus neurons have the capacity to stimulate cortical activation, to activate sympathetic and hypothalamo-pituitary-adrenal systems in the hypothalamus and to excite sympathetic and somatic motor neurons in the spinal cord. Therefore, even though it may not be necessary for maintaining a waking state, the noradrenergic system is important for stimulating a coordinated response to significant sensory stimuli or conditions and thus promoting a maximally attentive and active waking state.

Drugs that diminish noradrenaline, such as alpha-methyl tyrosine (AMT), through inhibition of its synthetic enzyme decrease arousal and enhance both SWS and REMS (King and Jewett, 1971). Drugs that act as agonists at postsynaptic alpha1 receptors

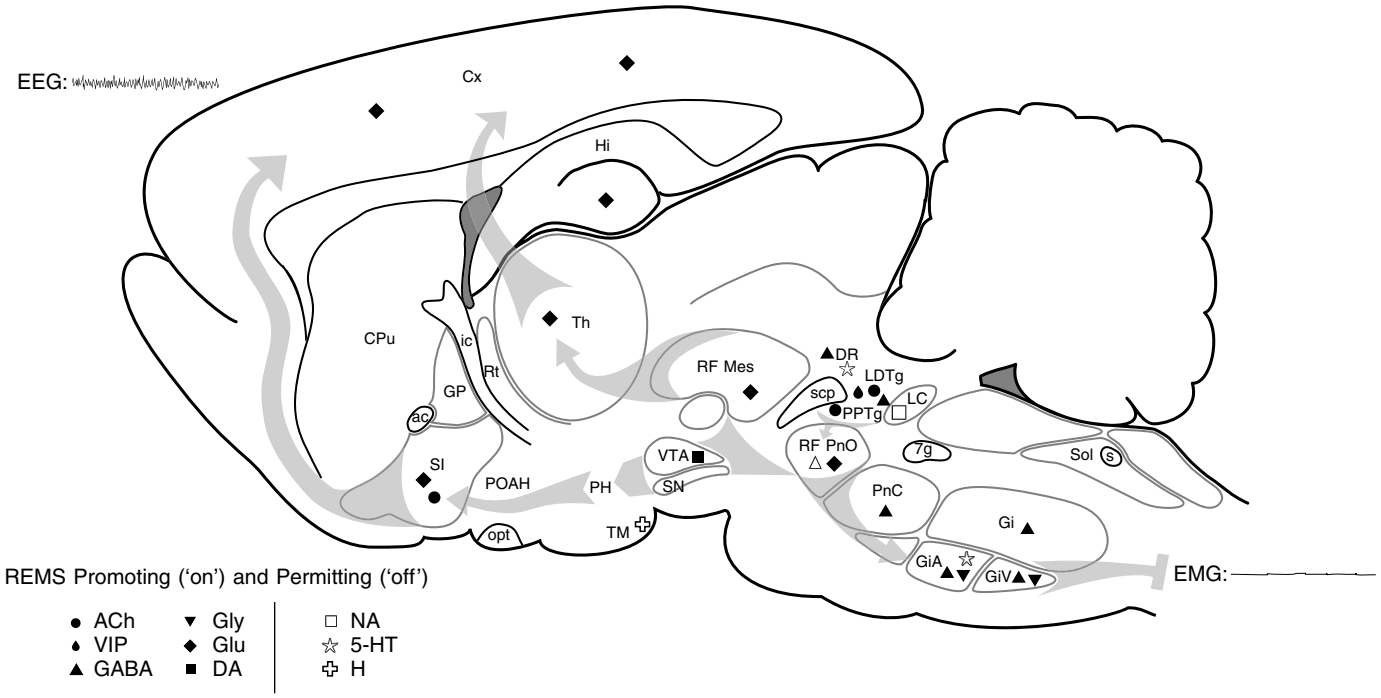

Figure XXIV-2.3 REMS-promoting and REMS-permitting systems. Schematic sagittal view of rat brain showing the major neuronal systems and their (excitatory, ending as arrows, and inhibitory, ending as blocks) pathways involved in promoting the EEG fast activity (upper left) and EMG muscle atonia (lower right) characteristic of rapid eye movement or paradoxical sleep (REMS or PS). Neurons essential for REM sleep are located in the pontine reticular formation, particularly the oral part (RF PnO). The major ascending pathways (indicated by arrows for excitation) promoting cortical activation are similar to those of the waking state; however, the extrathalamic relay into the limbic system may be more important in REMS. The descending motor inhibition (indicated by a block) is triggered by neurons in the oral pontine reticular formation (RF PnO) and partly relayed through neurons in the alpha and ventral gigantocellular fields (GiA and GiV) of the medullary reticular formation en route to the spinal cord. The major transmitter systems that promote REMS and discharge ('on') during REMS are represented by filled symbols, whereas those that are permissive to REMS by stopping their discharge ('off') during the state are represented by empty symbols. Cholinergic neurons that release acetylcholine (ACh) and are located in the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg) are critically involved in promoting REMS through their projections locally into the brainstem reticular formation, and importantly to neurons in the PnO (where they excite glutamatergic neurons and could also inhibit local GABAergic neurons), as well as rostrally into the forebrain. These may be joined in promoting REMS by neurons with similar projections in the region containing vasoactive intestinal peptide (VIP). Noradrenergic (NA) locus coeruleus (LC) and serotonergic (5-HT) dorsal raphe (DR) neurons (as well as histaminergic, H, tuberomammillary neurons, TM) that directly inhibit the cholinergic neurons permit REMS to occur by ceasing their discharge. The arrest of their discharge is effected by local GABAergic neurons, which may be excited by ACh. GABAergic neurons through the caudal pontine (PnC) medullary reticular formation (Gi, GiA, and GiV) may also inhibit local reticulo-spinal and serotonergic raphe-spinal neurons to effect a disfacilitation of motor neurons. In addition, GABAergic and glycinergic neurons in the ventral medullary reticular formation (GiA and GiV) that project to the spinal cord may both directly inhibit motor neurons. One unique group of GABAergic neurons in the PnO ceases discharge during REMS to disinhibit PnO glutamatergic neurons that propagate the ascending and descending correlates of the state. In the ascending pathways, dopaminergic (DA) neurons of the ventral tegmental area (VTA), which are excited by the cholinergic neurons, may be important in activation of the limbic system. The cholinergic basal forebrain neurons (SI and septum) are particularly important in activation of the limbic cortex (hippocampus [Hi]) and neo-cortex (Cx) during REMS (See Colour Plate XXIV-2.3)

stimulate wakefulness. These drugs appear to include modafinil, which stimulates prolonged cortical activation and behavioural arousal, since its effects are blocked by alpha1-receptor antagonists (Duteil *et al.*, 1990; Lin *et al.*, 1992). Through second messengers, alpha1-adrenergic receptors close potassium channels on thalamic and cortical neurons, resulting in their tonic depolarization and the increased excitation that underlies cortical activation (McCormick, 1992). Noradrenaline also acts on alpha2-adrenergic receptors, which commonly open potassium channels through second messengers to hyperpolarize neurons. These receptors are located presynaptically on noradrenergic neurons, as well as postsynaptically on other target neurons. For this reason, drugs that act upon alpha2 receptors are associated with complex, dose-dependent effects upon the sleep-waking cycle. However, the postsynaptic inhibition associated with alpha2 receptors may be important in inhibiting sleep-promoting neurons during waking (see below). Drugs that enhance synaptic levels of noradrenaline through stimulating release and/or blocking reuptake, such as desipramine or amphetamine and possibly also modafinil, enhance arousal, including both cortical activation and muscle tone, and thereby enforce wakefulness, while preventing the onset of both SWS and REMS (Mignot *et al.*, 1993).

Dopamine (DA), like noradrenaline, is principally a wake-promoting transmitter/modulator; however, unlike noradrenaline, it is particularly associated with the pleasurable and rewarding emotional states that may also occur during sleep. Dopaminergic neurons are located in the ventral mesencephalic tegmentum, within the substantia nigra (SN) and ventral tegmental area (VTA) (Figure XXIV-2.1). Dopaminergic neurons of the substantia nigra project predominantly to the striatum, whereas those of the ventral tegmental area project to limbic and medial cortical areas forming the respective nigro-striatal and meso-limbo-cortical projection systems. Lesions of the dopaminergic neurons in the ventral mesencephalic tegmentum result in reduced behavioural arousal; major lesions result in akinesia, along with anorexia and adipsia (Ungerstedt, 1971; Jones *et al.*, 1973). Whereas lesions of the

dopaminergic nigro-striatal neurons decrease locomotion, lesions of the meso-limbo-cortical neurons diminish attentive immobility and simultaneously decrease the high-frequency EEG activity associated with that behavioural state (Galey *et al.*, 1977; Montaron *et al.*, 1982). Lesions have also produced a decrease in REMS (Lai *et al.*, 1999). Surprisingly, however, dopaminergic unit activity has not been found to vary significantly across sleep-wake states when measured as average rate of discharge (Miller *et al.*, 1983; Trulson and Preussler, 1984). Similarly, the average release of dopamine has not been found to vary across the sleep-waking cycle (Trulson, 1985; de Saint Hilaire *et al.*, 2000; Shouse *et al.*, 2000). However, dopaminergic neurons discharge in different patterns such that although continuing at the same average firing rate, they may fire in either a tonic or a bursting pattern. Dopaminergic neurons discharge in bursts during arousing situations and particularly when presented with positively rewarding stimuli or conditions (Mirenowicz and Schultz, 1996). Moreover, dopamine release is greatest during waking when animals are aroused and in positively rewarding situations, including those associated with food and drug reward (Di Chiara and Imperato, 1988; Richardson and Gratton, 1996). Accordingly, dopaminergic neurons would be maximally active by bursting in highly aroused, motivated or rewarding situations during waking. They may also be differentially active during sleep, according to the evidence of a change in pattern of discharge during REMS relative to SWS (Miller *et al.*, 1983). Thus, unlike the other monoamine neurons, they do not cease firing during REMS and may discharge in a manner to contribute to the cognitive correlate of that state, as they do in waking (see below).

Drugs that diminish dopamine through inhibition of its synthesis also diminish noradrenaline, and their soporific effects must accordingly be attributed to the simultaneous depletion of both transmitters. Interpreting the effects of drugs acting upon different dopamine receptors is also difficult because of the presynaptic (D2) and multiple postsynaptic (D1/D2) sites of the receptors. More than EEG activity, dopamine-receptor antagonists most markedly alter behaviour and cognitive state. Neuroleptic drugs that block postsynaptic dopamine receptors, such as pimozide, induce a state of anhedonia (Wise *et al.*, 1978). Drugs that act to enhance synaptic levels of dopamine by blocking reuptake or stimulating release, particularly amphetamine and cocaine, have positively rewarding, pleasurable effects associated with their addictive properties (Di Chiara and Imperato, 1988). These drugs also enhance cortical activation and behavioural arousal (Wisor *et al.*, 2001). Drugs that are used in the treatment of hypersomnolence and narcolepsy, and that accordingly prevent both SWS and REMS, include the amphetamines and modafinil. Although to differing degrees, both these drugs most likely act simultaneously upon dopaminergic and noradrenergic transmission to enforce wakefulness (see above) (Wisor *et al.*, 2001).

Histamine (H) is a wake-promoting transmitter/modulator. The neurons containing histamine are located in the tuberomammillary nucleus (TM) of the posterior hypothalamus (Figure XXIV-2.1) and give rise to diffuse projections through the forebrain. Lesions or pharmacological inactivation of these neurons leads to an acute decrease in waking (Lin *et al.*, 1989; Sakai *et al.*, 1990) and increase in REMS as well as SWS (Sallanon *et al.*, 1988). Like noradrenergic neurons, presumed histaminergic neurons discharge at their maximal rate during waking, decrease their rate during SWS and cease firing during REMS (Sakai *et al.*, 1990).

Drugs that antagonize postsynaptic histamine receptors (particularly H1), the common antihistaminergic drugs used for antiallergy medication, as well as those that occupy the presynaptic autoreceptor (H3), which decrease histamine release, produce somnolence and a decrease in waking and vigilance (Monti *et al.*, 1986; Lin *et al.*, 1988, 1990). Conversely, drugs that act as agonists upon the postsynaptic receptors have the capacity to enforce attentive waking and prevent the onset of SWS (Lin *et al.*, 1988). Through these receptors, histamine blocks potassium channels on target thalamic and cortical neurons and thus stimulates a depolarization of those cells underlying cortical activation (McCormick, 1992; Reiner and Kamondi, 1994).

Serotonin (5-hydroxytryptamine [5-HT]) may promote waking, yet it appears to promote a quiet, satiated waking state that can also facilitate the passage into sleep. It is contained in neurons located in the raphe nuclei within the mesencephalon and pons (including the dorsal raphe [DR]), from which diffuse ascending projections into the forebrain arise, and the medulla, from which descending projections to the spinal cord arise (Figure XXIV-2.1). Lesions of the raphe nuclei have produced complete insomnia in association with a behaviourally agitated state (Jouvet, 1972), leading to the postulate that serotonergic neurons generate SWS. However, electrical stimulation of the midbrain raphe arrested waking behaviours, including eating, although it did not produce sleep (Jacobs, 1973). Moreover, most presumed serotonergic raphe neurons discharge during waking and decrease their discharge during SWS, becoming virtually silent during REMS (McGinty and Harper, 1976; Trulson and Jacobs, 1979). During waking, some presumed serotonergic neurons discharge in association with rhythmic motor activities, such as grooming in cats (Jacobs and Fornal, 1991). Accordingly, they may not generate SWS, although they can promote a quiet waking state that is more conducive to sleep onset. Release of serotonin is also, on average, lower during SWS than during waking (Wilkinson *et al.*, 1991; Portas *et al.*, 1998), but appears to increase during prolonged waking and prior to SWS onset (Python *et al.*, 2001).

Drugs that diminish serotonin levels through inhibition of its synthesis (by *p*-chlorophenylalanine [PCPA]) produce complete insomnia with acute administration (Jouvet, 1972) and a recovery of sleep with chronic administration (Dement *et al.*, 1973). Under both conditions, certain behaviours are increased, including eating, and sexual and aggressive activities, and appear along with a release of the phasic activity (ponto-geniculo-occipital [PGO] spikes) of REMS. The insomnia elicited by PCPA can be reversed with very low quantities of the 5-HT precursor, 5-hydroxytryptophan (5-HTP) (Jouvet, 1972). This effect is also present with local injections of the precursor into the preoptic region (Denoyer *et al.*, 1989). The effects of drugs that act upon individual serotonin receptors are difficult to interpret since they act at multiple presynaptic (5-HT1) and postsynaptic (5-HT1/2/3) sites. Increased synaptic levels of serotonin elicited by the serotonin reuptake inhibitors generally produce relatively quiet waking states, characterized by satiety and thus reduced appetite for food and sex, as well as the psychologically reported reduction in anxiety common in humans (Ursin, 1976, 1980; Sommerfelt and Ursin, 1991; Foreman *et al.*, 1992; Leibowitz and Alexander, 1998; Halford and Blundell, 2000; Kennedy *et al.*, 2000). Like the effects of the precursor, tryptophan, those of the reuptake blockers are best characterized as dampening cortical activation (Ursin, 1980). This effect may be mediated in part by serotonin's inhibitory effect upon cholinergic basal forebrain neurons (see below) through 5-HT1 receptors, which indirectly open potassium channels to hyperpolarize these cells (Khateb *et al.*, 1993). Local microinjections of serotonin into the region of the cholinergic neurons results in the decrease of high-frequency (gamma) cortical activity and the appearance of slow-wave activity with SWS (Cape and Jones, 1998). Through input to the septum, serotonin also dampens theta activity in the limbic system (Vertes *et al.*, 1994). It similarly inhibits cholinergic ponto-mesencephalic neurons that also serve to stimulate cortical activation through their thalamic and other subcortical relay stations (Luebke *et al.*, 1992). In the hypothalamus, serotonin appears to act as a satiety factor in the feeding system (Leibowitz and Alexander, 1998). In the brainstem and spinal cord, serotonin has the capacity to stimulate muscle tone in skeletal muscles (Kubin *et al.*, 1992). Accordingly, serotonin may promote a waking state

during which cortical activation is minimal, and feeding, sexual and aggressive behaviours are dampened, yet rhythmic motor activities and postural muscle tonus are maintained. Serotonergic systems may thus produce a sated, quiet waking state that precedes and facilitates SWS, but prevents REMS (see below).

Acetylcholine (ACh) promotes cortical activation that can be associated with either the state of waking or REMS (see below), as it also stimulates, through brainstem mechanisms, motor inhibition and muscle atonia. Cholinergic neurons are located in the ponto-mesencephalic tegmentum (laterodorsal and pedunculopontine tegmental nuclei [LDTg and PPTg]), as well as the medullary reticular formation, and, like neurons of the ascending reticular activating system, give rise to ascending projections to the thalamus, posterior hypothalamus and basal forebrain (Figure XXIV-2.1). Lesions of the cholinergic ponto-mesencephalic neurons have minimal effects upon waking but eliminate the state of REMS (Webster and Jones, 1988). Another major cholinergic cell group is located in the basal forebrain (substantia innominata [SI] corresponding to the nucleus basalis of Meynert, as well as the septum and diagonal band nuclei) and gives rise to widespread projections to the cerebral cortex (and hippocampus). Lesions of the cholinergic basal forebrain neurons lead to deficits in cortical activation and attention during waking (Stewart *et al.*, 1984; Dunnett *et al.*, 1991). Unit recording of presumed cholinergic neurons in the ponto-mesencephalic tegmentum has indicated that these cells discharge at higher rates during waking than during SWS; most also discharge during REMS and some at higher rates than during waking (El Mansari *et al.*, 1989; Kayama *et al.*, 1992). In the basal forebrain, unidentified but possibly cholinergic neurons discharge at higher rates during waking and REMS than during SWS (Detari *et al.*, 1984; Szymusiak and McGinty, 1986). Release of acetylcholine from both the thalamus and cortex is highest in association with cortical activation and high in both waking and REMS relative to slow-wave sleep (Celesia and Jasper, 1966; Jasper and Tessier, 1971; Williams *et al.*, 1994; Marrosu *et al.*, 1995). During waking, acetylcholine release is high in association with appetitive behaviours, such as eating (Inglis *et al.*, 1994). Acetylcholine release in the hippocampus is highest during REMS (Marrosu *et al.*, 1995); in the brainstem, it is also highest during REMS and the muscle atonia that accompanies that state (see below) (Kodama *et al.*, 1990; 1992).

Drugs that block the muscarinic postsynaptic action of acetylcholine, such as atropine, lead to the loss of fast cortical activity and its replacement by slow waves, despite the persistence of waking behaviours (Longo, 1966; Stewart *et al.*, 1984). This effect is marked behaviourally, nonetheless, by deficits in attention and memory. In addition to affecting glutamate receptors (see above), some anaesthetics, such as ketamine and halothane, appear to block nicotinic receptors (Flood and Krasowski, 2000; Mori *et al.*, 2001). Drugs such as eserine that enhance acetylcholine by blocking its catabolism through the acetylcholinesterase enzyme enhance and prolong cortical activation and diminish cortical slow waves and SWS (Jouvet, 1975; Vanderwolf, 1975). Direct administration of muscarinic agonists into the brain (done of necessity, since most do not cross the blood–brain barrier) also enhances cortical activation. Through second messengers, multiple muscarinic receptors (M1/M3) block potassium channels and lead to prolonged depolarization and increased excitation of their target neurons in the thalamus and cortex (McCormick, 1992). Nicotine also stimulates cortical activation and enhances vigilance (Domino, 1967; Koelega, 1993). Directly linked to sodium ion channels, the nicotinic receptor is associated with rapid depolarization and excitation of target neurons. The muscarinic-nicotinic mixed agonist, carbachol, when injected directly into subcortical sites, stimulates cortical, including limbic, activation accompanied by theta activation, and reduces locomotor activity (Brudzynski and Mogenson, 1986). Such injections can also induce REMS with muscle atonia, particularly when administered into the ponto-mesencephalic tegmentum (see below) (Hernandez-Peon and Chavez Ibarra, 1963; Baxter, 1969; Mitler and Dement, 1974; Amatruda *et al.*, 1975). Acetylcholine thus plays an important role in cortical and limbic activation associated with aroused, attentive, yet also immobile behavioural states, including REMS (see below).

Orexin (Orx), also known as hypocretin, is a wake-promoting neuromodulator that also stimulates eating, for which it was named. It is contained within neurons of the mid- and posterior hypothalamus (PH) that give rise to very diffuse projections through the brain, including the forebrain, brainstem and spinal cord (Figure XXIV-2.1). It appears to have the capacity to enforce waking with muscle tonus since in its absence or that of its postsynaptic receptor, as produced by genetic knockout, narcolepsy results in mice and dogs (Chemelli *et al.*, 1999; Lin *et al.*, 1999). As recently established in human cases, the Orx-containing cells in the hypothalamus are reduced in number with narcolepsy (Thannickal *et al.*, 2000). Orexin acts upon other activating systems including, importantly, the noradrenergic locus coeruleus (Bourgin *et al.*, 2000), histaminergic tuberomammillary (Huang *et al.*, 2001; Bayer *et al.*, 2002) and cholinergic basal forebrain neurons (Eggermann *et al.*, 2001), all of which it excites.

The drug which stimulates waking and vigilance and is used in the treatment of narcolepsy, modafinil, was recently shown by c-Fos immunostaining to activate Orx-containing cells (in addition to other neurons of the activating system) (Estabrooke *et al.*, 2001).

Corticotrophin-releasing hormone (CRH) is a wake-promoting neuromodulator and hormone. It is contained within neurons of the paraventricular nucleus of the hypothalamus (Figure XXIV-2.1) that stimulate release of adrenocorticotrophic hormone (ACTH) from the pituitary. A circadian rhythm of the activity of these neurons is presumably responsible for the circadian rise in human cortisol secretion that occurs in the morning, reaching a maximum upon waking, and more so in the presence of bright light (Leproult *et al.*, 2001). Also contained within other neurons in the hypothalamus and brainstem (not shown) with more widespread projections, corticotropin-releasing hormone may act upon many systems in the brain. Injected into the ventricles of animals, it promotes cortical activation and waking while diminishing slow-wave activity and SWS (Ehlers *et al.*, 1986). It also acts through other transmitter systems, including the locus coeruleus, to promote waking, cortical activation and arousal (Valentino *et al.*, 1993). Such circuits would be highly active during conditions of stress when an attentive waking state is maintained. In addition, however, CRH and ACTH may stimulate REM sleep in association with stress, as evident during REM sleep rebound following deprivation (Marrosu *et al.*, 1990; Gonzalez and Valatx, 1998).

In summary, multiple transmitter/modulator systems play partially redundant yet differentiated roles in promoting and maintaining the cortical activation and behavioural arousal of waking. Glutamatergic neurons, through the reticular activating system, diffuse thalamo-cortical projection system and basalo-cortical system, as well as the cortex, maintain fast excitatory activity through the brain during waking. In the most attentive and aroused conditions, including stress, noradrenergic locus coeruleus neurons, through diffuse projections, promote, maintain and enhance cortical activation and sympathetic and hypothalamo-pituitary-adrenal activation, as well as sensory-motor readiness. Histaminergic hypothalamic neurons, also through diffuse projections, maintain cortical activation and vigilance. These systems work mutually with corticotropin-releasing hormone and orexinergic hypothalamic neurons that serve to enforce a state of waking and prevent the onset of sleep. Serotonergic raphe neurons through diffuse projections also prevent the onset of REMS and loss of muscle tonus associated with that state, but they promote a quiet waking state that precedes and can expedite the onset of SWS. Cholinergic brainstem and basal forebrain neurons that project to the thalamus and cortex stimulate activation

of the limbic cortex and the neo-cortex during waking, but do not oppose and may actually stimulate the loss of muscle tonus that occurs during REMS, when they also promote cortical activation. Dopaminergic mesencephalic neurons may join cholinergic neurons during waking and REMS to stimulate limbic and cortical activation associated particularly with pleasurable states.

SLOW-WAVE SLEEP-PROMOTING TRANSMITTER SYSTEMS

GABA (gamma-hydroxy-butyric acid) is the most common inhibitory amino-acid transmitter in the central nervous system and, as such, is involved in almost all inhibitory processes that occur during different states. It is contained in interneurons through the cortex, hippocampus, thalamus, brainstem and spinal cord. It is also contained in certain long-projection neurons. In addition to suppressing discharge of neurons, GABAergic neurons can pace the activity of neurons in fast or slow rhythms. It should thus be mentioned that GABAergic interneurons and projection neurons are important in contributing to activating gamma and theta activities in the limbic cortex and the neo-cortex (Soltesz and Deschenes, 1993; Whittington *et al.*, 2000). The activity of particular GABAergic neurons is similarly important for pacing slower sleep rhythms while inhibiting activating systems (Figure XXIV-2.2). Through a bursting discharge, GABAergic neurons of the thalamic reticular nuclei play a critical role in the generation of thalamo-cortical spindle activity, by both inhibiting and pacing the thalamic relay neurons in association with the spindles and then slower delta activity through SWS (Steriade and Llinas, 1988). These GABAergic neurons appear to be responsible for closing the afferent gateway through the thalamus to the cerebral cortex during SWS (Hofle *et al.*, 1997). GABA is also contained within long-projection neurons, including those ascending to the cortex from the basal forebrain (Gritti *et al.*, 1997). Such long-projecting GABAergic neurons may be involved in dampening cortical activity in association with cortical slow-wave activity and SWS (Szymusiak and McGinty, 1989; Manns *et al.*, 2000). In addition, GABA is contained in locally projecting neurons within the basal forebrain that may inhibit the cholinergic neurons during SWS (Manns *et al.*, 2000; Szymusiak *et al.*, 2000). It is also contained in neurons projecting to the posterior hypothalamus and brainstem from the basal forebrain and the preoptic-anterior hypothalamic regions (Gritti *et al.*, 1994). It appears from c-Fos expression, in addition to neural firing profiles, that such GABAergic cells are most active with EEG slow-wave activity and SWS (Sherin *et al.*, 1996; Szymusiak *et al.*, 1998; Gong *et al.*, 2000; Manns *et al.*, 2000). Such cells have been identified in the substantia innominata and preoptic region, concentrated particularly in the ventro-lateral preoptic area (VLPO), as well as in the more medial and median preoptic nuclei. These GABAergic neuronal systems serve as gating mechanisms for state changes, since when they are on (closing the gate), neurons in the posterior hypothalamus and brainstem are inhibited, thus allowing sleep; when they are off (opening the gate), neurons in the posterior hypothalamus and brainstem are disinhibited, preventing sleep and promoting waking. Consistent with this view, GABA release is greatest in the posterior hypothalamus during SWS (Nitz and Siegel, 1996), and it is higher in brainstem dorsal raphe and locus coeruleus regions during SWS than waking (Nitz and Siegel, 1997a, b). Collectively, the preoptic GABAergic neurons form an integral part of the regulatory system for autonomic and hypothalamo-pituitary function, inhibiting sympathetic and hypothalamo-pituitary adrenal activity, while dampening the cortical activating system.

GABAergic neurons in the VLPO have been shown to be inhibited by noradrenaline (presumably through an alpha2-adrenergic receptor that opens potassium channels) (Gallopin *et al.*, 2000). Thus, in the absence of noradrenergic input, GABAergic preoptic area neurons may serve to decrease temperature, lower metabolism and decrease blood pressure while dampening activating systems and bringing on sleep. GABAergic neurons are also distributed within the reticular formation among the more numerous glutamatergic neurons (Jones, 1995) and among or around the cholinergic and noradrenergic neurons of the brainstem, from where they may exert a local inhibitory action to dampen the activity of these neurons at sleep onset and during SWS, as well as more markedly during REMS (see below). Local GABAergic neurons may inhibit neurons in the lower brainstem reticular formation to dampen the excitatory reticulo-spinal influence, resulting in the decrease in muscle tonus evident in the EMG during that state. Whereas many GABAergic neurons in the brainstem may be active during both SWS and REMS, others (such as those in the pontine reticular formation) may become inactive during REMS (see below).

Drugs that enhance GABAergic transmission include the major anaesthetic agents, such as pentobarbital (Schulz and Macdonald, 1981), and hypnotic drugs, including the benzodiazepines and benzodiazepine-like drugs (Mendelson, 1985; Smith, 2001). Most of these drugs act via $GABA_A$ receptors, which hyperpolarize and inhibit neurons through opening chloride channels. Whereas barbiturates and other anaesthetic agents directly stimulate this receptor, the benzodiazepines amplify the action of GABA upon the receptor, thus enhancing and prolonging its natural effect in the circuits within which it is released. Accordingly, benzodiazepines may serve to facilitate SWS by facilitating GABA's action within particular circuits that dampen cortical activation. Although the hypnotic drugs in this class increase SWS, they do not increase delta activity but instead increase spindling (Feinberg *et al.*, 2000).

Interestingly, in a case of idiopathic recurring stupor, the blocking of $GABA_A$ receptors could reverse the stupor and normalize the EEG, which was characterized by 14-Hz activity (Tinuper *et al.*, 1992). Gamma hydroxybutyrate (GHB) is another drug that facilitates delta SWS along with REMS within the natural sleep cycle (Broughton and Mamelak, 1980; Lapierre *et al.*, 1990). It restored sleep in a case of fatal familial insomnia (Reder *et al.*, 1995). It appears to act upon $GABA_B$ receptors that hyperpolarize neurons through second messengers that open potassium channels (Williams *et al.*, 1995). Transmitters or their agonists that open potassium channels allow a deeper hyperpolarization (to $\sim -90\,mV$) than those that open chloride channels (to $\sim -70\,mV$), perhaps explaining the enhancement of slow, delta activity with GHB. GHB, as well as other drugs acting upon $GABA_A$ receptors, such as clonazepam, also serve to diminish muscle tone during sleep (see below).

Somatostatin and cortistatin are related peptides that are colocalized with GABA in many neurons within the brain, including those of the reticular thalamic nucleus and the cerebral cortex (Figure XXIV-2.2) (Schmechel *et al.*, 1984; de Lecea *et al.*, 1997), and may be important in inhibiting through those neurons other thalamo-cortical systems, and promoting slow-wave activity during SWS (de Lecea *et al.*, 1996). Since somatostatin inhibits release of most hormones/transmitters, it could act to dampen the release of other transmitters with activating influences in multiple areas. Nonetheless, it should be kept in mind that somatostatin release in the hypothalamo-pituitary-somatic axis is specifically turned off during sleep to allow the SWS-dependent release of growth hormone.

Growth hormone-releasing hormone (GHRH) facilitates SWS (Ehlers *et al.*, 1986; Obal *et al.*, 1996; Zhang *et al.*, 1999). The neurons containing growth hormone-releasing hormone are located principally in the arcuate nucleus, from where they project to the median eminence (Figure XXIV-2.2). Together with the decrease in somatostatin release, growth hormone-releasing hormone is responsible for the increase in human growth hormone release from the pituitary that occurs in association with cortical slow-wave activity

at night during SWS (Van Cauter *et al.*, 1998). Growth hormone-releasing hormone is also contained in other neurons within the hypothalamus (not shown) to provide an innervation to other hypothalamic and preoptic nuclei. Intraventricular administration of the releasing hormone enhances slow-wave activity and SWS in animals (Ehlers *et al.*, 1986). Peripheral administration of a long-lasting analogue of somatostatin, which centrally blocks growth hormone release, decreases SWS, indicating that the releasing factor and the hormone itself may normally promote slow-wave activity and SWS through central mechanisms (Beranek *et al.*, 1997).

Adenosine may facilitate SWS (Benington *et al.*, 1995). It is the by-product of ATP catabolism as well as formation, and it is not associated with any particular group of neurons. Released with most synaptic vesicles, it is potentially released from most active nerve terminals. Acting through second messengers upon receptors (A1) that open potassium channels, adenosine has the capacity to hyperpolarize and inhibit neurons and nerve terminals. Like GABA, it could accordingly function within specific circuits to promote sleep. Its release is increased with prolonged waking episodes (Porkka-Heiskanen *et al.*, 1997).

The drug that is very well known to promote wakefulness and enhance vigilance (Koelega, 1993), caffeine, acts as an antagonist upon adenosine receptors, supporting the notion that adenosine normally acts to promote somnolence and sleep (Yanik *et al.*, 1987).

Insulin, cholecystokinin (CCK) and bombesin can facilitate SWS. These modulators/hormones are released peripherally in association with food intake and digestion. They may act upon the brain to promote SWS through transmission of impulses by the vagal nerve to the solitary tract nucleus or through access to the brain by circulation within regions of the brainstem and hypothalamus that are outside the blood–brain barrier (Danguir and Nicolaidis, 1984; DeMesquita and Hershel Haney, 1986; de Saint Hilaire-Kafi *et al.*, 1989). Cholecystokinin and bombesin are also contained in multiple central neurons in the brain (not shown), where they may also act in association with feeding. Their intraventricular administration facilitates sleep. Collectively, they mediate the postprandial satiety that facilitates sleep onset.

In summary, multiple transmitter/modulator/hormonal systems play differential roles in facilitating the onset of SWS or in generating and maintaining it. Whereas sleep onset is prevented by those systems stimulating the sympathetic and hypothalamo-pituitary-adrenal axis, as during conditions of strong motivational states or stress (see above), it is facilitated by those systems activating the parasympathetic and hypothalamo-pituitary-somatic axis, as during conditions of satiety. Insulin, cholecystokinin and bombesin released with food intake and digestion all facilitate sleep onset. In this manner, serotonin associated with low motivational states, satiety and quiescence may also facilitate sleep onset (see above). Adenosine, which may accumulate in the region of the most active neurons during waking, may selectively inhibit those neurons after prolonged waking to induce sleep. Ultimately, specific GABAergic neurons through the brainstem reticular formation, thalamus and hypothalamus/preoptic area, and basal forebrain are responsible for inhibiting activating and sympathetic systems, as well as enabling the slow rhythms within thalamo-cortical systems that characterize mammalian SWS. Colocalized with GABA in certain thalamic and cortical neurons, somatostatin/corticostatin may enhance this process.

REMS-PROMOTING AND REMS-PERMITTING TRANSMITTER SYSTEMS

Acetylcholine (ACh) is a REMS-promoting transmitter. The neurons containing acetylcholine within the ponto-mesencephalic tegmentum (Figure XXIV-2.3) are critically involved in the generation of REMS, as is evident by its loss following their destruction (Webster and Jones, 1988). In addition to projecting to the forebrain, they provide a dense innervation to the brainstem reticular formation, and particularly to the adjacent oral pontine reticular formation (RF PnO) (Jones, 1991). Electrophysiological studies have established that putative cholinergic neurons in this region discharge in both waking and REMS, and many discharge at their highest rates during REMS (El Mansari *et al.*, 1989). By the use of c-Fos expression as an indicator of neuronal activity, immunohistochemically identified cholinergic neurons were revealed to be maximally active as a population during REMS (Maloney *et al.*, 1999). Acetylcholine release is also maximal during REMS within the brainstem reticular formation (Kodama *et al.*, 1990; Kodama *et al.*, 1992). The cholinergic neurons of the basal forebrain can also promote REMS (Reid *et al.*, 1998; Cape *et al.*, 2000). Moreover, acetylcholine release from the hippocampus is maximal during REMS (Marrosu *et al.*, 1995).

Drugs that enhance acetylcholine levels by blocking its catabolism, such as eserine, promote waking under normal circumstances (see above). However, following depletion of the monoamines by reserpine (see below), eserine stimulates the appearance of REMS (Karczmar *et al.*, 1970). It can also stimulate REMS in depressed patients, who are believed to have increased pressure for REMS, perhaps due to enhanced cholinergic and/or deficient monoaminergic transmission (Sitaram *et al.*, 1976; Gillin and Sitaram, 1984). Carbachol, the muscarinic-nicotinic agonist, when injected into the oral pontine reticular formation (RF PnO), can trigger the state of REMS, including limbic theta activity, phasic oculomotor activity and tonic muscle atonia (Baghdoyan *et al.*, 1987; Vanni-Mercier *et al.*, 1989; Vertes *et al.*, 1993). It presumably mimics acetylcholine there, which is normally released by a rich fibre plexus therein emanating from the ponto-mesencephalic cholinergic neurons. Carbachol's effect, presumably like acetylcholine's, appears to depend upon nicotinic receptors and probably multiple muscarinic receptors, of which the M1/M3, which commonly indirectly close potassium channels, are thought to be important in REMS induction through direct excitation of particular reticular neurons (Velazquez-Moctezuma *et al.*, 1990; Sakai and Onoe, 1997). However, M2 receptors, which may indirectly open potassium channels to hyperpolarize neurons, could be important in selectively inhibiting certain neurons that would otherwise prevent the occurrence of the state and associated muscle atonia (see below).

Noradrenaline, serotonin and histamine (NA, 5-HT and H) play permissive roles in the occurrence of REMS (Figure XXIV-2.3). The importance of this role was first documented in the pharmacological studies (see above) showing that eserine could stimulate REMS, but only under conditions when the monoamines were depleted by reserpine (Karczmar *et al.*, 1970). It was subsequently discovered that the serotonergic neurons (McGinty and Harper, 1976), then found that the noradrenergic neurons (McCarley and Hobson, 1975), and more recently also found that the histaminergic (Vanni-Mercier *et al.*, 1984) neurons cease firing prior to and during REMS. It was proposed that the monoaminergic and cholinergic neurons discharge in a reciprocal manner across the sleep-waking cycle (McCarley and Hobson, 1975). Such a reciprocal relationship is apparent in c-Fos patterns of activation occurring in identified serotonergic and noradrenergic as opposed to cholinergic neurons in association with REMS deprivation as opposed to recovery (Maloney *et al.*, 1999). Since the cessation of firing by the noradrenergic, serotonergic and histaminergic neurons appears to be important for the occurrence of REMS, it is considered that they serve a permissive role in this state. The more recent evidence that serotonin and noradrenaline both hyperpolarize and inhibit the cholinergic ponto-mesencephalic neurons (through 5-HT1 and alpha2 receptors) lends further support to this thesis, indicating that these systems would normally exert an inhibitory influence

upon REMS-promoting cholinergic neurons (Luebke *et al.*, 1992; Williams and Reiner, 1993; Thanker *et al.*, 1998). Both noradrenaline and serotonin are also important for the maintenance of muscle tone characteristic of waking and thus prevention of the loss of muscle tone that occurs with REMS.

Drugs that enhance synaptic noradrenaline or 5-HT, such as amphetamines and tricycles, prevent the occurrence of REMS and associated loss of muscle tonus, as applied in their use in the treatment of narcolepsy (Noising and Mignot, 1997).

Dopamine (DA) is neither clearly REMS-permissive nor REMS-promoting as a transmitter. That dopaminergic neurons continue to discharge (Miller *et al.*, 1983) and dopamine to be released during REMS (see above) suggests that dopamine, in contrast to noradrenaline, does not prevent the occurrence of REMS. Since the dopaminergic neurons in the ventral mesencephalic tegmentum receive an excitatory input from the cholinergic ponto-mesencephalic neurons, which are active during REMS, it is not surprising that dopaminergic neurons are also active during REMS. Recent results from c-Fos studies indicate that immunohistochemically identified dopaminergic neurons of the ventral tegmental area are indeed highly active during REMS, such that they could be discharging in a manner during this state similar to that during highly motivated or rewarding waking states (see above) (Maloney *et al.*, 2002). Accordingly, the dopaminergic neurons could be responsible for some of the ascending components of REMS, including limbic and cognitive changes that could accompany dreaming (Maloney *et al.*, 2002) (Figure XXIV-2.3). The role of dopamine in hallucinations, to which dreams have often been likened, might reflect similar processes underlying these cognitive states (Yeoman, 1995).

Most drugs, like the amphetamines, that enhance dopamine release, as well as noradrenaline and serotonin release, stimulate waking and arousal (see above), and not REMS. However, reports of increased vivid dreams and nightmares, along with increased incidences of hallucinations and psychosis, have been reported with amphetamine and also in the treatment of Parkinsonism with l-DOPA (Moskovitz *et al.*, 1978; Thompson and Pierce, 1999).

GABA serves as a critical transmitter in gating the state of REMS in the brainstem and probably contributing to the muscle atonia of REMS there and in the spinal cord (Figure XXIV-2.3) (Maloney *et al.*, 2000). Many GABAergic neurons appear from c-Fos to be active during REMS, including those surrounding the monoaminergic cells, which they probably are responsible for inhibiting during REMS (Gervasoni *et al.*, 1998; Maloney *et al.*, 1999). Consonant with this action, GABA release is maximal during REMS in the locus coeruleus and dorsal raphe (Nitz and Siegel, 1997a, b). Active GABAergic neurons through the brainstem reticular formation may contribute to muscle atonia indirectly by inhibiting excitatory inputs from pontine and medullary reticulo-spinal and serotonergic raphe-spinal neurons to motor neurons, and thus effecting a disfacilitation of the motor neurons (Maloney *et al.*, 2000). Furthermore, spinally projecting GABAergic neurons in the ventral medullary reticular formation (alpha and ventral gigantocellular fields [GiA and GiV]) may contribute directly to the inhibition of spinal motor neurons. A unique group of GABAergic neurons located in the oral pontine reticular formation (PnO) appear to be less active (or 'off') during REMS relative to SWS (Maloney *et al.*, 2000). Injections of bicuculline into this region were shown to trigger a state of REMS (Xi *et al.*, 1999). In this area, GABAergic neurons may thus act as a gate that, when open (GABA neurons off), allows REMS to occur, but, when closed (GABA neurons on), prevents the occurrence of REMS by inhibiting local reticular neurons that drive REMS. These GABAergic cells could in turn be inhibited by cholinergic neurons or other ponto-mesencephalic GABAergic neurons during REMS.

Drugs that enhance GABA transmission through $GABA_A$ receptors (see above), such as clonazepam, are used in the treatment of REMS behaviour disorder to improve the deficient motor inhibition underlying that condition (Mahowald and Schenck, 1989). Gamma hydroxybutyrate (GHB), which serves as an agonist on the $GABA_B$ receptor (see above), has been used to enhance REMS with muscle atonia, in addition to SWS, during the night in narcoleptic patients in order to diminish narcoleptic attacks during the following day (Broughton and Mamelak, 1980).

Glycine (Gly) is responsible (probably together with GABA) for the postsynaptic inhibition of cranial and spinal motor neurons during REM sleep (Figure XXIV-2.3) (Chase *et al.*, 1989). Together with GABA, it is the other major inhibitory neurotransmitter within the spinal cord. It is also contained in neurons within the medullary reticular formation (alpha and ventral gigantocellular fields [GiA and GiV]) that project to the spinal cord. The drug that blocks glycine, strychnine, can reverse the motor inhibition during REM sleep when applied locally in the region of the motor neurons (Chase *et al.*, 1989).

Glutamate (Glu) plays an important role in the excitation and excitatory action of neuronal systems involved in REMS (Figure XXIV-2.3). It is contained in the majority of neurons within the brainstem reticular formation (above) and in those neurons within the oral pontine reticular formation (PnO) that appear to be critically involved in the generation of REMS and muscle atonia. Glutamate agonists can stimulate REMS when injected into the ponto-mesencephalic tegmentum (Onoe and Sakai, 1995).

Vasoactive intestinal peptide (VIP) is a peptide with REMS-promoting activity, as, when injected into the pontine reticular formation in regions where carbachol stimulates REMS, it can also do so (Riou *et al.*, 1982; Bourgin *et al.*, 1997). Often a cotransmitter with acetylcholine, and located in the region of the cholinergic neurons in the ponto-mesencephalic tegmentum (Figure XXIV-2.3), VIP may act upon similar systems in a more prolonged manner.

In summary, multiple transmitter/modulator systems participate by a particular progression and constellation of events in the generation of REMS. First, the activity of multiple wake-promoting systems is progressively dampened during the SWS that normally precedes REMS. This dampening is effected by particular inhibitory GABAergic neurons that must increase their activity to effect a complete inhibition of noradrenergic, serotonergic and histaminergic cell groups in order to permit the onset of REMS. Lifted from the inhibitory influence of noradrenergic and serotonergic neurons, cholinergic ponto-mesencephalic neurons (and vasoactive intestinal peptide neurons) increase their discharge. Perhaps by a direct inhibitory influence of acetylcholine or by an indirect one through an excitatory influence of acetylcholine on other inhibitory interneurons, specific GABAergic neurons in the oral pontine reticular formation are turned off, opening a gate for the occurrence of REMS through the activity of the oral pontine reticular neurons that release glutamate. These neurons are also directly excited by acetylcholine. Through selective inhibition and excitation of particular reticular neurons, ascending pathways for cortical activation and descending pathways, including glycinergic as well as GABAergic neurons, for motor inhibition are activated. One important ascending pathway may involve dopaminergic neurons that would contribute to the limbic activation and special cognitive components underlying dreaming.

In conclusion, multiple neurotransmitter systems are involved in redundant and interactive ways in promoting waking, SWS and REMS. Glutamate, as the basic excitatory transmitter of the activating system, as well as its forebrain and spinal targets, is critical to the waking state, while noradrenaline, dopamine, histamine, serotonin, acetylcholine and orexin modulate the activating system and

its targets in ways that enhance and prolong but also shape particular waking behaviours and motivational states. GABA, as the basic inhibitory transmitter through the forebrain and brainstem, is critical for SWS and acts through particular neurons in the pacing as well as inhibiting of projection neurons of the activating system and its targets. GABA is also critically involved in REMS, effecting the inhibition of noradrenergic, serotonergic, and histaminergic neurons, which by their cessation permit the occurrence of the state. Released from monoaminergic inhibition, cholinergic neurons by their activity promote REMS, and in this process they excite glutamatergic oral pontine reticular neurons, which are also released from GABAergic inhibition, to propagate the unique ascending forebrain activation and descending motor inhibition of that state.

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XXIV-3

Neuroendocrinology of Sleep Disorders

Axel Steiger

INTRODUCTION

The two major methods for the investigation of human sleep are the sleep electroencephalogram (EEG) and the collection of nocturnal hormone profiles. The combination of these electrophysiological and neuroendocrinological methods in young and elderly, female and male normal control subjects; in patients with psychiatric, endocrine and sleep disorders, under baseline conditions and after administration of synthetic and endogenous central nervous system (CNS) active compounds, particularly neuropeptides and neuroactive steroids; and in related animals models has shown that, firstly, during sleep a considerable activity of various endocrine systems occurs, and, secondly, a bidirectional interaction exists between the electrophysiological and neuroendocrine components of sleep. The aim of this chapter is to summarize the current knowledge in this field, which is thought to be the prerequisite for major achievements in the therapy of sleep disorders and of psychiatric disorders as well.

SLEEP EEG AND SLEEP-ASSOCIATED HORMONE SECRETION IN YOUNG NORMAL HUMAN ADULTS

When subjects spend a night in the sleep laboratory, their sleep EEG is analysed either visually by coding each 30-s interval of the night as a sleep stage according to standard guidelines (Rechtschaffen and Kales, 1968), resulting in a hypnogram (Figure XXIV-3.1), or by EEG spectral analysis (Steiger *et al.*, 1993a; Trachsel *et al.*, 1992). The hypnogram shows the cyclic occurrence of periods of non-REM sleep (NREMS) and rapid-eye-movement sleep (REMS). In young normal subjects during the first period of NREMS, the major portion of slow-wave sleep (SWS) occurs. Correspondingly, in EEG spectral analysis, the major portion of slow-wave activity (SWA) is detected. The amounts of SWS and SWA are relatively low during the second half of the night. During this interval, stage 2 sleep preponderates during NREMS. The mean value for the occurrence of the first period of REMS is about 90 min. This first REMS period is relatively short. During 8 h of night sleep, 3–6 sleep cycles are found. The duration of the REMS periods increases throughout the night. Correspondingly, the amount of REMS is higher in the second half of the night than in the first half.

Near to sleep onset in a rather strict, although not absolute, association with the first period of SWS, the major peak of growth hormone (GH) secretion in 24 h is found (Quabbe *et al.*, 1966; Steiger *et al.*, 1987; Takahashi *et al.*, 1968). This GH surge appears to be widely sleep dependent and is suppressed during sleep deprivation (Beck *et al.*, 1975; Sassin *et al.*, 1969). However, in sleep-deprived, but relaxed young men in a supine position, an unchanged nocturnal GH peak was observed (Mullington *et al.*, 1996). During the second half of the night, GH concentrations are low. The pattern of cortisol secretion is inverse to that of GH. After sleep onset, cortisol reaches its nadir. Between 0200 and 0300, the first pulse of cortisol release occurs, and it is followed by further pulses until awakening (Weitzman, 1976). Adrenocorticotrophic hormone (ACTH) is the prime stimulus of nocturnal cortisol secretion in man. Nevertheless, the secretion of ACTH and cortisol may dissociate (Fehm *et al.*, 1984). In summary, during the first half of the night, SWS, SWA and GH preponderate while ACTH and cortisol levels are low. In contrast, during the second half of the night, the amounts of REMS, ACTH and cortisol are high while SWS and GH secretion are low. This pattern suggests

(1) a reciprocal interaction of the hypothalamo-pituitary-somatotrophic (HPS) and the hypothalamo-pituitary-adrenocortical (HPA) systems (the corresponding peripheral end points are GH and cortisol, respectively)
(2) the existence of common regulators of sleep EEG and sleep-associated hormone secretion.

Indeed, there is now a good evidence that a reciprocal interaction of the key hormones of the HPS and HPA systems, GH-releasing hormone (GHRH) and corticotrophin-releasing hormone (CRH), plays a major role in sleep regulation, as indicated in more detail below.

Prolactin rises after sleep onset and reaches its peak during the second or the last third of the night. In males, testosterone rises constantly throughout the night (Weitzman, 1976). Melatonin secretion is related to the light-dark cycle and has its peak during the early morning (Zhdanova *et al.*, 1997).

The secretion of thyroidea-stimulating hormone (TSH) and of the thyroid hormone thyroxin is related to circadian rhythm (Brabant *et al.*, 1987; Chan *et al.*, 1978). The minimum TSH level is found during daytime. TSH rises during the night and reaches its maximum by midnight. Then the levels decline during the early morning hours. The course of thyroxin release is inverse to that of TSH. Thyroxin levels are low during the night and increase during daytime. One study reported declining TSH levels during REMS periods (Follenius *et al.*, 1988).

The hormone most clearly linked to the NREMS-REMS cycle is renin. Plasma renin activity oscillates throughout the night and reaches its peak during NREMS and its acrophase during REMS (Brandenberger *et al.*, 1988) (Figure XXIV-3.6).

Most sleep-endocrine studies have been done on males. A sexual dimorphism is reported in young normal humans. Cortisol secretion is higher in females than in males. A lower amount of SWS during the second half of the night and a greater decrease in SWS and SWA from the first to the second half of the night were found in young normal women (Antonijevic *et al.*, 1999).

Leptin, the protein product of the obese *(ob)* gene, is released from adipocytes in the periphery. It acts within in the hypothalamus (Elmquist *et al.*, 1997) and reduces food intake, probably by

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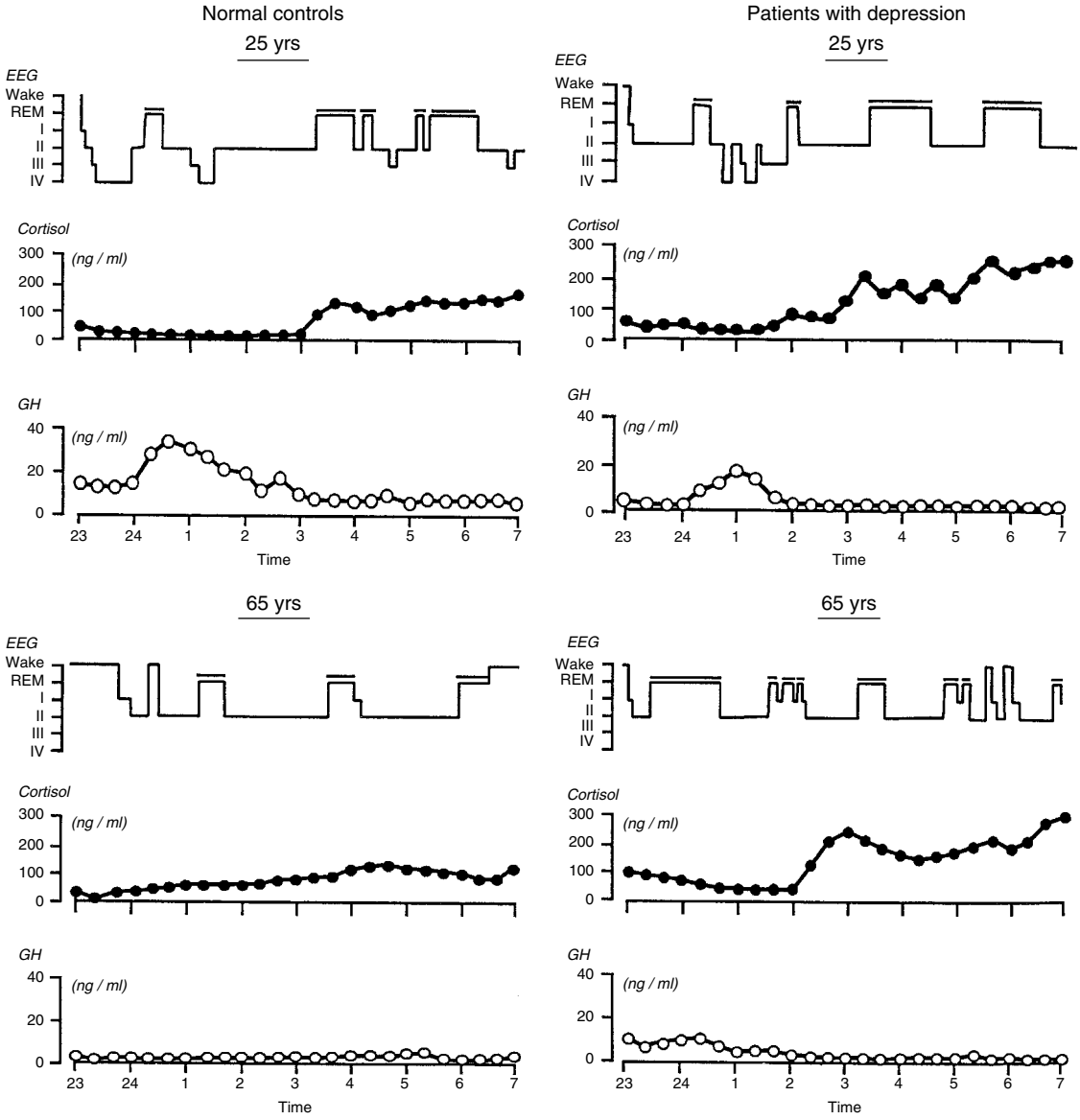

Figure XXIV-3.1 Individual hypnograms and patterns of cortisol and growth hormone (GH) secretion in four male subjects (young and old patients with depression and normal controls)

inhibition of neuropeptide Y (NPY) release (Hanson *et al.*, 1997; Tomaszuk *et al.*, 1996). A circadian rhythm of serum leptin with a maximum between 0000 and 0400, an inverse relationship between leptin and cortisol (Licinio *et al.*, 1997), and a sexual dimorphism with higher leptin levels in women than in men were reported (Deuschle *et al.*, 1996; Saad *et al.*, 1997). Higher nocturnal leptin levels in normal female control subjects than in males were confirmed in a recent study, whereas an inverse correlation between leptin and cortisol was observed in women rather than men (see Figure XXIV-3.2) (Antonijevic *et al.*, 1998).

CHANGES OF SLEEP DURING AGEING

Sleep EEG and nocturnal hormone secretion change throughout the lifespan. There is a controversy whether deterioration of sleep due to ageing starts during puberty or during early adolescence (Bliwise, 1993). It is well established, however, that as early as the third decade of the lifespan, distinct parallel decreases of SWS, SWA and GH secretion start. Near to the onset of the fifth decade, the GH pause occurs. In women, the menopause is a major turning point towards impaired sleep in older age (Ehlers *et al.*, 1993), whereas in men the sleep quality declines continuously during ageing. Correspondingly, in the elderly the amounts of SWS and SWA are low, or SWS may even be totally absent (Figure XXIV-3.1). Furthermore, sleep continuity is disturbed, as is evident from, prolonged sleep-onset latency, increases in the number and duration of nocturnal awakenings, and decreases in sleep efficiency and sleep period time. Finally, REMS time and REMS latency decrease in older age. Controversial reports exist on the effects of age on HPA hormones. Elevated and unchanged cortisol levels have been reported in the elderly. Most studies agree that the amplitude of the cortisol rhythm is blunted. Accordingly, cortisol concentrations during the first half of the night and at the

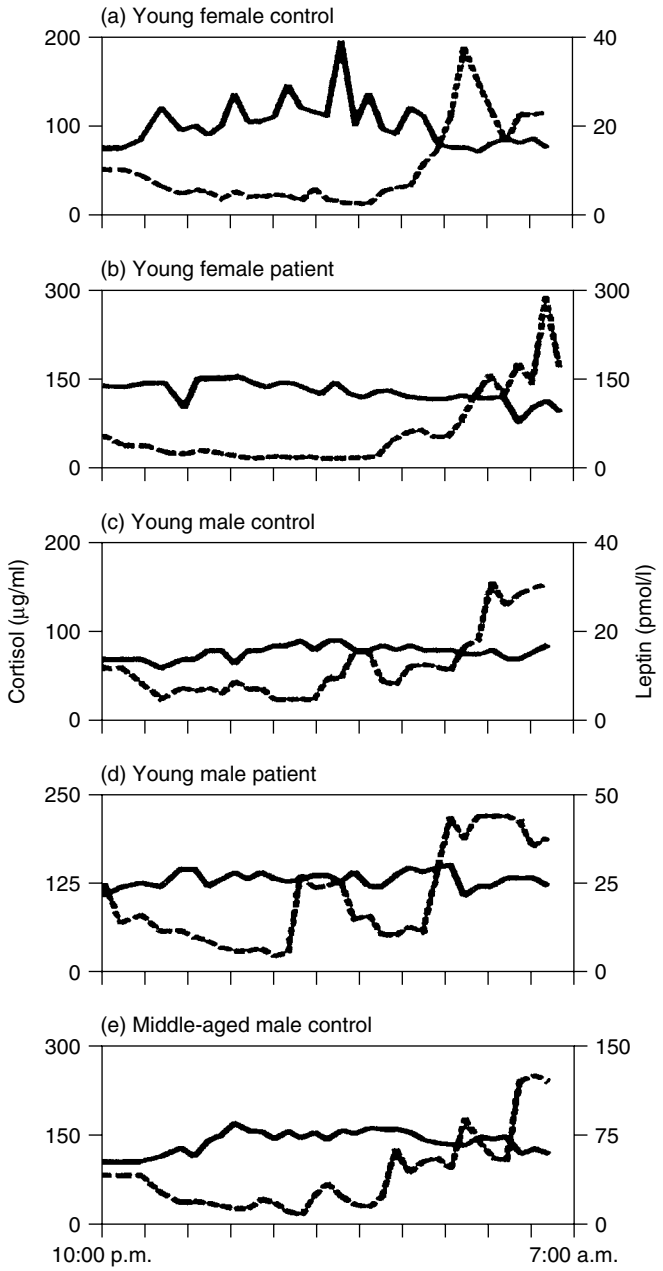

Figure XXIV-3.2 (A–E) Nocturnal profiles of leptin and cortisol. Representative profiles of nocturnal serum levels of leptin and cortisol are shown and correlation analysis between the two hormones was performed: A) young female control ($r = -0.61$, $P < 0.001$); B) young female patient ($r = -0.64$, $P < 0.001$); C) young male control ($r = -0.08$, n.s.); D) young male patient ($r = -0.22$, n.s.); E) middle-aged (42 years old) male control (-0.44, $P < 0.01$). Left y-axis represents plasma cortisol concentrations (dotted line); right y-axis represents serum leptin concentrations (black line) (Reproduced from Antonijevic *et al.*, 1998 with permission of Elsevier Science)

nadir are elevated. Similar results derive from the analysis of the largest sample ($n = 149$) of normal male control subjects (aged 16–83 years) investigated so far (Van Cauter *et al.*, 2000). These authors reported a modest effect of ageing on the 24-h mean cortisol level. Ageing was associated with an elevation of the evening cortisol nadir, but morning maximum cortisol values remained stable across all age ranges. Increases in evening cortisol levels became apparent after the age of 50 years, when sleep became more fragmented and REMS declined. Furthermore, a trend to an association between lower amounts of REMS and higher evening cortisol levels independent of age was found.

Melatonin and, in males, testosterone levels also decrease during ageing. The melatonin levels of older people who reported sleep maintenance problems in a questionnaire did not differ from those of older people reporting normal sleep (Baskett *et al.*, 2001). In contrast to most other hormones, prolactin levels remain widely unaffected by ageing (Van Coevorden *et al.*, 1991).

This complex pattern of age-related changes of sleep-endocrine activity mirrors a central process of ageing with major consequences to mental and physical health. The changes of sleep EEG correspond to a high incidence of insomnia and a high prescription rate of hypnotics in the elderly. A lack of GH is linked to risk of frailty. Elevated HPA activity impairs the capacity to cope with stress and elevates the risk of depression and cardiovascular diseases in higher age.

CHANGES OF SLEEP-ENDOCRINE ACTIVITY RELATED TO DISEASES

Psychiatric Disorders

Depression in Adult Patients

Disturbed sleep is a frequent symptom in most psychiatric disorders. Interestingly, sleep-endocrine changes in patients with depression are similar to those during normal ageing. Characteristically, sleep-EEG findings in depressed patients include disturbed sleep continuity, a decrease of SWS (in younger patients, a shift of the major portion of SWS from the first to the second sleep cycle) and REMS disinhibition (shortened REMS latency, prolonged first REMS period and elevated REMS density, a measure of the amount of rapid-eye movements during REMS) (reviewed by Benca *et al.*, 1992; Reynolds and Kupfer, 1987). Several endocrine aberrations are well documented in affective disorders, particularly HPA overactivity (reviewed in Holsboer, 1999) and HPS dysfunction (reviewed in Steiger *et al.*, 1989). Elevated cortisol and ACTH levels were reported in most sleep-endocrine studies in patients with depression throughout the night or during 24 h, respectively, in comparison to normal control subjects (Antonijevic *et al.*, 2000c; Linkowski *et al.*, 1987) (Figure XXIV-3.1). The circadian pattern of cortisol secretion is preserved during depression. Particularly in females, a positive correlation between age and cortisol levels was found (Antonijevic *et al.*, 2000c). Recently, enhanced cortisol plasma and norepinephrine levels but normal ACTH plasma and CRH cerebrospinal fluid (CSF) levels throughout 30 h were reported (Wong *et al.*, 2000). GH secretion was blunted in most (Jarrett *et al.*, 1990; Sakkas *et al.*, 1998; Steiger *et al.*, 1989; Voderholzer *et al.*, 1993), but not in all (Linkowski *et al.*, 1987) studies. These findings suggest a crucial relationship between shallow sleep, blunted GH and HPA overdrive in depression. In adult patients, ageing and depression exert synergistic effects on sleep-endocrine activity (Antonijevic *et al.*, 2000c). As a result of this synergism, sleep-endocrine changes are most distinct in elderly patients with depression (Figure XXIV-3.1).

As depression is frequently associated with loss of appetite, reduced food intake and weight loss, the secretion of leptin in patients with depression is of interest since leptin is involved in the regulation of food intake. In drug-free patients with major depression, nocturnal serum leptin was significantly higher than in age- and sex-matched controls (Antonijevic *et al.*, 1998). The sexual dimorphism with higher leptin levels in females than in males as described before was confirmed in patients and in controls.

Serum leptin was correlated with body mass index in controls, but not in patients, supporting an altered regulation of leptin secretion in depression. Neither in patients with depression nor male controls was a clear increase in leptin between 0000 and 0400 (as reported by Licinio *et al.*, 1997) or at any other time during the night observed. However, in the group of young female controls (younger than 35 years), an increase in leptin levels between 0000 and 0400 was found, showing the trend to be greater than in young female patients (see Figure XXIV-3.2). This finding suggests that in young female patients with depression the nocturnal leptin surge is blunted. As expected, cortisol levels were enhanced in the patients. As glucocorticoids can prevent the fasting-induced decline of serum leptin, we suggest that hypercortisolism in depression might counteract the reduction in leptin release due to decreased food intake and weight loss. Elevated leptin levels in depression might, in turn, further promote the release of CRH, as shown in animals (Raber *et al.*, 1997; Schwartz *et al.*, 1996), and contribute to HPA system overactivity in depression.

Simultaneous investigation of nocturnal TSH and ACTH levels in drug-free male patients with depression and matched controls showed a blunted TSH and elevated ACTH secretion in the patients. ACTH was negatively correlated to TSH in the first half of the night. These data support the hypothesis that both hypophyseal hormones reflect a common dysregulation of the HPA and the hypothalamo-pituitary thyroid systems in depression, probably as a result of impaired action of the corticotropin-release inhibiting factor (Peteranderl *et al.*, in press).

During antidepressant therapy with tricyclics (amitriptyline, imipramine), a decrease of nocturnal cortisol levels and of REMS and an increase of SWS were found in comparison to baseline values. At this examination, the Hamilton Depression Rating Scale (HAMD) score was improved by about 50% in comparison to the examination before treatment (Steiger *et al.*, 1993b). The effect of subchronic treatment with several antidepressants on sleep EEG and sleep-related hormone secretion was examined in normal male control subjects. The volunteers received first placebo for 3 days, then increasing dosages of antidepressants (the tricyclics amitriptyline, clomipramine and trimipramine, and the selective, reversible, short-acting inhibitors of monoamine oxidase type A, moclobemide and brofaromine for 7–10 days), and finally again placebo after drug withdrawal for 4–8 days (Steiger *et al.*, 1993b). Furthermore, the effects of acute oral administration of the selective serotonin-uptake inhibitor, fluoxetine, and placebo were compared (von Bardeleben *et al.*, 1989). As shown in Table XXIV-3.1, active antidepressant treatment decreased REMS after all substances except trimipramine. Whereas cortisol levels increased after some of the drugs, a distinct blunting of cortisol was observed after trimipramine. After cessation of trimipramine, cortisol levels distinctly exceeded baseline values. It was suggested previously that REMS suppression is the way of action of antidepressants (Vogel *et al.*, 1975). But this hypothesis is challenged by the fact that some antidepressants, including trimipramine, do not suppress REMS. However, normalization of HPA activity appears to be a common mechanism in the action of all antidepressants (Holsboer, 2000; Holsboer and Barden, 1996). This view is supported by the blunting of cortisol after trimipramine, which is known to be an effective antidepressant, although the substance does not share the classical neurobiological effects of most other antidepressants such as REMS suppression, beta-downregulation and inhibition of synaptic uptake of monoamines. In a similar vein, corticosteroid receptor density increased in the rat brain when the animals were treated during several weeks with various antidepressants from several classes, including trimipramine (Reul *et al.*, 1993).

Finally, in a double-blind protocol, the effect of treatment by trimipramine or imipramine on sleep EEG, hormone secretion and psychopathology was compared in patients with depression (Sonntag *et al.*, 1996). Both treatments produced rapid and significant clinical improvement. Imipramine and trimipramine differed, however, in their effects on neurobiological variables. REMS was suppressed and sleep continuity impaired after imipramine, whereas REMS and SWS increased and the sleep quality improved under trimipramine (see Figure XXIV-3.3). Cortisol levels were blunted throughout the night and prolactin was increased after trimipramine, whereas imipramine showed no effect on hormone secretion (see Figure XXIV-3.4).

Sleep-endocrine activity was studied longitudinally between acute depression and recovery in two studies in adult patients. A decrease of ACTH and cortisol throughout 24 h and a normalization of REMS were reported after remission (Linkowski *et al.*, 1987). Since not all of the patients in this study were drug-free at the retest, it is difficult to distinguish between the effects of remission and of antidepressants. Intraindividual comparison of drug-free patients (Steiger *et al.*, 1989) confirmed a decrease of cortisol after remission. In the same study, the prolactin levels of the patients did not differ from those of younger normal controls and remained unchanged after recovery (Steiger and Holsboer, 1997a). Similarly, nocturnal prolactin levels were not distinguishable between patients with depression and matched controls (Jarrett *et al.*, 1987). Furthermore, testosterone levels increased after remission (Steiger *et al.*, 1991). The pathological sleep EEG and blunted GH levels, however, remained unchanged. Sleep EEG showed even a further deterioration, as stage 4 sleep decreased and the number of awakenings increased (Steiger *et al.*, 1989) (see Figure XXIV-3.5). Both studies (Linkowski *et al.*, 1987; Steiger *et al.*, 1989) corroborate that hypersecretion of HPA hormones is a symptom of acute depression in adults.

The decline of cortisol levels after remission (Linkowski *et al.*, 1987; Steiger *et al.*, 1989) is similar to the normalization of results of challenge tests of the HPA system and of CRH CSF levels after recovery (reviewed by Holsboer, 1999). The persistence of most sleep-EEG changes (Kupfer *et al.*, 1993) and of blunted GH levels (Jarrett *et al.*, 1990) in remitted depressed patients has been confirmed over a period of 3 years. Obviously, HPA activity

Table XXIV-3.1 Changes of sleep EEG and nocturnal hormone secretion in normal control subjects after subchronic treatment with antidepressants

				Hormone secretion			
	REMS	NRREMS stages 1 and 2	SWS	Under medication		After withdrawal	
Brofaromine	↓	↑	↓	GH ↓		GH↓	
Moclobemide	↓	ø	ø	Cortisol ↑		Cortisol ↑	
Clomipramine	↓	↑	↓	Cortisol ↑		Cortisol ø	
Amitriptyline	↓	ø	↑	Cortisol ↑	GH ↓	Cortisol ø	GH ↓
Trimipramine	ø	ø	ø	Cortisol ↓	PRL ↑	Cortisol ↑	PRL ↓
Fluoxetine	↓	ø	ø	Cortisol ↑			

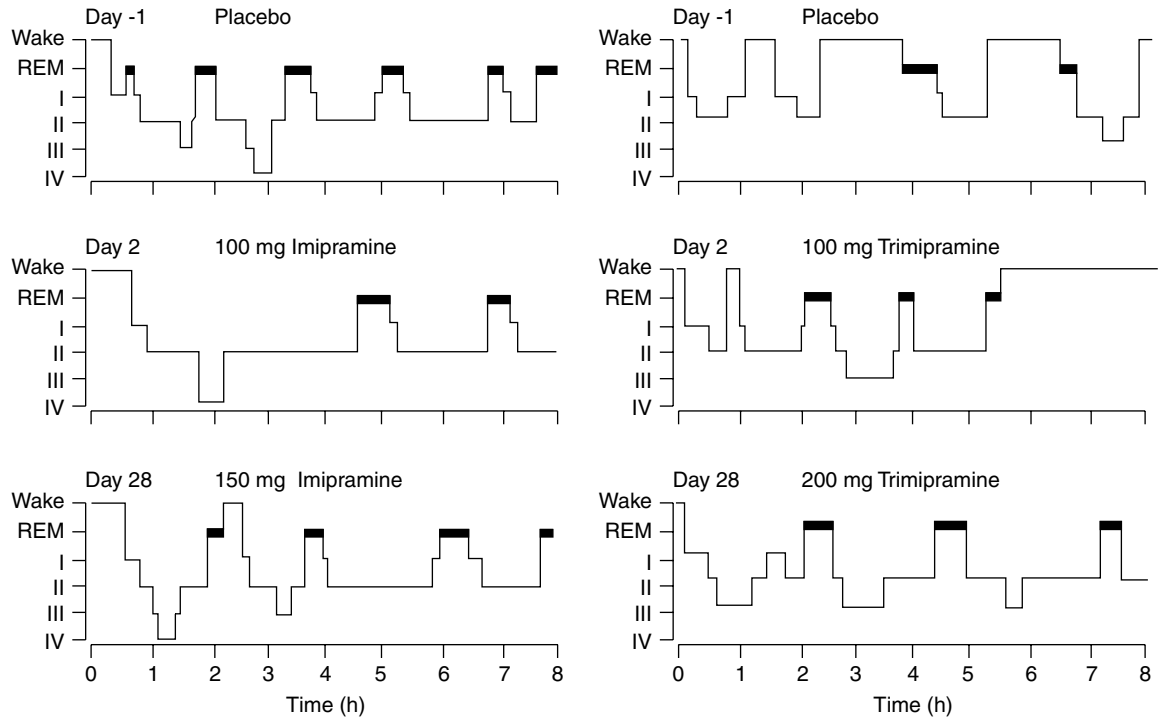

Figure XXIV-3.3 Changes in plasma cortisol concentration during treatment with trimipramine or imipramine (Reproduced from Sonntag *et al.*, 1996. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

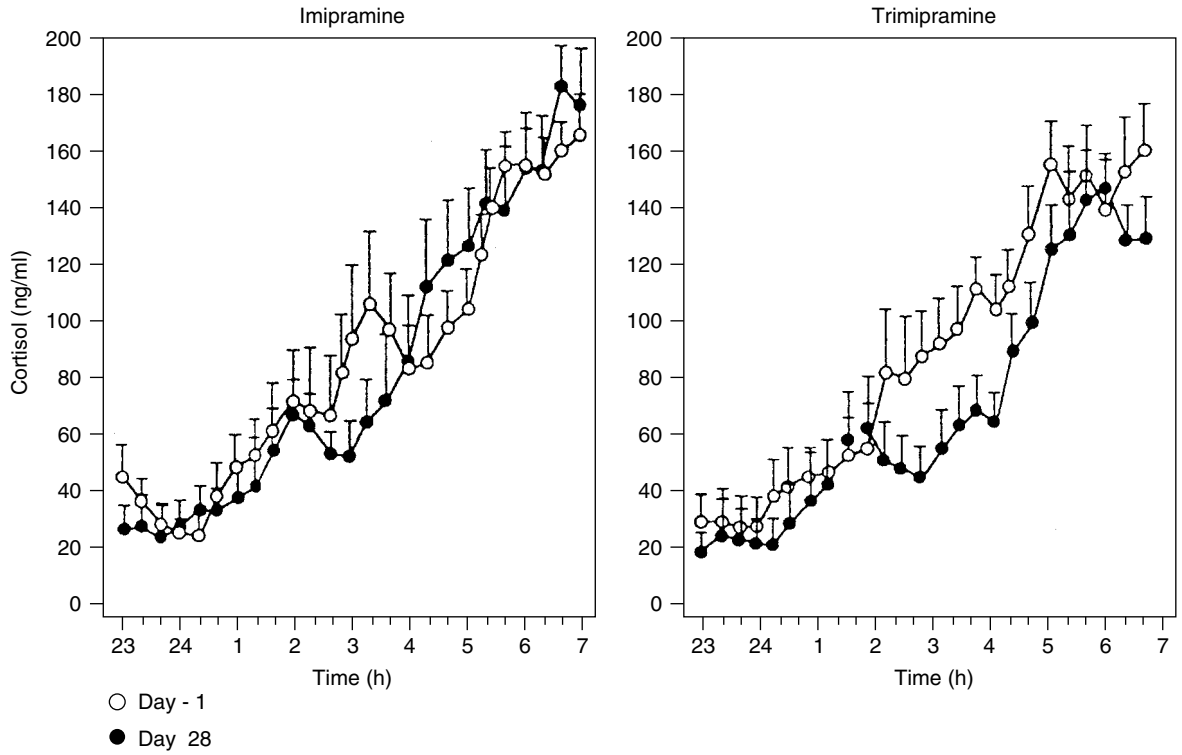

Figure XXIV-3.4 Changes in sleep-EEG variables during treatment with trimipramine or imipramine (Reproduced from Sonntag *et al.*, 1996. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

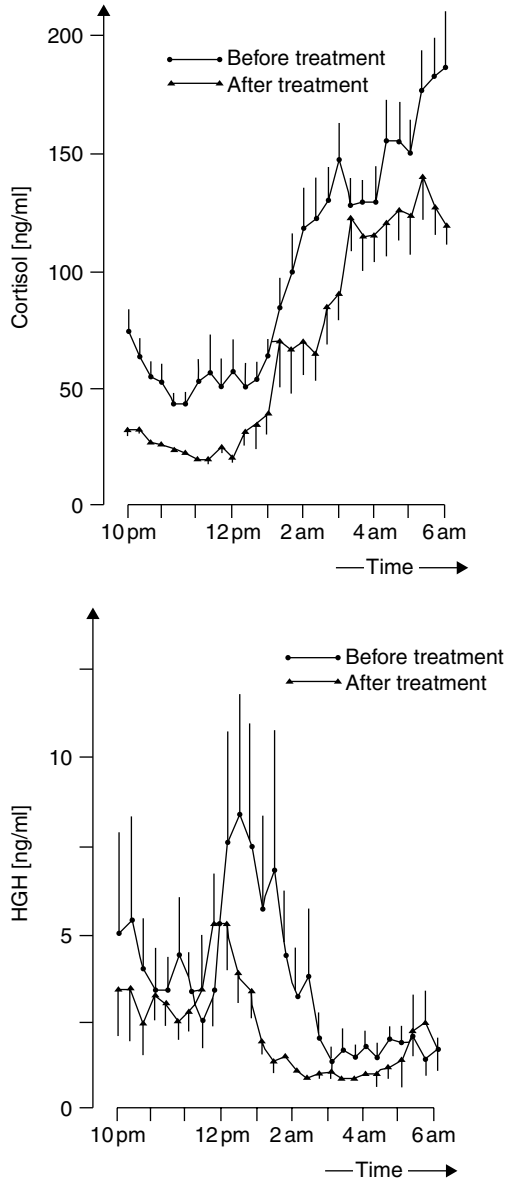

Figure XXIV-3.5 Courses of the mean nocturnal concentrations ± SEM of cortisol and GH in 10 depressed patients before treatment and after remission (Reproduced from Steiger *et al.*, 1989 with permission of Elsevier Science)

normalizes independently of sleep architecture. This observation clarifies that hypercortisolism in depression is not a consequence of shallow sleep. Blunted testosterone levels in males during acute depression, however, appear to be secondary to HPA overdrive. Finally, prolactin is not affected by either depression or ageing. The persistence of aberrations of sleep EEG and GH secretion in remitted patients may be explained as a biological scar due to the metabolic aberrations during acute depression. An alternative explanation is that these changes represent a trait in depressed patients. This issue will remain unclear as long as no data are available for comparing intraindividually the premorbid and depressed state in patients. Interestingly, in healthy relatives of patients with depression, some depression-like neurobiological changes were observed. In the Munich Vulnerability Study, healthy subjects with high familial risk of affective disorders were investigated by assessing the neuroendocrine, polysomnographic and psychometric status. Like patients with an acute episode of major depression, the healthy relatives exhibited signs of HPA system overactivity, as shown by the dexamethasone-CRH test, as well as reduced SWS in the first sleep cycle and increased REMS density. On a single case level, 32% of these high-risk probands exhibited depression-like features in at least two of the three areas assessed (Krieg *et al.*, 2001).

Depression in Children and Adolescents

Most but not all sleep-EEG studies in depressed children and adolescents did not find differences between patients and age-matched controls (reviewed by Lauer *et al.*, 1991). Similarly, changes of sleep-related hormone secretion are more subtle in depressed children and adolescents than in adults. In one study in prepubertal children with depression, sleep EEG and cortisol profiles throughout 24 h did not differ from those of children with other psychiatric disorders and controls. Only four of the 45 depressed children showed hypercortisolism. In a retest after recovery, cortisol hypersecretion persisted in only one of the four young patients (Puig-Antich *et al.*, 1989). In another study, prepubertal depressed children had lower cortisol levels during the first 4 h after sleep onset than controls. ACTH, prolactin and GH concentrations did not differ between patients and controls. Examination of clinical characteristics in patient groups revealed lower nocturnal ACTH in depressed inpatients than depressed outpatients and in depressed, sexually abused than depressed, non-abused children (De Bellis *et al.*, 1996). Depressed children who had experienced at least one clearly adverse, stressful life event during the prior year hypersecreted GH at night compared to depressed children without the experience of such an event and normal controls with and without stressful life events (Williamson *et al.*, 1996). This finding is in line with an elevated responsiveness of GH to CRH in adult patients with depression during daytime (Lesch *et al.*, 1988).

In a set of studies, the relationship between the longitudinal clinical course and sleep-endocrine findings in adolescent patients with depression was studied. In one study on sleep and cortisol, the patients and matched controls were retested about 7 years after the initial study. Although initial group comparisons failed to detect significant differences in sleep-endocrine variables, analyses incorporating clinical follow-up showed that changes in sleep EEG and cortisol are associated with differential longitudinal course. Normal controls who would develop depression had shown initially higher REM density and, by trend, shortened REM latency compared to controls without psychiatric disorder. Depressed patients with a recurrent unipolar course showed elevated cortisol levels around sleep onset compared to depressed patients with no further episodes during the follow-up interval (Rao *et al.*, 1996). Nocturnal GH secretion was studied in a large sample of adolescent depressed patients and matched controls. Clinical follow-up was performed approximately one decade later. The original adolescent nocturnal GH data were analysed in the light of the information obtained regarding clinical course into adulthood. Normal controls who developed at least one episode of major depression or dysthymia during the follow-up period differed from depression-free controls by a more rapid increase of nocturnal GH secretion after sleep onset. Of the subjects who had at least one major depressive episode during the follow-up, those who would go on to make suicide attempts released greater amounts of GH during the first 4 h of sleep. Adults with lifetime depression showed blunted GH in the 100 min preceding sleep onset (Coplan *et al.*, 2000).

Mania and Schizophrenia

Only two studies deal with sleep-endocrine activity in psychiatric disorders other than depression. Elevated cortisol levels were also found in acute mania (Linkowski *et al.*, 1994). Cortisol levels were enhanced in the first 3 h of the night, and prolactin was elevated distinctly around sleep onset in patients with schizophrenia. The sleep latency was prolonged, and the sleep efficiency and the time spent in REMS were reduced (Van Cauter *et al.*, 1991).

Sexual Dysfunction

Blunted sleep-related testosterone levels were reported in males with reduced sexual interest in comparison to normal controls (Schiavi *et al.*, 1986).

Dyssomnias

Experts have generated a host of diagnoses of sleep disorders. So far sleep-endocrine data are available only on some of these disorders. 'Dyssomnia' is defined as a disturbance of initiating or maintaining sleep or as excessive sleepiness (American Sleep Disorders Association, 1997). It should be kept in mind that hypersomnia during the daytime in the same patient can be the consequence of disturbed sleep during the night.

Insomnia

In patients with primary insomnia, elevated nocturnal cortisol levels and a shorter quiescent period of cortisol secretion were found in comparison to controls (Rodenbeck *et al.*, 1999). In a sample of younger adult patients with insomnia, a positive correlation between total wake time and 24-h urinary free cortisol was found (Vgontzas *et al.*, 1998). Furthermore, in a group of 10 patients with insomnia, wakefulness was increased and ACTH and cortisol levels were enhanced throughout 24 h in comparison to controls. The most distinct elevations were observed between 1400 and 1730 and between 2100 and 0300 (Vgontzas *et al.*, 2000). These observations point to an elevated activity of the HPA system in primary insomnia. There appear to be similarities in the pathophysiology of primary insomnia and of depression. This is of particular interest, since several epidemiological studies reported a highly increased risk of the development of a depressive episode in patients with persistent insomnia (Vollrath *et al.*, 1989). Unfortunately, so far, the literature offers no studies that integrate sleep-endocrine data in patients with primary insomnia and with depression.

Sleep Apnoea

Sleep apnoea is a disorder characterized by repetitive upper airway occlusion resulting in progressive hypoxaemia that induces arousals and termination of the obstruction. Consequently, marked sleep fragmentation and decrease of SWS are associated with this disease (Bradley and Phillipson, 1985). Nasal continuous positive airway pressure (CPAP) therapy has been shown to eliminate sleep apnoea and related hypoxaemia (Sullivan *et al.*, 1981). In one study, the effect of nasal CPAP therapy on sleep EEG and hormone secretion was investigated in obese patients with obstructive sleep apnoea syndrome. Under baseline conditions, the time spent in SWS and REMS was low, the number of apnoeas was elevated and the arterial oxygen saturation was reduced. With nasal CPAP therapy, all these variables turned significantly to normalization over one night. Furthermore, GH secretion, which was blunted at baseline, significantly increased on the CPAP night. Cortisol secretion did not differ significantly between both conditions (Cooper *et al.*, 1995). Similarly, cessation of nasal CPAP in eight patients regularly using this device led to an immediate recurrence of sleep apnoea, whereas ACTH and cortisol levels remained unchanged (Grunstein *et al.*, 1996). Krieger *et al.* (1991) compared sleep EEG, plasma renin activity and aldosterone levels in male patients with obstructive sleep apnoea at baseline and during one night with CPAP therapy. In untreated patients, frequent awakenings, absence of SWS, and few and short REMS periods were reported. CPAP therapy led to improvements in sleep depth and REMS. In some treated patients, regular NREMS-REMS cycles occurred; in others, the sleep structure remained irregular. Plasma renin activity profiles reflected the pattern of NREMS-REMS distribution as known from normal controls. Increasing plasma renin activity levels coincided with NREMS phases, and declining levels with REMS. When sleep cycles were regular in some of the treated patients, the oscillating plasma renin activity levels also became regular. Irregularities in sleep EEG, such as short sleep cycles and varying length of sleep-phase duration, led to fluctuations of smaller amplitude and to a general non-oscillatory pattern of plasma renin activity. The mean levels of plasma renin activity and aldosterone were significantly enhanced by nasal CPAP treatment.

Narcolepsy

Narcolepsy is thought to be a disorder of REMS regulation. Accordingly, patients with narcolepsy show several abnormalities of their sleep-wake organization throughout 24 h and of the internal sleep structure. Prominent symptoms are cataplexy and excessive daytime sleepiness, which often results in sleep attacks. The sleep EEG of these patients frequently shows sleep-onset REMS episodes, e.g., the occurrence of REMS directly at or during the first 10 min after sleep onset, frequent awakenings and REMS fragmentation. Normal cortisol and melatonin secretion was reported in narcoleptic patients. Blunted prolactin levels were found, and studies on GH release are contradictory, reporting either high or blunted secretion (reviewed by Schulz *et al.*, 1992). Schulz *et al.* (1992) investigated 24-h plasma renin activity and sleep EEG in patients with narcolepsy and in normal control subjects. The mean concentrations of plasma renin activity was similar in patients and controls. The individual hormone profiles reveal that the already mentioned association between renin oscillations and the NREMS-REMS cycle was preserved. Plasma renin activity profiles exactly reflected the irregularities and disturbances in the sleep structure of the patients with narcolepsy (see Figure XXIV-3.6). In contrast to controls, in these patients no upward trend of plasma renin activity was found. The authors argued that this phenomenon is probably induced in the control subjects by the repetitive occurrence of longer episodes of NREMS. Due to marked sleep fragmentations in the narcoleptic patients, the duration of NREMS was often insufficient to stimulate plasma renin activity. This hypothesis is supported by the more recent finding that SWA stimulates plasma renin activity (Luthringer *et al.*, 1995). In accordance with this observation, sleep-onset REMS episodes that are not preceded by NREMS are not accompanied by relative decline of plasma renin activity.

In patients with narcolepsy, leptin levels were examined in CSF and serum from a blood sample collected in the morning. When these were compared to two control groups of patients with depression and patients with a non-inflammatory neurological disorder, respectively, leptin serum levels were blunted, whereas CSF levels did not differ significantly between groups (Schuld *et al.*, 2000). The authors suggested that reduced leptin production in narcolepsy may be caused by deficiency of hypocretin. It is thought that hypocretins are involved in the pathophysiology of narcolepsy, because mice whose hypocretin gene has been inactivated exhibit a narcolepsy-like phenotype. Furthermore, CSF levels of hypocretins are blunted in patients with narcolepsy (Sutcliffe and de Lecea, 2000).

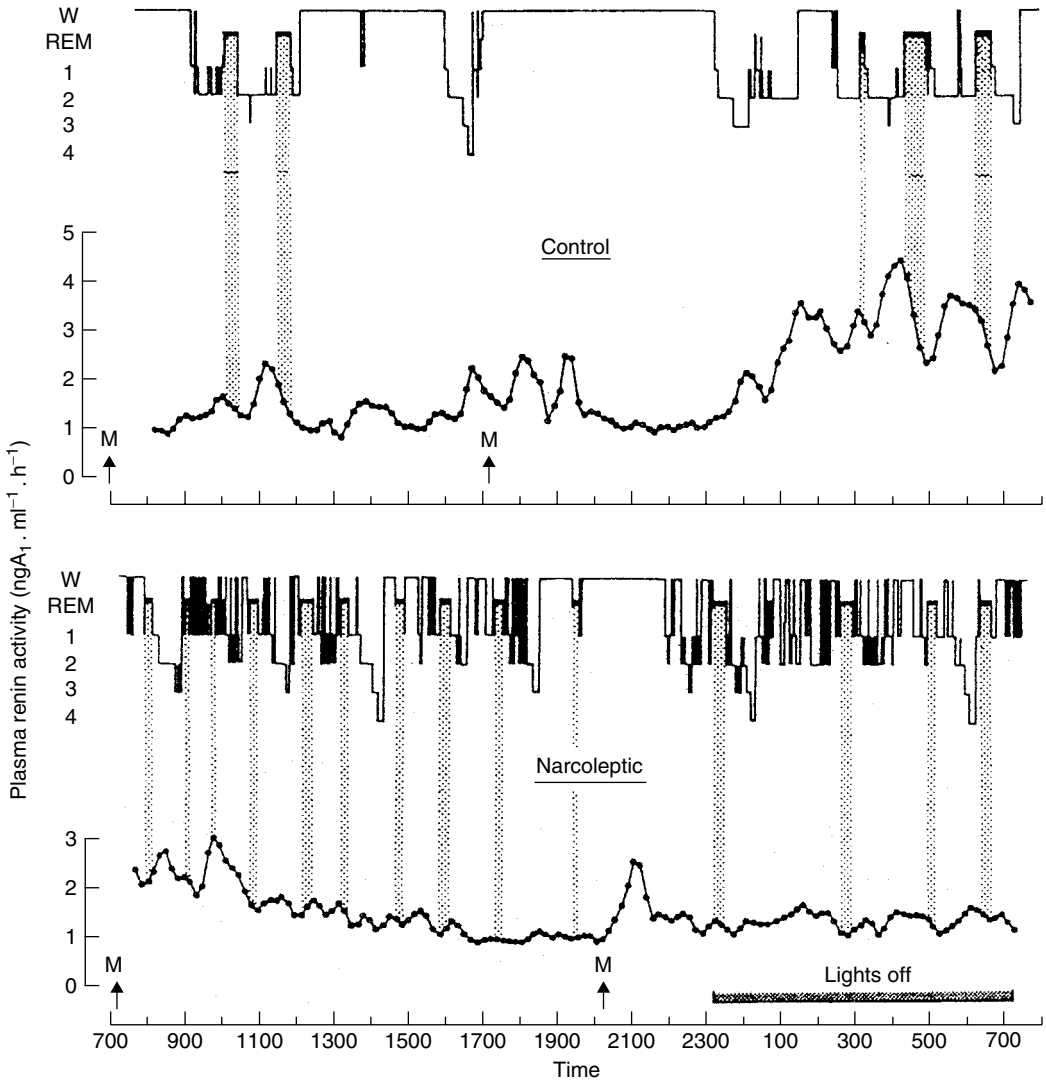

Figure XXIV-3.6 Individual 24-h profiles of plasma renin activity and the corresponding sleep-stage pattern for one control subjects and one patient with narcolepsy (Reproduced from Schulz *et al.*, 1992 by permission of SLEEP)

Restless Legs Syndrome

Restless legs syndrome is a clinical entity characterized by uncomfortable dysaesthesia or paraesthesia in the legs and less frequently in the arms, occurring primarily at rest in the evening hours. Ten male, never-medicated patients with chronically mild to moderate symptoms of this disorder did not show aberration of sleep EEG and of 24-h hormone profiles of cortisol, GH and prolactin, in comparison to age-matched male controls (Wetter *et al.*, in press).

Sleep-Wake Rhythm Disorders

The circadian rhythm of circulating melatonin and the sleep-wake rhythm were investigated in five patients with chronic sleep-wake rhythm disorder and matched controls (Rodenbeck *et al.*, 1998). All the patients showed altered circadian melatonin rhythm variables. The melatonin onset to sleep onset interval varied between the patients, and the melatonin acrophase of sleep- onset interval was prolonged in four patients. The authors concluded that individual phase relations exist between the circadian melatonin rhythm and the sleep-wake cycle in patients with chronic sleep-wake rhythm disorders. Since a prolonged melatonin acrophase to sleep-onset interval was the most consistent finding regardless of aetiology, this abnormality is thought to be a maintaining factor in this disorder as a result of the reduced phase-resetting properties of the circadian pacemarker. Furthermore, rather low circadian melatonin amplitudes and a subsensitivity to daylight are thought to maintain the disorder, at least in some patients.

Familial Advanced Sleep-Phase Syndrome

Familial sleep-phase syndrome is a rare disorder with disabling early evening sleepiness and early morning awakening. Three kindreds with a profound phase advance were identified. In the patients, polysomnographic measures of sleep phase were advanced by almost 4 h compared with those of control subjects. The melatonin and temperature rhythms were also both phase advanced by 3–5 h. The patients tended to fall asleep during solar clock times

corresponding to the 'maintenance of wakefulness zone' in healthy subjects. They also tended to wake up during solar clock times corresponding to the circadian peak of sleepiness in controls. These findings define a hereditary circadian rhythm variant associated with a short endogenous period (Jones *et al.*, 1999).

Disturbed Sleep Associated to Medical Disorders

Fatal Familial Insomnia

Fatal familial insomnia is a rare disease with selective thalamic degeneration that results in chronic sleep loss (Lugaresi *et al.*, 1986). Cortisol levels have been reported to be elevated from the early stages of the disease, further increasing with disease progression. ACTH concentrations, however, remained normal even in later stages (Montagna *et al.*, 1995). In two patients, 24-h profiles of GH and prolactin were studied longitudinally (Portaluppi *et al.*, 1995). The nocturnal GH peak disappeared simultaneously with sleep loss whereas the physiological rhythm of prolactin secretion with normally placed nocturnal acrophases was still present for months after total disruption of the sleep-wake cycle. Complete obliteration of the prolactin rhythm was found only in the advanced stages of the disease as the result of a progressive decrease in the circadian amplitude of variation.

Blindness

A high prevalence of sleep disorders is known in blind people. Subjects with no conscious light perception (NPL) have a higher occurrence and more severe sleep disorders than those with some degree of light perception. It was shown that patients with NPL are likely to have free running circadian rhythms of melatonin, cortisol and sleep (Skene *et al.*, 1999).

Brain Injury

As mentioned before, the persistent changes of sleep EEG and GH in depressed patients after recovery (Steiger *et al.*, 1989) may represent a biological scar due to the metabolic aberrations during acute depression. A recent study on young male patients who survived severe brain injury supports this view (Frieboes *et al.*, 1999). Several months after the injury, the cortisol concentrations of these patients did not differ from normal controls. The GH levels and sleep-stage 2 time of the patients, however, were lower than in the controls. These findings point to similarities between the sleep-endocrine activity of patients who survived brain injury and of those who recovered from depression. In the survivors of brain injury, it appears likely that either HPA overactivity due to stress under the intensive care situation or treatment with glucocorticoids in a subgroup contributed to the changes of NREMS and GH.

Obesity

Several studies report blunted nocturnal GH secretion in obese patients (Ferini-Strambi *et al.*, 1991). Ferini-Strambi *et al.* (1991) examined sleep EEG and sleep-related GH secretion in obese patients without obstructive sleep apnoea before and after weight loss due to a hypocaloric diet. They were compared with lean control subjects. Sleep-EEG parameters did not differ significantly between groups. Before weight loss, there was nearly a total lack of GH secretion in the obese subjects. After weight loss, the mean body mass index in these patients decreased from 37.1 to 31.4 (controls 22.3). Although the sleep architecture in obese patients was unchanged after weight loss, GH concentrations increased, suggesting a partial restoration of GH secretion.

Whipple's Disease

In a patient with long-lasting nearly complete sleep loss due to cerebral Whipple's disease (Lieb *et al.*, 1999), GH secretion was nearly flattened over 24 h. The methodological limitation of this case report is the fact that GH concentrations were determined every 30 min. Therefore, it appears possible that some minor peaks were not detected. Nevertheless, these data suggest that severe chronic sleep loss is accompanied by marked blunting of GH release (Lieb *et al.*, 2000).

Sleeping Sickness

The sleep-wake cycle is distinctly changed in human African trypanosomiasis (sleeping sickness). Sleep EEG and 24-h hormone profiles were compared in patients with sleeping sickness and controls (Brandenberger *et al.*, 1994). A marked sleep fragmentation was found in the patients. The circadian rhythm of cortisol was attenuated in all but one of the six patients. In patients and normal subjects, SWS was associated with the declining phases of cortisol secretion. In the patients, profiles of prolactin and plasma renin activity did not show the increase normally associated with long sleep periods but reflected the disturbances of sleep and wakefulness throughout 24 h. In both patients and controls, REMS began during the descending phases of prolactin pulses. In both groups, plasma renin activity reflected the sleep-stage distribution, with NREMS occurring during the ascending phases and REMS during the descending phases of the oscillations. However, in the patients, sleep fragmentation often did not allow sufficient time for plasma renin activity to increase, as known in regular sleep cycles.

Disturbed Rhythms due to Environmental Influences

Modern civilization influences the physiological sleep-wake schedule of millions of people throughout the world. Intercontinental air travel, shift work and prolonged awake time due to artificial light exert distinct effects on sleep-endocrine activity.

Jet Lag

In an experiment, sleep, EEG and hormone secretion were studied in normal subjects before and after a flight from Europe to the USA. The GH secretion adapted quickly to the new sleep schedule, but it took 2 weeks for the cortisol pattern to be totally adjusted (Desir *et al.*, 1981). Dissociation of sleep and cortisol rhythm during the first few days after long-distance travel may contribute to the symptoms of jet lag.

Light

The major environmental factor influencing the timing of neuroendocrine rhythms via the suprachiasmatic nucleus is light. This view is further supported by studies in which 24-h hormone profiles were sampled in normal control subjects on two separate occasions, once after they were chronically exposed to simulated short (8-h) 'summer nights' and once after they were chronically exposed to simulated long (14-h) 'winter nights'. The duration of the period of rising cortisol levels, active melatonin secretion and high prolactin secretion was longer during the 'winter nights' than during the 'summer nights' (Wehr, 1998).

Shift Work

In shift workers, a long-lasting resistance of the cortisol rhythm to total adaptation to an inverted sleep schedule was detected. Young male night workers who had been permanently on night shift for at least 2 years were compared to day-active control subjects. Sleep EEG and hormone secretion were examined in each group during the usual sleep time (0700–1500 in the night workers, and 2300–0700 in the control subjects, respectively). Furthermore, hormone secretion was investigated during the usual work time. Sleep-EEG variables did not show major differences between the groups. In contrast, cortisol levels were enhanced and TSH levels were blunted in the night workers during the usual sleep time. However, cortisol levels were blunted in the night workers during their work hours. During this interval, a transient increase of prolactin occurred (Weibel and Brandenberger, 1998). Another study from the same laboratory (Weibel *et al.*, 1997) found a more random distribution of GH pulses during sleep in the daytime sleep of night workers than controls.

Sleep in Endocrine Disorders

Hypo- and hypersecretion of various hormones is regularly linked to disturbed sleep. Insomnia is the most frequent sleep-related symptom in these disorders. Only in patients with prolactinoma has enhanced sleep depth been reported so far.

Disorders of HPS System

Given the strong association between SWS and GH secretion, it was interesting to investigate patients with inborn or acquired lack of GH secretion and those with excessive GH levels in acromegaly.

In eight patients, isolated GH deficiency was initially diagnosed by slow growth velocity, an abnormal insulin hypoglycaemia test and retarded bone age. In comparison to normal controls, SWS in these patients was reduced, whereas total sleep time and time spent in stages 1 and 2 were increased (Åström and Lindholm, 1990). The same laboratory reported a decrease in SWA in these patients (Åström and Jochumsen, 1989). Guilhaume *et al.* (1982) examined a small sample of children with psychosocial dwarfism. At the initial examination, the amount of SWS was low. After several weeks in a new environment, an improvement of sleep quality was observed during recovery of growth, particularly an increase of SWS.

In patients with acromegaly, obstructive sleep apnoea syndrome is frequent due to hyperplasia of the upper airway soft tissue (Hart *et al.*, 1985). But also patients with acromegaly but without sleep apnoea have daytime sleepiness and an abnormal sleep structure. One year after adenectomy, REMS time and SWS time increased in patients with acromegaly (Åström and Trojaborg, 1992).

HPA System Aberrations

The capacity of the adrenal glands to produce corticosteroids is severely reduced in Addison's disease (Gillin *et al.*, 1974). There exist only a few case reports on these patients, and these reports do not suggest major changes of their sleep EEG. Garcia-Borreguero *et al.* (2000) compared patients with Addison's disease under two conditions, either continuous hydrocortisone replacement therapy or shorter hydrocortisone withdrawal. Under hydrocortisone replacement, REMS latency was shortened, and REMS time and intermittent wakefulness were increased in comparison to hydrocortisone withdrawal. The authors suggest that cortisol may be needed to facilitate the initiation and maintenance of REMS.

Excessive cortisol levels are produced in patients with Cushing's disease, of either central or peripheral origin. SWS is decreased in these patients (Krieger and Glick, 1974; Shipley *et al.*, 1992). In one study, increases of sleep latency and intermittent wake time and disinhibition of REMS (shortened REM latency, elevated REMS density) were also reported (Shipley *et al.*, 1992). Particularly the latter report suggests that sleep-EEG changes in Cushing's disease and in depression are similar.

Hypothyroidism and Hyperthyroidism

Changes of sleep-wake behaviour are characteristic symptoms of diseases of the thyroid gland. From clinical practice, it is well known that hyperthyroidism is linked with insomnia, whereas fatigue is frequently observed in patients with hypothyroidism. Astonishingly, there are only a few data on sleep EEG in these diseases. One study reported that SWS was reduced in patients with hypothyroidism in comparison to normal controls. The sleep EEG normalized after therapy (Kales *et al.*, 1967).

Prolactinoma

Comparison of patients with hyperprolactinoma and normal controls showed a separate increase of SWS in the patients (Frieboes *et al.*, 1998).

EXPERIMENTAL MODELS OF CHANGES OF SLEEP-ENDOCRINE ACTIVITY

A host of preclinical, human and clinical studies have investigated the interaction of sleep EEG and endocrine activity. The applied methods include transgenic animals; manipulation of endocrine activity in animals by surgery; manipulation of sleep-wake behaviour in normal human control subjects, as by partial or total sleep deprivation; and exogenous administration of hormones, particularly peptides and steroids, to animals and humans. The knowledge accumulated from these studies helps us to understand the pathophysiology of the aberrations reported before in this chapter. Ehlers and Kupfer (1987) were the first to advance the hypothesis that the key peptides of the HPS and the HPA systems, GHRH and CRH, play a major role in sleep regulation. Today this view is corroborated by many data. Besides stimulating GH release, GHRH has been shown to promote NREMS, particularly SWS, in various species including the human (male subjects) (reviewed in Krueger and Obál, 1993), and to blunt HPA hormones in human males (Steiger *et al.*, 1992; Antonijevic *et al.*, 2000b; Antonijevic *et al.*, 2000c). In contrast to GHRH, CRH stimulates the HPA hormones ACTH and cortisol, blunts GH release, impairs sleep and enhances vigilance. Furthermore, a REMS-promoting effect of CRH has been discussed (Steiger, in press). It is thought that changes of the GHRH/CRH ratio result in changes in sleep-endocrine activity. Besides GHRH and CRH, there are other endocrine factors which appear to participate in sleep regulation (see Figure XXIV-3.7).

HPA Hormones

The synthesis and release of CRH is reduced by a hypothalamic gene defect in the Lewis rat. Lewis rats spend less time awake and show more SWS than intact control rats. After intracerebroventricular (ICV) administration of CRH, waking increased

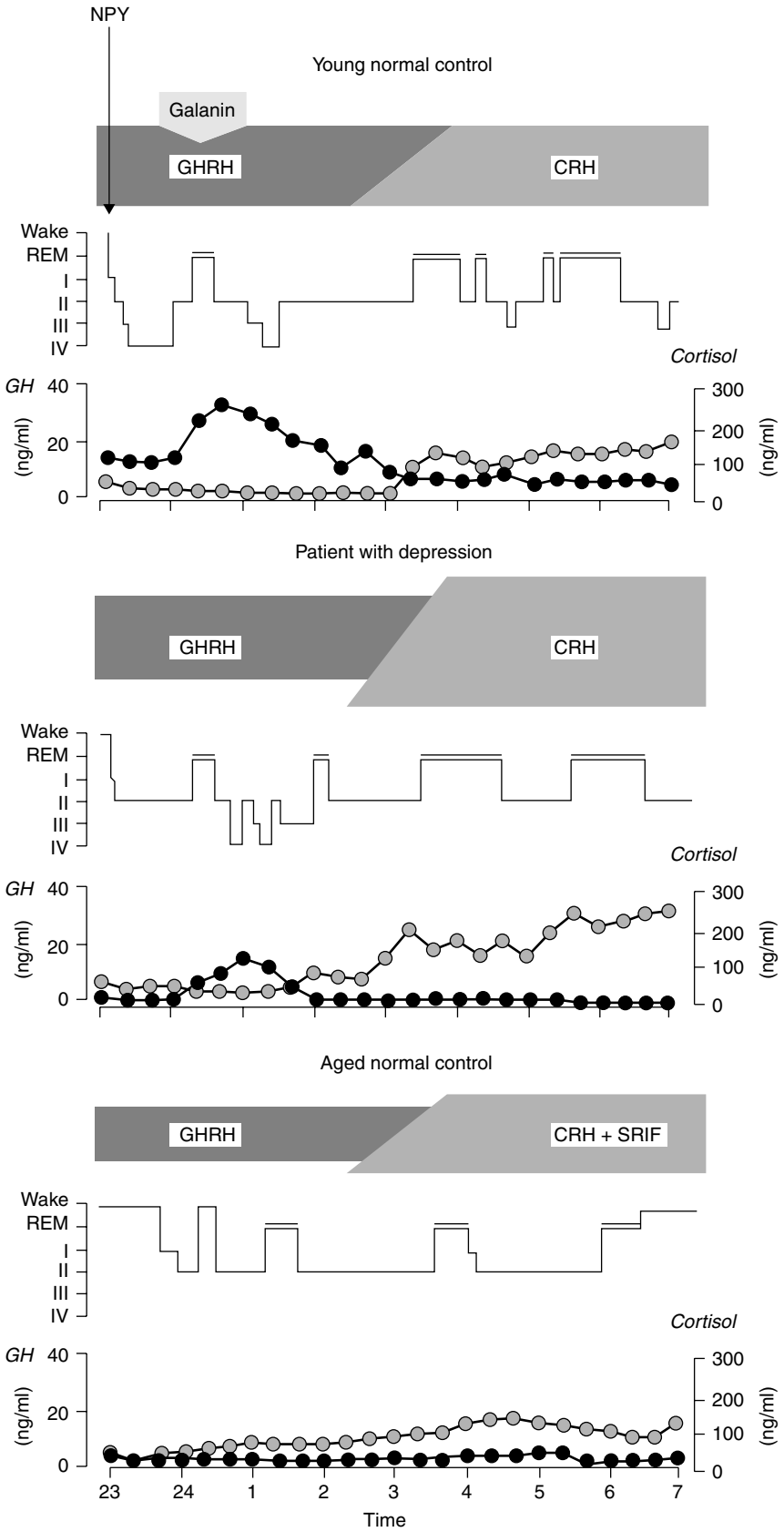

Figure XXIV-3.7 Model of peptidergic sleep regulation

similarly in Lewis and control rats. The sleep-disturbing effects of CRH are suggested by this study (Opp, 1997). In rats, after adrenalectomy and corticosterone replacement, the concentration of this hormone remained about $100\,ng\,ml^{-1}$ throughout 24 h. In the sham-operated control group, however, corticosterone levels ranged between peak levels of about $240\,ng\,ml^{-1}$ in the evening and the minimum of about $20\,ng\,ml^{-1}$ in the morning. Sleep EEG differed only slightly between groups. Slightly more, but shorter, REMS episodes were observed in the adrenalectomized group than the control rats. Obviously, the tonic levels of corticosterone exerted only minor effects on spontaneous sleep-wake behaviour (Langebartels and Lancel, 2000).

ICV administration of CRH diminished SWS in rats (Ehlers *et al.*, 1986) and rabbits (Opp *et al.*, 1989). Moreover, after 72 h of sleep deprivation, SWS was reduced in rats. Furthermore, sleep latency and REMS time increased (Marrosu *et al.*, 1990). Similarly, in young, normal, male control subjects after pulsatile intravenous (IV) administration of $4 \times 50\,\mu g$ human CRH between 2200 and 0100, SWS decreased during the second half of the night, whereas REMS decreased. Furthermore, the GH peak was blunted and during the first hours of the night, cortisol concentration was elevated (Holsboer *et al.*, 1988). In human sleep-endocrine studies, the time, the dosage and the protocol of the administration of peptides are crucial methodological issues. Repetitive administration around sleep onset (Holsboer *et al.*, 1988) mimics the physiological partial release of CRH. In contrast, after continuous nocturnal infusion of CRH, no effect on the sleep EEG was found (Fehm *et al.*, 1993). Moreover, after hourly IV injections of $10\,\mu g$ CRH between 0800 and 1800, sleep EEG remained unchanged during the following night (Kellner *et al.*, 1997). In this study, melatonin levels were blunted after CRH. This finding suggests that the low melatonin syndrome in patients with depression is secondary to CRH overdrive. The responsiveness of sleep to CRH appears to increase during ageing. This theory is suggested by a study comparing the influence of a single dose of ovine CRH given 10 min after sleep onset between young and middle-aged normal control subjects (Vgontzas *et al.*, 2001). In young men, sleep EEG remained unchanged, whereas wakefulness increased and SWS decreased in the middle-aged subjects.

Two studies reported conflicting results after administration of CRH antagonists to rats. In one study, two different antagonists, alpha-helical CRH and astressin, were given before the dark period. These substances reduced wakefulness in a dose-dependent manner, whereas the time courses of these effects differed between drugs (Chang and Opp, 1998). Administration of the substances before the light period had no effect on sleep. The authors concluded that CRH contributes to the regulation of physiological waking. In contrast, in another study (Gonzalez and Valatx, 1997), alpha-helical CRH was effective only in stressed animals. In these rats, REMS was elevated and declined to values of the nonstressed condition after the drug. In sleep-deprived rats, REMS rebound, but not SWS rebound, was diminished by alpha-helical CRH during recovery sleep. The authors suggested that stress acting via CRH may be the major factor inducing the REMS rebound after sleep deprivation (Gonzalez and Valatx, 1998). Some of the cited preclinical work (Gonzalez and Valatx, 1997; Gonzalez and Valatx, 1998; Marrosu *et al.*, 1990) suggests that CRH promotes REMS. From the study in young human controls, the role of endogenous CRH in REMS regulation is uncertain, however, since CRH diminished REMS (Holsboer *et al.*, 1988).

Studies on the effects of ACTH and cortisol on sleep may help to differentiate the central and peripherally mediated sleep-EEG changes after CRH in human subjects. As reviewed elsewhere (Steiger, in press), suppression of REMS is a common effect of the acute administration of CRH, ACTH and cortisol in humans, whereas a synthetic ACTH (4-9) analogue affected neither cortisol release nor REMS (Steiger and Holsboer, 1997b). Furthermore, inhibition of cortisol synthesis by metyrapone in young normal men reduced SWS and cortisol while REMS remained unchanged (Steiger *et al.*, 1998). It is likely that endogenous CRH was enhanced in this study since ACTH was elevated. Interestingly, the effects of subchronic treatment with steroids differ from acute administration in normal controls. Sleep EEG was investigated in female patients with multiple sclerosis at baseline and after 2 and 9 days of administration of the glucocorticoid receptor agonist methylprednisolone. After 2 days, no major effects were found. After 9 days, REMS latency was shortened, REMS density increased and a major portion of SWS shifted from the first to the second NREMS period. These effects are similar to sleep-EEG aberrations in patients with depression (Steiger and Antonijevic, 2001).

Furthermore, the administration of the mixed glucocorticoid and progesterone receptor antagonist, mifepriston, is a method to modulate the HPA system. In a single case study in a normal control after oral mifepriston, ACTH and cortisol were enhanced. Sleep quality was disturbed markedly as sleep latency and intermittent wakefulness increased and SWS and REMS decreased (Wiedemann *et al.*, 1992). The sleep-disturbing effects of mifepriston were further corroborated by a set of studies from the same group (Wiedemann *et al.*, 1994; Wiedemann *et al.*, 1998). Whereas HPA hormones are widely dependent on a circadian rhythm, most studies report increases of ACTH and cortisol after awakenings and during sleep deprivation (reviewed by Steiger, in press).

HPS System

Hormones of the HPA system can modulate sleep. GHRH is the endogenous substance with the best-documented sleep-promoting activity. ICV administration of GHRH to rats and rabbits increases SWS (Ehlers *et al.*, 1986; Obál *et al.*, 1988). The same effect is seen when GHRH is injected into the medial preoptic area in rats (Zhang *et al.*, 1999) or IV in rats (Obál *et al.*, 1996). NREMS increases when GHRH is inhibited by receptor antagonists (Obál *et al.*, 1991) or by negative feedback inhibition of GHRH after administration of GH (Mendelson *et al.*, 1980; Stern *et al.*, 1975) or insulin-like growth factor-1 (Obál *et al.*, 1999). Hypothalamic GHRH mRNA is dependent on a circadian rhythm. In the rat, the highest concentration is found at the beginning of the light period when sleep propensity reaches its maximum in these nocturnal animals (Bredow *et al.*, 1996). Furthermore, hypothalamic GHRH contents display sleep-related variations (Gardi *et al.*, 1999). A major role of GHRH in sleep promotion by sleep deprivation is indicated since GHRH antibodies antagonize this effect in the rat (Obál *et al.*, 1992). Furthermore, hypothalamic GHRH mRNA has been found to be increased after sleep deprivation in rats (Toppila *et al.*, 1997; Zhang *et al.*, 1999). The sleep rebound following sleep deprivation was inhibited by microinjections of a GHRH antagonist into the area preoptica of the rat (Zhang *et al.*, 1999). Very big 'supermice' slept more than normal mice (Lachmansingh and Rollo, 1994). However, dwarf rats with deficits in the central GHRHergic transmission and reduced hypothalamic GHRH content had less NREMS than control rats (Obál *et al.*, 2001). Taken together, these data suggest that GHRH is a common stimulus for NREMS, particularly SWS and GH secretion.

We showed that GHRH promotes sleep also in humans. After repetitive IV administration of GHRH during the first few hours of the night, SWS and GH secretion increased in young normal male controls and cortisol secretion decreased (Steiger *et al.*, 1992). Sleep promotion in young men by GHRH was confirmed after IV(Kerkhofs *et al.*, 1993; Marshall *et al.*, 1999) and intranasal

(Perras *et al.*, 1999a) administration. We investigated the effects of GHRH on sleep-endocrine activity in three states with a change of the GHRH/CRH ratio in favour of CRH: first, the second half of the night in young normal men; second, in elderly normal men and women; and, third, in patients with depression.

In the first case, in young normal men, administration of GHRH during the early morning hours (0400–0700) prompted no major effects on sleep EEG, particularly no change of SWS. GH increased whereas HPA hormones remained unchanged (Schier *et al.*, 1997). In the second case, in the normal elderly, during the daytime, the response of GH to GHRH was blunted (Iovino *et al.*, 1989). Similarly, we found only a weak sleep-promoting effect of GHRH in the elderly. The first NREMS period was prolonged and the time of awakenings decreased, whereas SWS remained unchanged (Guldner *et al.*, 1997). In the third case, the influence of pulsatile IV administration of GHRH during the first few hours of the night was tested in 42 drug-free patients of both sexes with major depression (age range 19–76 years) and matched controls. Interestingly, a sexual dimorphism in the response to GHRH was found. In male patients and controls, GHRH inhibited ACTH levels during the first half of the night and cortisol levels during the second half of the night. In contrast, these hormones were enhanced in females, regardless whether they were healthy or depressed. Similarly, NREMS and stage 2 sleep increased in male patients and controls, whereas opposite sleep-impairing effects occurred in women. These data confirm a reciprocal antagonism of GHRH and CRH in males, whereas a synergism of GHRH and CRH is suggested in females. The latter issue may be an explanation for the increased prevalence of mood disorders in women (Antonijevic *et al.*, 2000b; Antonijevic *et al.*, 2000c).

The major antagonist of GHRH in the regulation of GH is somatostatin. In rats, systemic administration of the somatostatin analogue octreotide decreased NREMS (Beranek *et al.*, 1999). Similarly, SWS was reduced in young normal men after subcutaneous administration of octreotide (Ziegenbein *et al.*, 2000). Octreotide is known to be more potent than exogenous somatostatin. This is shown by the fact that repetitive IV administration of somatostatin impaired sleep in normal elderly controls (Frieboes *et al.*, 1997), whereas it had no effect in young normal men (Steiger *et al.*, 1992).

Galanin

Under repetitive IV administration of galanin to young normal men, SWS and the duration of REMS periods increased, whereas the secretion of GH and cortisol remained unchanged (Murck *et al.*, 1999).

Neuropeptide Y (NPY)

Besides GHRH, NPY appears to be a physiological antagonist of CRH. Originally, this theory was based on the opposite effects of CRH and NPY in animal models of anxiety (reviewed in Steiger and Holsboer, 1997b). This view is supported by recent studies on the sleep-EEG effects of NPY. After ICV administration of NPY to rats, EEG spectral activity changed in a way similar to the effects of benzodiazepines (Ehlers *et al.*, 1997a). Furthermore, the prolongation of sleep-onset latency by CRH was antagonized dose-dependently by NPY in rats (Ehlers *et al.*, 1997b). Similar effects were observed in young normal men. Repetitive IV administration of NPY prompted decreases of sleep latency and the first REMS period, and increases of stage 2 sleep and sleep period time, and blunted cortisol and ACTH secretion (Antonijevic *et al.*, 2000a). Moreover, in patients with depression of both sexes with a wide age range and age-matched controls, the sleep latency was shortened after NPY, whereas cortisol and ACTH levels and the first REM period remained unchanged (Held *et al.*, 1999). These data suggest that NPY participates in sleep regulation, particularly in the timing of sleep onset as an antagonist of CRH acting via the $GABA_A$ receptor.

Vasoactive Intestinal Polypeptide (VIP)

Pulsatile IV administration of vasoactive intestinal polypeptide (VIP) in young normal male control subjects decelerated the NREMS-REMS cycles. Each cycle was prolonged, the cortisol nadir appeared to be advanced and the GH surge was blunted (Murck *et al.*, 1996). These findings suggest that VIP exerts a specific effect on the temporal organization of sleep-endocrine activity, including the timing of the cortisol nadir. It appears likely that VIP affects the circadian clock, resulting in prolonged sleep cycles and earlier occurrence of the cortisol nadir. Blunted GH surge may be explained as a result of the advanced elevated HPA activity.

HORMONES AS THERAPY IN SLEEP DISORDERS

Several approaches were applied to use hormones or synthetic analogues in the treatment of sleep disorders. In most cases, the rational was the substitution of declining hormone levels.

Oestrogen Replacement Therapy

In postmenopausal women oestrogen replacement therapy by skin patch (50 μg of estradiol per day) enhanced REMS and reduced intermittent wakefulness during the first two sleep cycles. The normal decrease in SWS and SWA from the first to the second sleep cycle was restored by oestrogen (Figure XXIV-3.8) (Antonijevic *et al.*, 2000d). These data suggest that oestrogen treatment after the menopause can help to restore the normal sleep-EEG pattern in women.

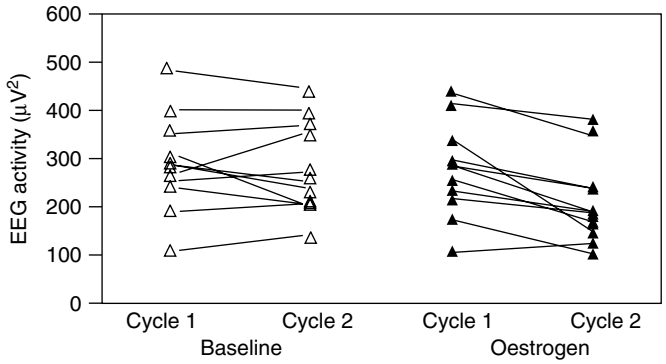

Figure XXIV-3.8 Changes in delta electroencephalographic activity from first to second cycle during baseline and oestrogen replacement therapy (ERT). A change in delta electroencephalographic (EEG) activity from first to second non-rapid-eye movement during baseline (open circles) was not consistently observed, whereas during ERT (filled circles) a normal decrease in delta activity from first to second non-rapid-eye movement period was observed in most subjects (expressed as percentage change of the respective value from first to second non-rapid-eye movement period: $-2.4\% \pm 5.4\%$ during baseline versus $-20.4\% \pm 5.7\%$ during ERT; univariate F test after one-factorial (treatment) analysis of variance, including covariate short-term versus long-term ERT; $n = 11$; F1, $9 = 8.8$; $P < 0.05$)

GHRH and GH Secretagogues

Acute repetitive IV (Guldner *et al.*, 1997) and intranasal (Perras *et al.*, 1999b) administration of GHRH in elderly subjects had only a relatively weak sleep-promoting effect compared to the increase of SWS in young men after this peptide. A pilot study tested the hypothesis that after priming (e.g., daily IV administration of GHRH every 2 days for 12 days) the sleep-promoting effect of GHRH would be restored in the elderly. The study results in two subjects do not support this hypothesis (Murck *et al.*, 1997). GH secretagogues are synthetic peptides. These substances share the capacity of GHRH to stimulate GH release, although they act via a specific GH secretagogue receptor, and not at the GHRH receptor. As in the observations after GHRH, oral administration of one GH secretagogue, MK-677, for 1 week, had a distinct sleep-promoting effect in young men and only a weak effect in elderly controls (Copinschi *et al.*, 1997). Obviously, the capacity of GHRH and GH secretagogues to promote sleep in the elderly is reduced. It might be necessary to start with the substitution of GHRH or related analogues earlier during the lifespan in order to counteract the sequelae of their declining endogenous activity.

Vasopressin

After 3 months' daily intranasal administration of vasopressin to elderly subjects, SWS was enhanced markedly, whereas intermittent wakefulness and self-rated sleep quality remained unchanged (Perras *et al.*, 1999b).

Melatonin

Study results on a beneficial effect of melatonin in young and elderly subjects are ambiguous (Baskett *et al.*, 2001). A possible side effect of long-term treatment with melatonin is a blunting of sexual steroids in men and women. So far, there is a lack of sufficient data from clinical studies to recommend melatonin as an effective alternative sleeping pill. Some studies suggest that, as a result of its phase-shifting properties, melatonin may be helpful in the treatment of rhythm disturbances, such as jet lag and disturbed rhythms in blind patients (Sack *et al.*, 2000; Zhdanova *et al.*, 1997).

CONCLUSIONS

The data reported in this chapter corroborate the bidirectional action of sleep EEG and endocrine activity. Shallow sleep and sleep loss due to various causes are frequently linked to elevated secretion of cortisol and ACTH and blunted GH levels. Another clear link has been shown between changes of the sleep-wake rhythm and the pattern of melatonin activity. The link between the NREMS-REMS cycle and the oscillations of plasma renin activity known from normal controls appears to be preserved in disrupted sleep patterns. Although it is so far unknown what factors are the common regulators of sleep and plasma renin activity, the role of GHRH and CRH as links between sleep EEG and peripheral hormone secretion is well established. At least in male subjects, GHRH appears to stimulate GH and SWS and to inhibit HPA hormones. CRH exerts opposite effects. Changes of the GHRH/CRH ratio occur during normal ageing (reduced GHRH activity) and in depression (CRH overactivity). Common features of these states are shallow sleep, blunted GH and elevated cortisol levels. Sleep-EEG changes in dwarfs (low GHRH) and subjects with acromegaly (feedback inhibition of GHRH due to excessive GH levels) can be similarly explained. Gender differences in sleep regulation are suggested by the opposite effects of GHRH in normal and depressed women in comparison to men. This issue needs further clarification.

Besides GHRH and CRH, other peptides and steroids are thought to participate in sleep regulation. Like CRH, somatostatin is sleep-impairing factor, and, like GHRH, NPY and galanin promote sleep. VIP appears to be involved in the temporal organization of sleep. The similarities between sleep-EEG in patients with depression and with Cushing's disease and in patients with multiple sclerosis after subchronic administration of a glucocorticoid agonist suggest, that besides CRH, elevated glucocorticoid levels also contribute to the sleep-EEG changes in depression. So far, the use of hormones in the therapy of sleep disorders is rare. Since the hypnotics of today do not induce physiological sleep (Steiger and Lancel, 2000), novel substances are needed that correspond better to human physiology. The progress in sleep-endocrine research should help to develop such therapeutic strategies. In the related field of antidepressants, the recent development of CRH antagonists (Holsboer, 1999; Zobel *et al.*, 2000) is an encouraging example.

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XXIV-4

Neuroimmunology of Sleep

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INTRODUCTION

Sleep consumes a third of our lives, but its functions are unknown. Excess, inhibited, or fragmented sleep often accompanies psychiatric disorders or their treatments. The fatigue and confusion associated with the abnormal sleep may constitute a major complaint of the patient. Before we can begin to understand the pathological sleep associated with psychiatric disorders, it is essential that we have a better understanding of normal sleep and its functions. Ironically, the major insights we have gained into the regulation of normal sleep come from another class of pathologies, acute infectious diseases. This chapter will summarize what we have learned about sleep regulation through analysis of the molecular changes associated with infections.

Acute infections are generally detected clinically by the manifestations of fever and malaise. Included in the concept of malaise is the subjective feeling of profound fatigue and the overwhelming need to sleep. Sleep is detected and measured primarily through analysis of electroencephalographic (EEG) patterns, as has been described elsewhere in this text. Over the last 20 years we have characterized the EEG changes that occur in response to several acute infections. These changes will be described in more detail later in this chapter, but consistent features are the manifestation of increased slow-wave or non-rapid-eye-movement sleep (NREMS), increased slow-wave amplitudes, and often the reduction of total REM sleep (Krueger and Majde, 1994). These sleep characteristics are seen regardless of whether the stimulus is a purely microbial component or an actual infection. A major breakthrough in our conceptualization of sleep regulation is the realization that the peripheral cytokines produced in response to an infectious or other inflammatory stimulus are responsible for triggering the subjective need to sleep as well as the characteristic sleep changes. Furthermore, these same cytokines are actively involved in regulating physiological sleep.

Cytokines are a large class of protein hormones produced primarily by cells of the immune system or by damaged epithelial cells. Over 100 cytokines have been identified to date. One subclass of cytokines, the chemokines, appears to act primarily in a paracrine fashion to regulate inflammation at the site of tissue damage. However, a large and loosely defined subclass of cytokines, the proinflammatory cytokines, act not only locally but also systemically to trigger all of the characteristic responses to inflammatory challenge, including fever, anorexia, and somnolence. Thus, at least one function of proinflammatory cytokines appears to be signalling the brain that the host is threatened by invading micro-organisms and that adaptive responses are required (Hart, 1988). The best-characterized proinflammatory cytokines are the interleukin-1s (IL1α and IL1β), IL6, and tumour necrosis factor-α (TNF-α). The array of physiological, behavioural, haematological, and biochemical responses initiated by these cytokines is termed the acute-phase response (APR). In addition to proinflammatory cytokines, growing evidence points to an important systemic role of anti-inflammatory cytokines such as IL10, IL4, IL13, and transforming growth factor-β (TGF-β) in the inhibition of the APR, including the excess sleep component. In the sections to follow, we will outline the evidence that proinflammatory and anti-inflammatory cytokines regulate both pathological and physiological sleep.

In addition to cytokines, several classical hormones, such as growth hormone-releasing hormone (GHRH), corticotrophin-releasing hormone (CRH), and prolactin, have been implicated in sleep regulation. Growth factors such as nerve growth factor (NGF), neurotropins, epidermal growth factor, and fibroblast growth factor are involved as well (reviewed by Krueger and Obál, 1997). The gaseous neurotransmitter nitric oxide (NO) has also been implicated (Kapás *et al.*, 1994; Kapás and Krueger, 1996). The regulation of these hormones and NO synthesis by cytokines may form the basis for the association of cytokines and sleep.

INDIGENOUS MICROBIAL FLORA AND CYTOKINE STIMULATION IN THE HEALTHY INDIVIDUAL

It is widely recognized from such conditions as rheumatic heart disease that bacteraemia can occur in response to dental work. Profound immunodeficiency disorders such as AIDS have also demonstrated that the massive microbial flora that lines our mucosal surfaces, particularly the intestine, can escape protective mucosal barriers when normal immune defences are impaired. Much less is known about our day-to-day exposure to these micro-organisms because this exposure is not perceptible. However, studies of the intraintestinal lymphoid tissue, the Peyer's patches, reveal specialized epithelial cells that can transport bacteria to adjacent macrophages for degradation (Owen *et al.*, 1986). Bacteria and their breakdown products accumulating in the Peyer's patches then can enter the portal circulation or the mesenteric lymph (Sartor *et al.*, 1988). Though not quantified, it is thought that millions of bacteria may be cleared by this mechanism daily.

The first insights into the relationship of microbial products to sleep were gained from structural studies on a sleep factor isolated from human urine (Krueger *et al.*, 1982a). This factor proved to share the unique chemical properties of the peptidoglycans found in the cell walls of bacteria (Krueger *et al.*, 1982a). Peptidoglycans comprise 90% of the cell wall of Gram-positive bacteria and 5–20% of the cell wall of Gram-negative bacteria (Krueger and Majde, 1990). Subsequent sleep studies with various natural and synthetic peptidoglycans revealed that the minimally active unit is a glycopeptide consisting of the sugar *N*-acetylmuramic acid (found only in bacteria) and the dipeptide L-alanine-D-alanine, termed muramyl dipeptide (MDP) (Krueger *et al.*, 1982b). MDP and certain derivatives have immunological

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adjuvant properties. Peptidoglycans also contain the bacterially unique amino acids D-glutamic acid (eukaryotes employ primarily L-amino acids) and diaminopimelic acid, as well as L-lysine. Many peptidoglycan derivatives are pyrogenic as well as somnogenic.

In addition to the peptidoglycans found in all bacterial cell walls, the lipopolysaccharide (LPS) moiety of the endotoxin component of Gram-negative bacterial cell walls also potently alters sleep through induction of proinflammatory cytokines (Krueger and Majde, 1994). A majority of studies on cytokine induction by microbial products have employed LPS as the stimulus. Other bacterial components such as lipoteichoic acid and lipoproteins also stimulate proinflammatory cytokine induction, although their effects on sleep have not been analysed. Recently, it has been recognized that structural differences in bacterial DNA are recognized by the immune system with the production of cytokines (Sweet *et al.*, 1998). It is not yet known whether bacterial DNA plays a role in bacterial illness or in sleep responses to bacteria.

It has recently been determined that bacterial and fungal cell-wall components, although structurally distinct, are detected by phagocytes through activation of Toll-like receptors (TLRs), a receptor type first demonstrated as a host-defence mechanism in fruit flies (Aderem and Ulevitch, 2000). Activated TLRs then signal the phagocyte to release proinflammatory cytokines.

A common feature of all microbial components (with the possible exception of bacterial DNA) capable of inducing cytokines is their chemical resistance to biodegradation (Krueger and Majde, 1994). While mature macrophages and neutrophils carry enzymes capable of degrading peptidoglycans (lysozyme, *N*-acetylmuramyl-L-alanine amidase, Hoijer *et al.*, 1997), these enzymes do not always achieve complete degradation and fragments may be secreted extracellularly (Pabst *et al.*, 1999). These secreted partial degradation products can then serve as steady stimulators of cytokine release by neighbouring phagocytes. Little direct evidence exists for this cytokine source from the intestine, however, because the portal blood and mesenteric lymph of healthy individuals are rarely accessed. Furthermore, it is clear that soluble receptors and other binding factors exist in body fluids that are capable of neutralizing and facilitating the clearance of cytokines (Dinarello, 2000). Low levels of cytokines, especially IL1s, routinely circulate in association with intrinsic binding factors. These binding factors are likely to play a significant role in determining the levels of cytokines free to bind to cell receptors and activate neighbouring or distant cells.

While there are substantial gaps in our knowledge of physiological cytokine regulation, indirect evidence supports a role for indigenous flora in the regulation of both body temperature and sleep, in that animals with reduced indigenous flora have lower body temperatures and sleep less (Krueger and Majde, 1994). Rats treated with antibiotics that reduce intestinal flora have reduced NREMS but normal REMS (Brown *et al.*, 1990). Furthermore, neutralization of the proinflammatory cytokines IL1β and TNFα with antibodies or endogenous binding factors also reduces spontaneous sleep (see section below on *IL1β and TNFα*).

Whereas much remains to be elucidated regarding the exact source of the cytokines regulating physiological sleep, the means by which cytokines produced in the periphery can affect the brain have been extensively investigated. Three possible routes to the brain for peripheral cytokines have been implicated: brain circumventricular organs (Blatteis and Sehic, 1997), specific transporters in the blood–brain barrier (Banks *et al.*, 1991), and sub-diaphragmatic vagal afferents (Kapás *et al.*, 1998; Hansen and Krueger, 1997). Of these three routes, stimulation of the vagal nerve has been most closely linked with sleep regulation. The liver paraganglia contain IL1 receptors (Goehler *et al.*, 1997). Systemic bacterial LPS and IL1 both induce increased IL1β mRNA production in the brain, and this effect is blocked by vagotomy (Hansen *et al.*, 1998a; Layé *et al.*, 1995). Excess NREMS in response to systemic IL1β is also blocked by vagotomy (Hansen and Krueger, 1997). Other events that promote NREMS such as excessive food intake are also dependent on vagal innervation (Hansen *et al.*, 1998b). The relative importance of these three brain access routes in regulating both physiological and pathological sleep under different conditions is unknown, although direct action on the brain via circumventricular organs and/or blood–brain barrier transporters would require elevation of unbound cytokines in the circulation.

CHANGES IN SLEEP DURING ACUTE INFECTIONS OR CHALLENGE WITH MICROBIAL COMPONENTS

Acute infections such as influenza are associated with an overwhelming need to sleep and an increase in total sleep time. Our knowledge of polysomnographic sleep changes in acute infections is derived from animal studies, as only subjective sleep reports have been acquired during acute infections in man (Smith, 1992). As mentioned previously, acutely infected animals demonstrate increased NREMS and diminished REMS as well as increased amplitudes of EEG slow waves, which are considered an index of the intensity of sleep (Krueger and Majde, 1994). These gross changes occur in bacterial, fungal, and viral infections, but with different kinetics depending on the infective organism, dose, and route (Toth, 1999).

The sleep alterations in acute bacterial, fungal, and viral infections have been recently reviewed in detail (Toth, 1999) and in summary form (Krueger *et al.*, 2001). Studies with bacteria in rabbits include both human pathogens (*Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*) and the rabbit pathogen *Pasturella multocida* (Toth, 1999). Challenge of rats with baker's yeast or rabbits with *Candida albicans* induces sleep alterations similar to those seen in animals challenged with Gram-positive bacteria (Toth, 1999). Viral sleep alterations have been analysed in an abortive rabbit model of influenza virus and an abortive mouse model of Newcastle disease virus. NREMS and delta-wave amplitudes are increased and REMS decreased during the febrile period in all of these infections (Toth, 1999). A similar but more prolonged sleep alteration is seen in an active influenza infection in mice when virus is administered in such a manner as to cause pneumonitis, whether or not a lethal infection ensues (Fang *et al.*, 1995a; Toth, 1999). When the same amount of virus is delivered only to the upper respiratory tract, no changes in sleep or other symptoms are seen (Fang *et al.*, 1996).

The same fundamental sleep changes occur in response to challenge with bacterial cell-wall peptidoglycans or LPS, with biodegradation-resistant fungal cell-wall polysaccharides, or with synthetic or virus-associated double-stranded RNA derived from viral replication intermediates (Krueger and Majde, 1994). These fungal and viral components stimulate proinflammatory cytokine induction similarly to that induced by bacterial cell-wall components, though by different mechanisms.

It is the central thesis of this chapter that microbially upregulated cytokines initiate the sleep changes associated with acute infections, presumably because the amounts of cytokines produced exceed the levels that can be bound and cleared by the body. Unbound cytokines produced in the periphery then act upon the brain, probably via vagal afferents (Hansen *et al.*, 1998a), to induce IL1β in relevant brain regions. This thesis is supported by the observation that excess NREMS, fever, and other behavioural responses induced by systemic LPS, muramyl peptides, or IL1β are attenuated by central inhibition of IL1β (Kent *et al.*, 1992; Klir *et al.*, 1994; Takahashi *et al.*, 1996). The extensive evidence

that IL1 and TNF play a role in physiological and pathological sleep regulation is discussed in the section below on humoral regulation.

It should be noted that, whereas we have focused on cytokines as triggers of the APR, other inflammatory mediators such as the eicosenoids may also play a role in the CNS response to infection. It is widely recognized that prostaglandin E_2 (PGE_2) is an important mediator of the fever response to infection (Kluger, 1991). PGD_2 and PGE_2 have also been implicated in sleep regulation (Hayaishi, 1988; Terao *et al.*, 1998). IL1 induces brain astrocytes to produce both prostaglandins (Hayaishi, 1988), and IL1 promotes sleep when injected into a PGD_2-sensitive brain region (Terao *et al.*, 1998). Thus, IL1 within the brain may regulate sleep in part through eicosenoid induction.

The proinflammatory cytokine response to microbes or their stimulatory components is grossly indistinguishable from the proinflammatory cytokine response to other inflammatory stimuli such as tissue damage, although infections are more commonly associated with sleep alterations than are other acute inflammatory states. Whether the relative potency of infections is qualitative or quantitative is unknown.

SLEEP ALTERATIONS IN CHRONIC INFECTIONS AND CHRONIC INFLAMMATORY STATES

Chronic infections with such agents as human immunodeficiency virus (HIV) or hepatitis virus are much more difficult to dissect than are acute infections because they are inevitably associated with diffuse chronic inflammation. In addition to the proinflammatory cytokine cascade characteristic of acute inflammation, chronic inflammation has the added complexity of manifesting a simultaneous anti-inflammatory cytokine cascade. Sleep responses have been examined following administration of single anti-inflammatory cytokines (Krueger and Majde, 1994), and IL10, IL4, and TGFβ all have been found to reduce sleep (Kushikata *et al.*, 1998). However, no studies have attempted to characterize the sleep effects of the complex mixture of pro- and anti-inflammatory cytokines that occurs in chronic inflammation. Studies of sleep in chronic infections suggest, however, that the proinflammatory cytokines dominate sleep regulation in clinical disease.

The chronic infections that have been most extensively investigated with respect to sleep changes are the immunodeficiency virus infections of humans and cats; trypanosome infections of rabbits, rats and humans; and prion infections of mice, rats, cats, and humans (reviewed by Toth, 1999). All of these infections directly affect the brain, and their associated sleep alterations may reflect inflammatory changes within the brain. Because patients infected with the human immunodeficiency virus HIV-1 or the prion causing Creutzfeldt-Jakob disease often manifest psychiatric disorders, what is known about their sleep changes is summarized here.

Sleep changes in HIV-1 infections have been studied during asymptomatic and symptomatic phases. Asymptomatic HIV-1-infected men express increased NREMS during the second half of the night, sleep fragmentation, and abnormal REMS architecture (reviewed by Toth, 1999). These altered sleep patterns precede neurological involvement or onset of secondary infections (Darko *et al.*, 1995). Progression to overt AIDS is marked by reduced NREMS, marked sleep fragmentation, and profound disruption of sleep architecture.

Prions, infectious agents associated with profound dementia, also cause alterations in sleep. Rats inoculated with the scrapie prion demonstrate unusual spiking patterns in the EEG during wakefulness 4 months after inoculation (Toth, 1999). Later NREMS and active wakefulness are reduced and drowsiness is increased (Toth, 1999). Cats challenged with Creutzfeldt-Jakob brain homogenate demonstrate increased NREMS, reduced wakefulness, and abnormal REMS 20 months after inoculation (Toth, 1999). The condition known as fatal familial insomnia is associated with prion-related thalamic neurodegeneration (Toth, 1999). Deletion of the prion protein gene results in altered sleep and circadian rhythms in mice (Toth, 1999).

Less is known about the specific sleep alterations associated with chronic inflammatory diseases, although sleep disorders are prominent components of such diseases as rheumatoid arthritis, fibromyalgia, and chronic fatigue syndrome. Sleep alterations in these three diseases will be briefly described here, as they may affect the psychiatric patient or cause the patient to seek psychiatric help.

Fatigue is a common symptom of all chronic inflammatory diseases. In autoimmune disorders such as rheumatoid arthritis, the fatigue is probably due to the associated sleep fragmentation with frequent movement of the extremities and frequent arousal (Mahowald *et al.*, 1989). Alpha-delta sleep patterns where EEG NREMS intrudes on delta sleep are characteristic. Increasing sleep though treatment with the hypnotic drug triazolam reduces morning stiffness and daytime sleepiness without improving sleep fragmentation (Walsh *et al.*, 1996). Rats experiencing chronic adjuvant arthritis display fragmented sleep, sleep less during the normal sleeping period, and thus lose their normal diurnal sleep rhythms (Landis *et al.*, 1988). Non-steroidal anti-inflammatory analgesics have complex effects on the sleep in these animals (Landis *et al.*, 1989). The pain associated with rheumatic diseases does not appear to cause the fragmented sleep (Hirsch *et al.*, 1994), but the disrupted sleep may contribute to the pain (Moldofsky *et al.*, 1993).

Syndromes associated with chronic fatigue, subjective cognitive impairment, and diffuse myalgia rather than focal inflammation (joint swelling) of the type seen in rheumatic diseases include fibromyalgia and chronic fatigue syndrome. Fibromyalgia is distinguished from chronic fatigue syndrome clinically by its associated marked fibrositis. Depression is also commonly associated with both these disorders. Recently, the depression in chronic fatigue syndrome was shown to be associated with a characteristic REM latency that has been associated with other depressive disorders (Morehouse *et al.*, 1998). Most patients with these syndromes complain of non-restorative sleep, and sleep fragmentation similar to that in rheumatoid arthritis is characteristic of fibromyalgia (Branco *et al.*, 1994). One study has indicated that the association of these two syndromes with sleep disorders is no greater than in patients with psychiatric disorders not associated with pain (Buchwald *et al.*, 1994). Both syndromes are frequently preceded by a febrile illness, but fibromyalgia is also commonly associated with both psychological distress and primary sleep disorders such as sleep apnoea and periodic limb movement (Moldofsky, 1993).

The sleep disorder in chronic fatigue syndrome is essentially the same as that in fibromyalgia (Moldofsky, 1993), although the association with primary sleep disorders such as sleep apnoea is not seen. Circadian rhythm disruption of melatonin secretion and core temperature is seen in chronic fatigue syndrome, which may or may not be a consequence of the sleep disorder (Williams *et al.*, 1996). It has been proposed that fibromyalgia and chronic fatigue are both clinical manifestations of the reciprocal relationship between the immune and sleep-wake systems. Interference with either the immune system (by an infection generating large amounts of cytokines) or the sleep-wake system (by sleep deprivation) affects the other system and will be accompanied by symptoms of chronic fatigue (Moldofsky, 1993). Interestingly, a study of the chronic fatigue and arthralgia syndrome that commonly follows infection with the tick-borne spirochete causing Lyme disease reveals sleep

features similar to those of other chronic inflammatory diseases (Bujak *et al.*, 1996).

HUMORAL REGULATION OF SLEEP — THE INVOLVEMENT OF IMMUNE SYSTEM PRODUCTS

While our discussion above has focused on microbial products and inflammatory mediators in sleep regulation, we would be remiss if we failed to emphasize that sleep is ultimately regulated by both neural and humoral mechanisms that interact and are inseparable. Neural mechanisms of sleep regulation have been extensively reviewed elsewhere (Steriade and McCarley, 1990); here we will focus on humoral mechanisms.

The involvement of specific neurotransmitters in sleep regulation is often included within the concept of humoral regulation of sleep. Almost all well-characterized neurotransmitters have been implicated in one or more aspects of sleep-wake regulation. In this section we will focus on neuromodulator involvement in sleep regulation, specifically IL1β, TNFα, and GHRH in physiological NREMS regulation, and prolactin (PRL) and vasoactive intestinal peptide (VIP) in REMS regulation, because for these substances there is extensive evidence for their involvement in physiological sleep regulation. In the section below on central targets in the brain, we will present evidence for the concept that IL1, TNF, and other somnogenic growth factors such as NGF induce sleep via nuclear factor kappa B (NFκB)-dependent mechanisms; several events downstream from NFκB activation, such as cyclooxygenase-2 (COX-2) and inducible-nitric oxide synthase (iNOS) induction, also seem to be involved in sleep regulation.

IL1β and TNFα

IL1β or TNFα given centrally or systemically induce prolonged large increases in the amount of time spent in NREMS in every species thus far tested (rats, rabbits, mice, monkeys, sheep, and cats). For example, 3 μg of TNF given intraperitoneally to mice induces about 90 extra min of NREMS during the first 9 h after injection (Fang *et al.*, 1997). The excess NREMS induced by IL1β or TNFα is associated with high-amplitude EEG slow waves (Krueger *et al.*, 1984). Similar supranormal EEG slow waves occur during NREMS after sleep deprivation (Pappenheimer *et al.*, 1975) or during the initial sleep responses to infectious challenge (Toth and Krueger, 1988). These supranormal EEG slow waves are posited to reflect a higher intensity of NREMS (Borbély and Tobler, 1989). Low somnogenic doses of IL1β and TNFα have little effect on REMS, whereas doses that induce large increases in NREMS often reduce duration of REMS. In rats (Lancel *et al.*, 1996; Opp *et al.*, 1991) and cats (Susic and Totic, 1989), higher doses of IL1β inhibit sleep.

The excess NREMS induced by either IL1β or TNFα appears to be physiological in the sense that after low somnogenic doses, sleep architecture remains normal; animals continue to cycle through stages of sleep and wakefulness, although within each cycle there may be more NREMS. After IL1β or TNFα treatment, sleep remains readily reversible; for example, the entry of the experimenter into the room quickly awakens the cytokine-treated

Table XXIV-4.1 Putative sleep-regulatory substances

Sleep-promoting substances	Sleep-inhibitory substances
Interleukin-1β[1,4] (ILβ)	Hypocretin
Interleukin-1α[4] (IL1α)	Corticotropin-releasing hormone[4] (CRH)
Interleukin-2[4] (IL2)	Adrenocorticotropin hormone[4] (ACTH)
Interleukin-6[4] (IL6)	Alpha melanocyte-stimulating hormone[4] (αMSH)
Interleukin-15[4] (IL15)	Interleukin-4[4] (IL4)
Interleukin-18[4] (IL18)	Interleukin-10[4] (IL10)
Epidermal growth factor[4] (EGF)	Interleukin-13[4] (IL13)
Acidic fibroblast growth factor[4] (aFGF)	Transforming growth factor β[4] (TGFβ)
Nerve growth factor[3,4] (NGF)	Somatostatin (SRIH)
Brain-derived neurotropic factor[3] (BDNF)	Insulin-like growth factor-1[4] (IGF-1)
Neurotropin-3 (NT3)	Soluble TNF receptor[4] (STNFR)
Neurotropin-4 (NT4)	Soluble IL1 receptor[4] (sIL1R)
Interferon-α[4] (IFNα)	Interleukin-1-receptor antagonist[4] (IL1RA)
Interferon-γ[4] (IFNγ)	Glucocorticoid[4]
Tumour necrosis factor α[1,4] (TNFα)	Prostaglandin E_2[4] (PGE_2)
Tumour necrosis factor β[4] (TNFβ)	
Oleamide	
Growth hormone-releasing hormone[1,3,4] (GHRH)	
Adenosine[1,3,4]	
Prostaglandin D_2[1,3,4] (PGD_2)	
Nitric oxide[1,3,4] (NO)	
Nuclear factor kappa B[4] (NFκB)	
Uridine	
Vasoactive intestinal peptide[1,2] (VIP)	
Growth hormone[2,4] (GH)	
Prolactin[1,2,4] (PRL)	
Insulin[4]	

[1] Substances for which there is extensive evidence for their involvement in physiological sleep regulation.
[2] Promotes REMS only.
[3] Promotes both NREMS and REMS.
[4] Also involved in host defence.

animals. After such a disturbance, the return to NREMS is generally more rapid after IL1β- or TNFα-treatment than in control animals. After somnogenic doses of either IL1β or TNFα, there are no gross behavioural abnormalities; sleep postures remain normal and no motor dysfunction is evident. Changes in sleep-coupled autonomic functions also remain intact; for example, changes in brain temperature associated with sleep state persist in IL1β- or TNFα-treated animals.

As mentioned earlier, inhibition of either IL1 or TNF reduces spontaneous NREMS, thereby strongly implicating these substances in physiological sleep regulation. Thus, anti-TNF or IL1 antibodies, soluble IL1 or TNF receptors, and the IL1 receptor-antagonist cytokine inhibit spontaneous NREMS and the expected NREMS rebound induced by sleep deprivation (Krueger and Majde, 1994). The latter data suggest that IL1 and TNF are also involved in sleep-deprivation-induced excess NREMS. In contrast, inhibition of IL18 (related to IL1) does not affect spontaneous NREMS, although it does attenuate bacterial cell-wall product-induced sleep (Kubota *et al.*, 2001). Other physiological inhibitors of IL1 or TNF also inhibit spontaneous sleep; the list includes IL4 (Kushikata *et al.*, 1998), IL10 (Opp *et al.*, 1995), IL13, TGFβ, PGE_2, α-melanocyte-stimulating hormone, glucocorticoids, and CRH (reviewed by Krueger & Obál, 1997) (Table XXIV-4.1) (Figure XXIV-4.1). Many of these substances form part of a feedback mechanism dampening the proinflammatory cytokine cascade (Figure XXIV-4.1).

There are diurnal variations within the brain of IL1β mRNA (Taishi *et al.*, 1997) and TNF mRNA levels (Bredow *et al.*, 1997), as well as IL1β (Nguyen *et al.*, 1998) and TNFα (Floyd & Krueger, 1997) protein levels. These diurnal variations occur in the hypothalamus (an area involved in sleep regulation) and several other brain areas including the hippocampus and the cerebral cortex. After sleep deprivation, hypothalamic IL1β mRNA levels (Taishi *et al.*, 1998; Mackiewicz *et al.*, 1996) and TNF mRNA levels (Veasey *et al.*, 1997) increase. In contrast, related molecules such as the IL1 receptor accessory protein are not affected by sleep deprivation (Taishi *et al.*, 1998). IL1β also seems to be involved in the excess NREMS associated with excess food intake; rats on a cafeteria diet express increased IL1β mRNA in the hypothalamus during peak food-induced sleep responses (Hansen *et al.*, 1998c). In humans, there is a diurnal rhythm in plasma levels of TNFα, and plasma levels of TNFα correlate with EEG-slow-wave activity (Darko *et al.*, 1995). Furthermore, in humans, plasma levels of TNFα increase after sleep deprivation (Yamusa *et al.*, 1992), and the ability of circulating white blood cells to produce TNFα increases after sleep deprivation (Uthgenannt *et al.*, 1995; Hohogen *et al.*, 1993).

Mutant mice lacking the TNF 55-kDa receptor do not exhibit sleep responses if given TNFα, although they are responsive to IL1β (Fang *et al.*, 1997). In contrast, mutant mice lacking the IL1 type I receptor are unresponsive if given IL1β, but do exhibit robust NREMS responses if given TNFα (Fang *et al.*, 1998). Both strains of mice sleep less than strain controls, although the sleep deficit in the TNF-receptor knockout mice occurs mostly during daylight hours while that in the IL1 type I receptor knockout mice occurs during the night-time. In humans, sleep deprivation induces enhanced levels of the 55-kDa soluble TNF receptor, but not the 75-kDa soluble TNF receptor (Shearer *et al.*, 2001).

Certain clinical conditions associated with excessive sleepiness also seem to involve cytokines; that is, sleep apnoea patients have elevated TNF plasma levels (Entzian *et al.*, 1996; Vgontzas *et al.*, 1997). HIV-AIDS patients have disrupted TNF rhythms (Darko *et al.*, 1995). Treatment of rheumatoid arthritis patients with the TNF 75-kDa soluble receptor alleviates the fatigue associated with the disease (Franklin, 1999). TNFα is elevated in chronic fatigue patients (Moss *et al.*, 1999). Elevated TNF also occurs in pre-eclampsia patients accompanied by excess sleep (Edwards *et al.*, 2000). Postdialysis fatigue is also associated with elevated TNF levels (Dreisbach *et al.*, 1998; Sklar *et al.*, 1998), and cancer patients receiving either TNFα or IL1β report fatigue (e.g., Eskander *et al.*, 1997). Infectious challenge induces a plethora of proinflammatory cytokines and many other substances implicated

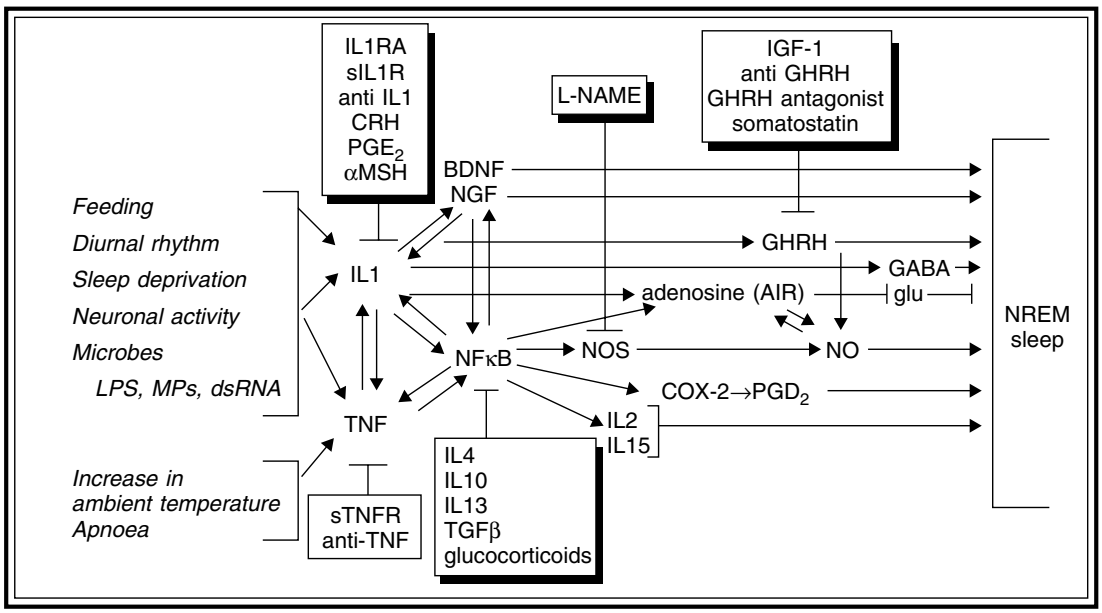

Figure XXIV-4.1 Biochemical cascades are involved in sleep regulation. Substances in boxes inhibit sleep and inhibit the production or actions of the sleep-promoting substances illustrated via feedback mechanisms. Inhibition of one step does not completely block sleep, since parallel sleep-promoting pathways exist. These redundant pathways provide stability to sleep regulation. Our knowledge of the biochemical events involved in sleep regulation is more extensive than that illustrated. For abbreviations, see Table XXIV-4.1

in sleep regulation. These same substances also play a role in host defence (Table XXIV-4.1).

The Somatotrophic Axis

In humans, a major peak in growth hormone (GH) release occurs during the first period of deep, slow-wave sleep (Takahashi *et al.*, 1968). Furthermore, abnormal GH secretory patterns are associated with sleep disorders (e.g., Aström and Linhölm, 1990). In addition, during extended wakefulness, GH release is suppressed, and when subjects are allowed to sleep, excess GH secretion occurs (e.g., Moldofsky *et al.*, 1988). Nevertheless, dissociation between GH release and sleep can occur (Carlson *et al.*, 1972), and it is thus posited that GHRH provides the link between GH release and sleep (Obál *et al.*, 1988; reviewed by Krueger *et al.*, 1999).

GHRH administration induces increases in the amount of time spent in NREMS in several species including rats, rabbits, and humans (reviewed by Krueger and Obál, 1997). For instance, in humans, systemic injection of GHRH (e.g., Steiger *et al.*, 1992) and a GHRH-containing nasal spray (Perras *et al.*, 1989) enhance NREMS. The GHRH-induced NREMS is characterized by supranormal EEG slow waves and, thus, is thought to be of greater intensity. GHRH can also induce increases in REMS (Obál *et al.*, 1988). However, in hypophysectomized animals, GHRH induces only NREMS (Obál *et al.*, 1996), and the REMS-promoting actions of GHRH are thought to be the result of pituitary GH release. The sleep induced by GHRH appears normal in the sense that it is easily reversible if animals are disturbed, and no abnormal behaviours are evident.

Inhibition of GHRH — for example, by using an anti-GHRH antibody or a GHRH peptide antagonist — inhibits spontaneous NREMS and sleep rebound after sleep loss (e.g., Zhang *et al.*, 1999). Transgenic mice that overexpress GH in the brain have less NREMS, but not REMS; these mice have less GHRH and more somatostatin (SRIH) in the brain (Zhang *et al.*, 1996). Furthermore, dwarf rats and lit/lit mice, which underexpress GHRH receptors in the hypothalamus, also have reduced NREMS and REMS (Obál *et al.*, 2001; Alt *et al.*, 2001). Inhibition of GHRH by antibodies also attenuates IL1β-induced excess NREMS, thus suggesting that IL1β elicits its effects on sleep, in part, via GHRH (reviewed by Krueger *et al.*, 1999).

Within the hypothalamus, GHRH-containing neurons are found in the arcuate nucleus and around the ventral medial nucleus. The former project to the median eminence and are probably involved in GH release. The ventromedial GHRHergic neurons, including those in the paraventricular nucleus, project to both the median eminence and to the basal forebrain. The preoptic area — basal forebrain — is involved in NREMS regulation; for example, damage of this area is associated with insomnia (von Economo, 1930), and rapid electrical stimulation of this area induces NREMS. This area also contains GHRH receptors. Microinjection of GHRH into the anterior hypothalamus induces excess NREMS, whereas injection of the GHRH peptide antagonist into the same area inhibits NREMS and attenuates sleep rebound after sleep loss (Zhang *et al.*, 1999). There is a diurnal rhythm of GHRH mRNA within the hypothalamus, but not other areas of brain; the highest levels occur during the onset of daylight hours, which is the peak sleep period in rats (Bredow *et al.*, 1996). After sleep deprivation, there also is an increase in GHRH mRNA levels in the hypothalamus (Zhang *et al.*, 1998). These changes in GHRH mRNA are found only in the extra-arcuate GHRHergic neurons (Toppila *et al.*, 1997). The GHRH content of the hypothalamus also has a diurnal rhythm, with the highest values at the beginning of the dark period, and decreased amounts associated with sleep loss (Gardi *et al.*, 1998), suggesting that GHRH release is associated with higher sleep propensity.

Other members of the somatotropic axis also influence sleep and GHRH secretion. SRIH inhibits GHRH and GH release, and it inhibits NREMS, presumably via its effects of GHRH. In contrast, SRIH stimulates REMS despite the fact that it inhibits GH release (Beranek *et al.*, 1997). GH by itself stimulates REMS (reviewed by Krueger and Obál, 1997), and the promotion of REMS by GHRH in thought to be mediated via release of pituitary GH. Furthermore, microinjection of GHRH into the basal forebrain induces NREMS, but not REMS. GH may, via its metabolic actions, have a NREMS-promoting activity; sleep is reduced in rats treated with anti-GH antibodies (Obál *et al.*, 1997a). In contrast, GH stimulates insulin-like growth factor-1 (IGF-1); IGF-1 has a brief inhibitory action on both REMS and NREMS (Obál *et al.*, 1998).

The role of the somatotrophic axis in sleep responses elicited by infectious agents remains relatively uninvestigated. However, GH release is increased during infection, and is induced by cytokines such as IL1 and TNF. GH also has many direct actions on immunocytes, and the effects of IL1 on sleep involve, in part, GHRH. Finally, preliminary data from our laboratory indicate that lit/lit mice that lack functional GHRH receptors sleep less after being challenged with influenza virus; normal mice sleep much more (Alt *et al.*, 2001). Collectively, such data suggest a role for the somatotropic axis hormones in sleep regulation in health as well as in disease.

Prolactin

REMS is enhanced in response to systemic administration of PRL in hypophysectomized pontine cats (Jouvet *et al.*, 1986). Since this initial anecdotal report, several additional lines of evidence have implicated PRL in the regulation of REMS (reviewed by Roky *et al.*, 1995). The major findings are as follows:

1. Administration of exogenous PRL stimulates REMS in cats, rabbits, and rats.
2. PRL-induced increases in REMS develop slowly, over 2–3 h, and are then maintained for several hours.
3. PRL-induced changes in sleep are restricted to REMS; that is, NREMS is not altered after bolus injection of PRL.
4. In rats, PRL enhances REMS only during the light period.
5. In contrast, REMS is suppressed during the light period and is enhanced at night in PRL-deficient rats (Valatx *et al.*, 1990).
6. Rats made chronically hyperprolactinaemic, by grafting pituitaries under the kidney capsule, have increases in REMS during the light period of the day (Obál *et al.*, 1997b).

Interestingly, small enhancements in NREMS were also observed in these animals. REMS and NREMS are also enhanced in hyperprolactinaemic pseudopregnant rats (Zhang, 1995). Slight increases in NREMS might be attributed to a GH-like effect of chronically increased PRL.

The modulation of REMS is a central action of PRL. Thus, intracerebral injection of PRL or anti-PRL antibodies stimulates or inhibits REMS, respectively (Roky *et al.*, 1995). Brain PRL may originate from two sources: it is produced by hypothalamic neurons, and PRL is also released by the anterior pituitary, and circulating PRL is transported into the brain via a specific receptor-mediated transport mechanism residing in the choroid plexus (Walsh *et al.*, 1987). The REMS-promoting activity of blood-borne PRL is suggested by several observations. REMS is enhanced after systemically injected PRL (Jouvet *et al.*, 1986; Obál *et al.*, 1989), excess PRL released from pituitary grafts (Obál *et al.*, 1989), or stimulation of endogenous PRL secretion from the pituitary (Obál *et al.*, 1994). Furthermore, ether stress induces increases in plasma levels of PRL and REMS; the latter increases do not occur if rats are hypophysectomized or if they are treated with anti-PRL antibodies before being exposed to the ether stressor (Bodosi *et al.*,

2000). Nevertheless, only small decreases in REMS occur when basal, non-stimulated blood PRL is immunoneutralized (Obál *et al.*, 1992). Increases in plasma PRL stimulate REMS, but, at normal concentrations, circulating PRL has only a slight effect on REMS regulation. The impact of increased blood PRL on REMS is, in part, attributed to a stimulation of the expression of PRL receptors in the choroid plexus because PRL enhances its own transport into the brain. It is likely that intracerebral PRL modulates REMS under physiological conditions whereas pituitary PRL provides additional stimulating influence when PRL secretion is high, as in stress. Furthermore, intracerebroventricular injections of VIP and PACAP promote REMS (Obál *et al.*, 1989; Fang *et al.*, 1995b) and elicit expression of PRL mRNA in the hypothalamus (Bredow *et al.*, 1994). Anti-PRL antibodies block VIP-induced REMS response. In conclusion, the observation that PRL is probably involved in modulating REMS and is also involved in host-defence provides another link between sleep and host-defence mechanisms.

CENTRAL TARGETS IN THE BRAIN: NFκB AND NO SYNTHASES

The biochemical regulation of NREMS is undoubtedly complex, involving many biochemical cascades operating in several cell types within the brain. Several of the sleep-regulatory substances (Figure XXIV-4.1) are either upregulated in response to NFκB activation or themselves induce NFκB activation. For instance, several sleep-promoting substances activate NFκB; the list includes IL1, TNF, NGF, interferon-α, epidermal growth factor, acidic fibroblast growth factor, insulin, and insulin-like growth factor (reviewed by Krueger and Majde, 1994; Krueger *et al.*, 1999). Furthermore, NFκB is involved in the expression of IL2, cyclooxygenase-2, inducible NOS (reviewed by Eizirik *et al.*, 1996), IL1, TNF (reviewed by Ballou *et al.*, 1996), NGF, and the adenosine A_1 receptor (Nie *et al.*, 1998), all of which are part of the biochemical network involved in sleep regulation (Figure XXIV-4.1). Other substances, such as IL4, IL10 (e.g., Clarke *et al.*, 1998), and glucocorticoids (see Barnes, 1997), directly or indirectly inhibit NFκB activation, and they inhibit sleep (Krueger *et al.*, 1999). Furthermore, sleep deprivation promotes NFκB activation in murine cerebral cortex, and cortical NFκB activation has a diurnal rhythm, with higher levels of activation occurring during daylight hours (the sleep period in mice) than during the dark period (Chen *et al.*, 1999). Thus, the activation of NFκB correlates with high sleep propensity. Furthermore, a NFκB cell-permeable inhibitor peptide, given centrally, inhibits spontaneous NREMS and REMS, and attenuates IL1β-enhanced NREMS (Kubota *et al.*, 2000). Finally, NFκB is involved in the production of several other transcription factors that could also be involved in sleep regulation, such as c-fos (Shiromani *et al.*, 1998).

NO also seems to function as an endogenous sleep-promoting substance. NO is produced by three distinct NO synthases (NOSs), all of which are found in the brain. NOS inhibitors injected systemically or intracerebroventricularly (i.c.v.) suppress REMS and NREMS in rats and rabbits (reviewed by Kápas *et al.*, 1993). Conversely, systemically or i.c.v. administered, the NO donors *S*-nitrosyl-*N*-acetylpenicillamine (SNAP) and 3-morpholinosydnonimine (SIN-1) mimic the effects of sleep deprivation, in that each induced prolonged increases in the duration and intensity of NREMS in rats after a 7–9-h delay (Kapás *et al.*, 1996). Furthermore, NREMS responses after sleep deprivation are diminished in rats given a NOS inhibitor prior to deprivation (Riberio *et al.*, 2000). Inducible NOS (iNOS or NOS-2) is stimulated by proinflammatory cytokines and is dependent on NFκB activation, making this isoform an attractive candidate for sleep regulation.

While the specific signalling mechanisms involved in sleep responses to NO stimulation remain incompletely understood, one effect probably involves modulation of cholinergic signalling within pontine nuclei linked to sleep-wake regulation. Thus, decreased acetylcholine release occurs in the pontine reticular formation (PRF) of cats injected into the PRF with NOS inhibitors (Leonard and Lydic, 1995; 1997), and is accompanied by decreased REMS. Injection of the pedunculopontine tegmental nucleus (PPT) with NOS inhibitors likewise affects sleep, with reduced REMS and NREMS observed in cats (Datta *et al.*, 1997) and rabbits (Williams *et al.*, 1997). Conversely, increases in REMS and NREMS occur after PPT injection of the NO precursor L-arginine in rats (Hars, 1999), or the NO donor SNAP (*S*-nitroso-*N*-acetylpenicillamine) in cats (Leonard and Lydic, 1997). Thus, manipulation of NO levels in this neuronal circuit appears to influence sleep and waking states through modulation of the cholinergic neurotransmission involved in ascending reticular activation.

NO may also affect sleep through interaction with other sleep-regulatory pathways. For instance, NO production is induced by a number of other sleep-regulatory substances, including IL1, GHRH, adenosine (Rosenberg *et al.*, 2000), and TNFα (reviewed by Krueger and Majde, 1994). The somnogenic effects of IL1 signalling may be exerted, in part, by its induction of NO, as enhanced sleep in rabbits after i.c.v. IL1 injection is abolished if the rabbits are given a NOS inhibitor (Kapás *et al.*, 1994).

It is hypothesized (L. Kapás, personal communication) that opposing influences of NO on circadian and homeostatic sleep-regulatory mechanisms are responsible for sleep-wake regulation. In the suprachiasmatic nucleus (SCN) of rats, NO sensitivity is highest during the dark, behaviourally active period. NO production in the SCN is enhanced by retinohypothalamic glutamate release, and functions to promote arousal, possibly via a cGMP-dependent enhancement of neurotransmission. Conversely, NO promotes sleep within the brain structures involved in homeostatic sleep regulation. Within these sites, NO sensitivity does not exhibit circadian variation. Rather, NO production progressively increases in a neuronal use-dependent manner, such that its tonic release may influence multiple potential effectors, which collectively may increase sleep propensity. NO is elevated in mouse influenza-infected lungs (Akaike *et al.*, 1996), but NOS activity in the brain has not been examined in influenza. However, mice lacking inducible NOS do not have as large a NREMS response after influenza viral challenge (Chen *et al.*, unpublished).

SLEEP DEPRIVATION AND IMMUNE CONSEQUENCES

Rats deprived of sleep for 2–3 weeks die (Rechtschaffen *et al.*, 1983). The probable cause of death is septicaemia; the bacteria cultured from the blood are primarily facultative anaerobes indigenous to the host (Everson, 1993). Whether death or septicaemia occurs in other species after sleep deprivation has not been determined. In any case, the results with rats suggest that loss of sleep leads to host-defence breakdown. Consistent with this notion are the more recent findings that after just a few days of sleep deprivation viable bacteria can be cultured from organs of filtration such as mesenteric lymph nodes (Everson & Toth, 1997; Landis *et al.*, 1997), and the number of bacteria in the intestine increases (Everson, 1993; Bergmann *et al.*, 1996b). The mechanisms responsible for these effects remain unknown, although, as described below, several immune system measures are altered by sleep deprivation. Furthermore, these sleep-linked changes in bacteria could be related to a normal endosymbiotic relationship between bacteria and the host. Rats treated with antibiotics (neomycin and metronidazole) have reduced NREMS but normal REMS (Brown *et al.*, 1990), thereby suggesting that bacterial products could affect everyday sleep (reviewed by Krueger and Majde, 1994), as discussed above in the section on indigenous microbial flora.

In contrast to long-term sleep deprivation, short-term sleep deprivation does not result in death and, under some circumstances, may even enhance host-defence mechanisms. For example, in one study, rats deprived of sleep had smaller tumours than corresponding control animals (Bergmann *et al.*, 1996a). Other studies suggest that short-term sleep deprivation has little effect on infection. In human studies of sleep deprivation, there has been a failure to demonstrate an increased incidence of infection. However, most of these studies involve healthy young volunteers in unchallenging environmental conditions. In fact, if studies were designed to increase the probability of infection, they probably would not be approved. Consistent with these human studies is a report that sleep deprivation of rabbits failed to exacerbate *E. coli*-induced clinical illness (Toth *et al.*, 1995). Nevertheless, there is a wealth of data indicating that short-term sleep deprivation is associated with changes in immune system parameters.

Several laboratories have measured natural killer cell (NK) activity in conjunction with sleep and sleep loss. In several of these studies, circulating NK activity decreased after sleep deprivation (Moldofsky *et al.*, 1989; Irwin *et al.*, 1994, 1996). In other studies, circulating NK activity increased after sleep deprivation (Born *et al.*, 1997; Dinges *et al.*, 1994). Although there is a reduction of NK activity in depressed insomniac patients (Irwin *et al.*, 1992), in normal individuals NK activity may decrease during sleep (Moldofsky *et al.*, 1989). In summary, it seems likely that sleep or sleep loss may affect NK activity, but the magnitude and direction of such effects is sensitive to specific experimental conditions and the subject population.

Sleep deprivation affects several other facets of the immune system, including antigen uptake (Casey *et al.*, 1974), lymphocyte DNA synthesis (Palmblad *et al.*, 1979), phagocytosis (Palmbald *et al.*, 1976), mitogen responses (Moldofsky *et al.*, 1989), circulating immune complexes (Isenberg *et al.*, 1981), circulating IgG levels (Renegar *et al.*, 1998), secondary antibody responses (Brown *et al.*, 1989), and a variety of lymphocyte subsets (Dinges *et al.*, 1994). Sleep deprivation is also associated with changes in cytokines. Sleep loss is associated with an enhanced ability of lymphocytes to produce interferon (Palmblad *et al.*, 1976), increased white blood cell and monocyte TNF production (Yamasu *et al.*, 1992; Uthgenannt *et al.*, 1995), increased production of IL1β and IFNα by cultures of whole blood (Hohagen *et al.*, 1993), and increased plasma IL1 activity in humans and rats (Moldofsky *et al.*, 1989; Opp & Krueger, 1994). In normal people, and in people with sleep disorders, plasma levels of cytokines are related to the sleep-wake cycle or sleep disturbance. For example, IL1 plasma levels peak at the onset of NREMS (Moldofsky *et al.*, 1986), and TNF levels vary with EEG-slow-wave amplitude (Darko *et al.*, 1995). Patients with obstructive sleep apnoea syndrome have altered plasma TNF (Entzian *et al.*, 1996; Vgontzus *et al.*, 1997). Patients with psychoses who have reduced sleep have enhanced IL1β plasma levels (Appelberg *et al.*, 1997).

Collectively, the data cited above strongly suggest that sleep and sleep loss affect immune function. Nevertheless, few studies have directly measured the effect of sleep on host outcome. Study results often leave one uninformed as to whether the effects of sleep or sleep loss is adverse or beneficial to the host. Despite such limitations, it does appear that sleep influences immune function. Furthermore, currently, it appears that short-term sleep loss may enhance host defences, whereas long-term sleep loss is devastating.

RECUPERATIVE PROPERTIES OF SLEEP

There is no direct evidence that sleep per se aids in recuperation from infectious diseases or inflammation, primarily because of the difficulty of isolating sleep as an independent variable. Thus, during manipulations of sleep, such as sleep deprivation many physiological parameters vary. For example, sleep deprivation, is associated with changes in hormonal and cytokine production, body temperature, food intake, metabolic rate, etc. However, there are some data consistent with the notion that sleep is beneficial during infectious disease. After microbial challenge, rabbits that had robust NREMS responses within the first 12 h had a higher probability of survival than animals that failed to exhibit NREMS responses (Toth *et al.*, 1993). Although this evidence is correlative in nature, it suggests that perhaps our grandmothers' folk wisdom of the curative and preventative properties of sleep may, in fact, be correct. In any event, it is likely that physicians will continue to prescribe bed rest and sleep because this is often just what the patient wants to do.

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XXIV-5

Psychophysiology of Sleep Disorders

R.T. Pivik

INTRODUCTION

Understanding the disordered presupposes a fundamental knowledge of the normal reference state or behaviour. It should come as no surprise, therefore, that our understanding of the psychophysiology of sleep disorders is limited, given that our knowledge of the characteristics and processes associated with normal sleep physiology and related variations in cognition can, in many respects, be considered elementary. Until relatively recent times, the development of knowledge about sleep was restricted by conceptual limitations and behavioural barriers. The emphasis on wakefulness as the state during which the most important behavioural and cognitive accomplishments occurred diminished the importance of sleep as a behaviour worthy of or even requiring investigation. This attitude was reflected in the view of the waking state as 'the sole portion of ... existence that "counts" in any way, sleep appearing as "time out" from the game of living' (Kleitman, 1963: p. 3) — an attitude reinforced by the behavioural inertness characteristic of the sleeping organism. Still, the influence of sleep on waking behaviour is undeniable and evident both in its timing and duration. The occurrence of sleep can be considered to be subject to limited volitional control since it is possible to remain awake for long periods of time or to select convenient times and environments for sleeping. However, at times the pressure for sleep is irresistible and sleep will occur despite the known likelihood of potentially deadly consequences, as while driving an automobile. The amount of sleep obtained has also been shown to be important for normal waking activities. The adverse effects of reduced sleep on alertness and performance are well documented (Monk, 1991; Gillberg and Äkerstedt, 1994), and, in the extreme, the long-term absence of sleep may lead to death (Horne, 1988; Everson, 1995; Bentivoglio and Grassi-Zucconi, 1997). From these observations, two important inferences can be drawn: 1) sleep is not simply a passive state during which physical activities are suspended; and 2) sleep processes interact with those occurring during waking in complementary and synergistic ways to maintain and extend life. Until the mid-20th century, insight into the nature of this codependence was prevented by the inability to obtain information regarding ongoing processes from the sleeping organism. Technologic developments during the past half-century have largely removed obstacles to these efforts. Notable among these developments has been the ability to conduct recordings of central nervous system and related autonomic and peripheral nervous system activities over the extended time periods that sleep typically occurs. More recently, these measures have been supplemented with assessments of state-related variations in brain metabolism using imaging procedures.

The interest in applying these technologic tools to chart variations in sleep physiology across the night was accompanied by an equally intense curiosity regarding what these measures might reveal about the occurrence of mental activity during sleep. The long-standing belief that experiences of mental activity continue during sleep in the form of dreams suggested that sleep was not a mental void. Furthermore, since these mental experiences occur during times of sustained disengagement from the environment, the nature of psychophysiological relationships under these circumstances could be different than those present during wakefulness. The advent of the new methods brought the study of these relationships within the range of effective experimental control. Investigations using these new procedures revolutionized the study of sleep, and the findings from this research have forced a redefinition of concepts regarding physiological and psychological activities and events that can be considered normal.

Although it is apparently the natural order that the states of sleep and wakefulness remain separate, their codependence renders them inseparable and interactive. Some aspects of these state interactions are fixed, and others more variable. Since the existence of higher mammals can be considered a closed system generally dichotomized into states of sleep and wakefulness (although conditions exist which do not clearly fall into either of these categories, such as coma and anaesthetic states), interactions based on state duration are constrained and essentially hydraulic in nature — that is, increases in time spent in one state are at the expense of time spent in the other.

It can be assumed that an important determinant of state duration is the time needed to satisfy functional requirements. Consequently, an important source of sleep-wakefulness interactions derives from the manner in which within-state behaviours (or processes) affect, or are reflective of, the realization of these requirements. Broad examples of such variations would include the nature of waking activity (e.g., resting vs. vigorous physical activity) or sleep quality (e.g., quiescent vs. disturbed). Such variations have both immediate within-state effects as well as longer-term, between-state consequences.

The relative inaccessibility of sleep processes to systematic study delayed the recognition of these interactions and consequences as significant determinants of behaviour. This delayed acknowledgement was evident in the absence of sleep-related variables among factors considered contributory to mental disorders in the initial publications of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) in 1917 and 1952 (DSM-I). The publication of DSM-I occurred near the time that rapid-eye-movement (REM) sleep was discovered (Aserinsky and Kleitman, 1953) — a revelation that effectively launched the new era of sleep research. Fifteen years later (1968), DSM-II appeared, and for the first time sleep disorders were included (referenced under 'Special Symptoms'). By 1979, an understanding of sleep had developed to the point that a 'Diagnostic Classification of Sleep and Arousal Disorders' could be published. Beginning with the publication of DSM-III one year later, the involvement of sleep disorders in the determination of mental status has been consistently and more fully represented.

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As background for a consideration of the associations between disordered sleep and mental processes, a brief overview of fundamental observations that have been made regarding normative sleep physiology and sleep-associated cognition will be provided.

SLEEP PHYSIOLOGY: DESCRIPTIVE ASPECTS

As previously mentioned, the technology that provided the window through which investigators could view sleep processes in progress was the ability to conduct long-term recordings of electrophysiological activities. Although this technology was available and being used to describe sleep-related variations in electroencephalographic (EEG) activities in the 1930s (Loomis *et al.*, 1937, 1938), it would be another 15 years before the discoveries that brought renewed attention to the study of sleep would begin to be made. These discoveries consisted of reports of recurring episodes of physiological activation during sleep that were related to the occurrence of dreaming (Aserinsky and Kleitman, 1953, 1955; Dement, 1955; Dement and Kleitman, 1957a, 1957b; Dement and Wolpert, 1958a, 1958b). Subsequent research has provided a detailed map of the physiological and psychological dimensions of sleep.

The work begun by Loomis *et al.* (1937, 1938) in describing sequential brain potential patterns during sleep has been expanded to include the additional physiological descriptors (measures of autonomic and muscle activities), and stages of sleep have been defined and their variations charted across the night (Dement and Kleitman, 1957a; Rechtschaffen and Kales, 1968). Examples of physiological variations characteristic of sleep-wakefulness and within sleep-stage differentiations are presented in Figures XXIV-5.1 and XXIV-5.2. Sleep-stage determinations in these figures are based on the standardized criteria for the

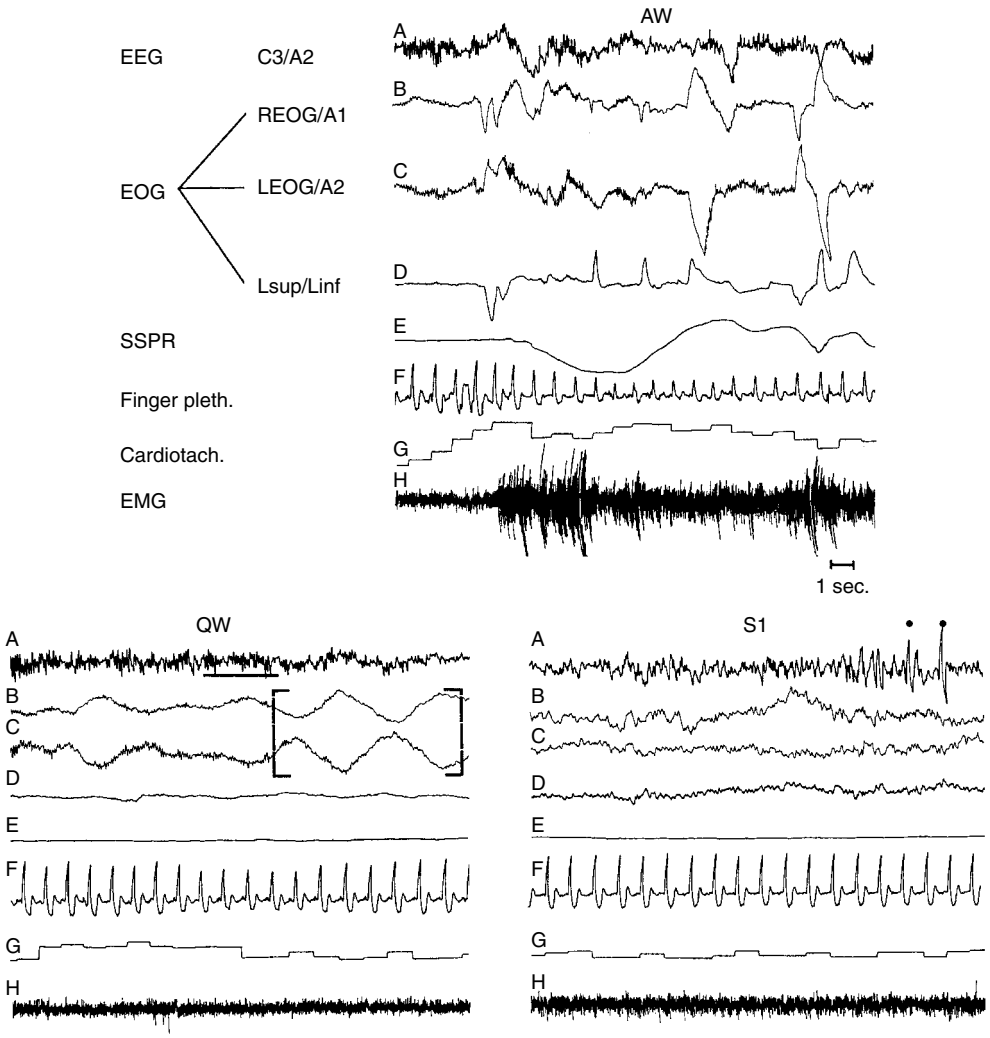

Figure XXIV-5.1 Polygraphic tracings of physiological measures associated with sleep-wakefulness state variations. Eight channels of activity (A–H) are depicted during active and quiet wakefulness (AW and QW, respectively) and the first stage of sleep (NREM stage 1, or S1). The recorded variables include the electroencephalogram (EEG; C3/A2, channel A), the electro-oculogram (EOG; horizontal [right and left outer canthi] and vertical [placements superior and inferior to right eye orbit], channels B, C, and D, respectively), autonomic activity (spontaneous skin potential response [SSPR], channel E), finger plethysmogram (channel F), cardiotachometer recordings (channel G), and the facial (orbicularis oris) electromyogram (EMG; channel H). In these examples, the passive decrease in EMG activity which commonly occurs at sleep onset (S1) does not become evident until slow-wave sleep (Figure XXIV-5.2, S3 and S4). Electrophysiological features of note are alpha activity (underscored, channel A) and slow, rolling eye movements (channels B and C) preceding sleep onset in QW, and vertex sharp waves in stage 1 (see dots, channel A). See text for discussion of sleep-stage definitions and electrophysiologic composition (Reproduced from Pivik, 2000, by permission of Cambridge University Press)

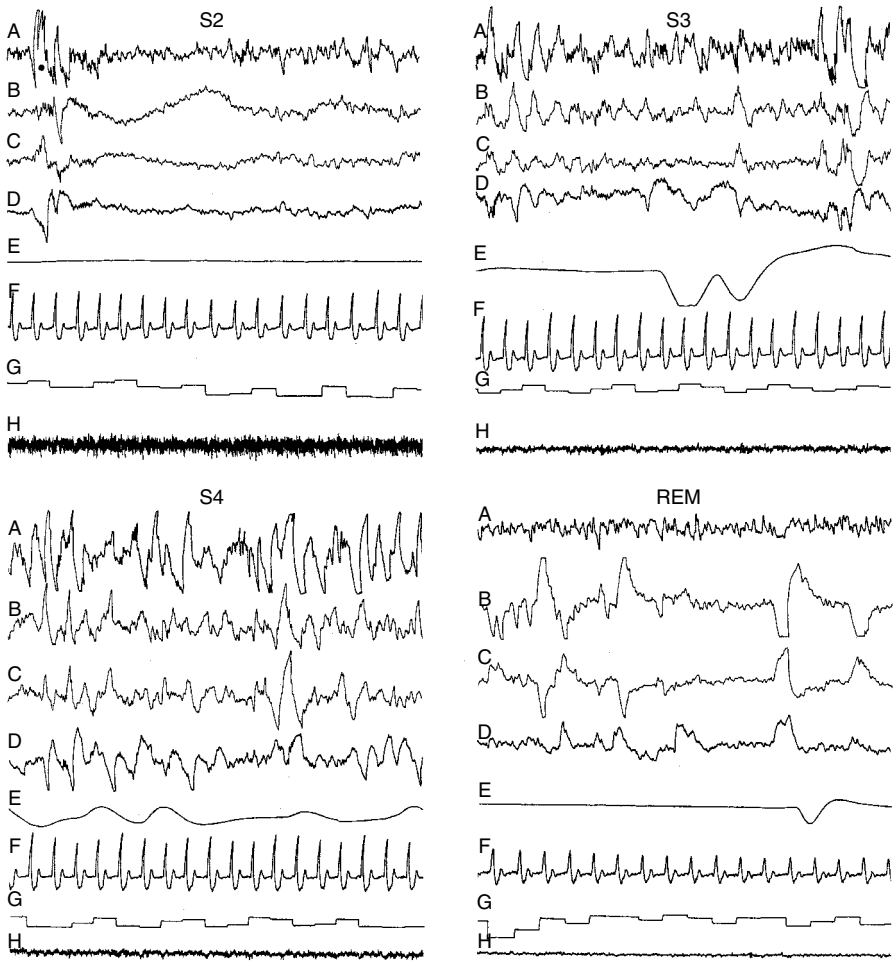

Figure XXIV-5.2 Polygraphic tracings of physiological measures associated with variations in sleep stages (NREM stages 2–4 [S2–S4] and REM). For explanation of channels A–H, see Figure XXIV-5.1. Of note is the occurrence of stage 2 K-complexes (see dot, channel A). See text for discussion of sleep stage definitions and electrophysiological composition (Reproduced from Pivik, 2000, by permission of Cambridge University Press)

sleep-stage analysis of adult human sleep published by Rechtschaffen and Kales (1968). These figures include the basic measures required for sleep evaluation (EEG, EOG, and EMG; channels A, B, C, and H), as well as optional measures, such as recordings of vertical eye movements (channel D) and autonomic activity (channels E, F, and G). In these illustrations, wakefulness (AW and W) is associated with a low-voltage, mixed-frequency EEG, blinking, rapid eye movements, and variations in the levels of tonic facial EMG activity.

As accurately described by Davis *et al.* (1938), 8–12 Hz alpha activity is diminished during the transition from wakefulness to sleep. This is accompanied by a slowing of EEG activity, an increase in 4–7 Hz theta activity, the sporadic occurrence of vertex sharp waves, and the appearance of slow horizontal eye movements, while facial muscle tonus may decrease relative to levels present during relaxed wakefulness (see Figure XXIV-5.1, S1). Stage 2 is defined by the intermittent occurrence of K-complexes and 12–14 Hz spindle activity against a background of relatively low amplitude, mixed-frequency EEG activity (Figure XXIV-5.2). Relative to stage 2, during stages 3 and 4 (slow-wave sleep) there are increasing amounts of delta activity (0.5–4 Hz) present in each scoring epoch. Stage 3 epochs must contain 20–50%, and stage 4 epochs more than 50%, of this activity (Figure XXIV-5.2). Collectively, stages 1–4 are referred to as non-REM (NREM) sleep. REM sleep is defined by a relatively low-voltage, mixed-frequency EEG, the absence of K-complexes or spindles, the sporadic occurrence of eye movements, and reduced levels of submental and facial EEG activity (Figure XXIV-5.2). Other distinctive EEG features which may be present during REM sleep are bursts of theta activity (sawtooth waves) preceding clusters of eye movements (Berger *et al.*, 1962), and alpha activity which is 1–2 Hz slower than subjects' waking alpha frequency (Johnson *et al.*, 1967).

Associated with these sleep stages are other variations in physiologic measures which, although not integral to stage determination, serve to reinforce and validate stage differentiation. Prominent among these are the presence of generalized physiologic activation during REM sleep, including increases in the rate and irregularity of respiratory (Snyder *et al.*, 1964; Aserinsky, 1965) and cardiovascular (Snyder *et al.*, 1963, 1964; Pivik *et al.*, 1996) activities, and increased density of eye movements as a function of time both within individual REM periods and within REM periods across the night (Aserinsky, 1969, 1971). Electrodermal activity in REM sleep is reduced and more similar in form to responses of this system during wakefulness (Broughton *et al.*, 1965; Hauri and Van de Castle, 1973b). These REM-associated variations occur against a background of centrally mediated inhibition of facial and submental musculature and spinal monosynaptic reflexes (Jouvet and Michel, 1959; Berger, 1961; Hodes and Dement, 1964; Jacobson *et al.*,

1964; Pompeiano, 1966, 1967). Other notable physiologic variations that occur in association with REM sleep include increases in body movements and K-complexes just prior to REM periods (Dement and Kleitman, 1957a; Pivik and Dement, 1968, Halasz *et al.*, 1977) and reduced numbers of K-complexes and increased spindle activity in the few minutes following REM periods (Pivik and Dement, 1968; Azumi *et al.*, 1975).

With the exception of unusual levels of autonomic, hormonal, and motor activation present during slow-wave sleep, physiological activation during most NREM sleep is unremarkable. During stage 4, for example, dramatic increases in electrodermal activity are common (Broughton *et al.*, 1965; Johnson and Lubin, 1966) (Figure XXIV-5.2). Electrodermal activation of this intensity would normally indicate enhanced arousal, yet the arousal threshold during stage 4 is higher than that of all other sleep stages (Goodenough *et al.*, 1965; Bonnet and Moore, 1982; Lammers and Badia, 1991; Busby *et al.*, 1994). An explanation consistent with these data attributes this overactivity to the release of subcortical control areas involved in the production of these responses from inhibition by higher centres, and not to a condition of enhanced physiological arousal (Johnson and Lubin, 1966; Johnson, 1973). During slow-wave sleep, approximately 80% of the total daily secretion of growth hormone is released (Takahashi *et al.*, 1968; Sassin *et al.*, 1969; Born *et al.*, 1988). It is also during this time that a variety of arousal disorders (termed parasomnias), characterized by varying degrees of motor and autonomic activity, occurs (Roffwarg, 1979). More detailed considerations of the nosology, description, and treatment of these and other sleep-related disorders are available (*International Classification of Sleep Disorders Diagnostic and Coding Manual* [*ICSD*, 1990] or *Principles and Practice of Sleep Medicine* [Kryger *et al.*, 2000]).

The nature and organization of the physiologic variations described above differ distinctly from those present during wakefulness, and these differences can be summarized as follows:

1. The presence of waveforms unique to sleep — for example, endogenously determined K-complexes, 12 to 14 Hz spindle activity, vertex sharp waves, and frontal sawtooth waves.
2. The prevalence and concentration of activities — for example, the enhancement of slower EEG frequencies (delta and theta), and the concentration of these and other activities, such as eye movements or galvanic skin responses (GSRs), at specific times of the night. With respect to EEG activity, computerized analyses have shown that in only rare instances is the EEG composed of a single frequency; even in the desynchronized low-voltage EEG of wakefulness in normal individuals, there is a small but nonetheless real component of delta activity present (Lubin *et al.*, 1969; Hoffman *et al.*, 1979). The shift away from the higher frequencies associated with arousal during wakefulness and the concentration on slower activities are what make sleep unique.
3. The predictable constellations of physiological patterns that occur — for example, concentrations of delta activity are associated with high GSR activation during slow-wave sleep, and indices of cortical, ocular-motor, and autonomic arousal are associated with sustained muscular inhibition during REM sleep (Pivik, 1986: p. 384).

In the course of studying the global patterns of state change during sleep another fundamental observation was made, that is, that sleep-stage pattern variations across the night were largely predictable from night to night, indicating the existence of the sleep cycle (Dement and Kleitman, 1957a) (Figure XXIV-5.3). Sleep profiles such as those illustrated in Figure XXIV-5.3 are useful for representing general sleep characteristics, such as latencies, cyclicity, stage distribution, and relative amounts of sleep disturbance. These graphs underscore the concentration of stage 4 in the first third of the night and REM sleep in the last third (Williams

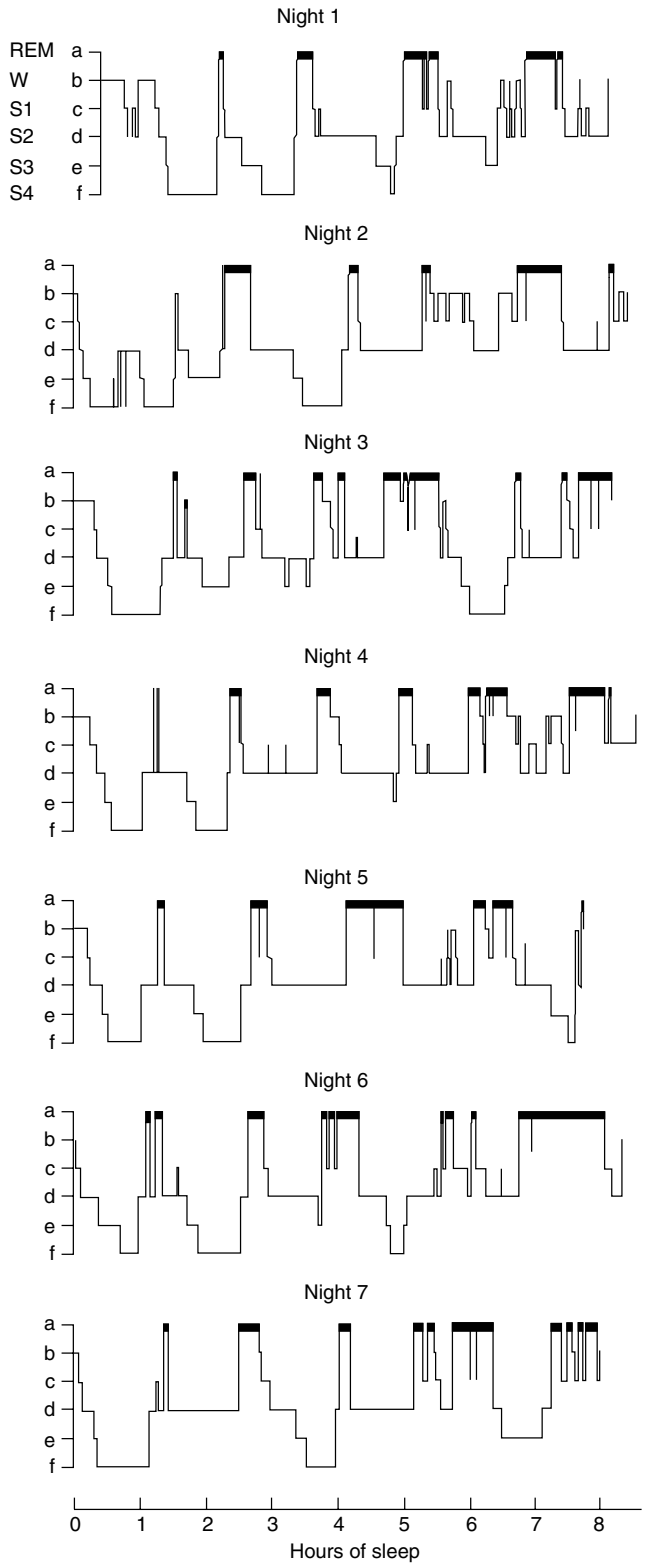

Figure XXIV-5.3 Sleep profiles depicting variations in sleep stages (ordinate) as a function of time asleep (abscissa). These profiles, based on seven consecutive nights of baseline sleep in a young adult, illustrate the stability of sleep patterns across nights, the presence of patterned oscillations between REM (darkened rectangles) and NREM sleep (i.e., sleep cycle), and the decrease in stage 4 and increase in REM sleep as a function of sleep time within a given night (Reproduced from Pivik, 2000, by permission of Cambridge University Press)

et al., 1964, 1966) — observations consistent with the exponential decrease in delta activity across the night as determined from computerized analyses of EEG sleep data (Feinberg *et al.*, 1978; 1980). Figure XXIV-5.3 also illustrates the relative difficulty in initiating REM mechanisms early in the night, as indicated by the latency to REM sleep (normally about 90 min) and the general brevity of REM periods that may occur during the first 2 h of sleep (Dement and Kleitman, 1957a; Berger and Oswald, 1962; Roffwarg *et al.*, 1966).

SLEEP COGNITION: DESCRIPTIVE ASPECTS

The discovery that dreaming is associated with a physiological state that recurs predictably across each night and which normally accounts for one quarter of each night of sleep in the adult, forced a reconsideration of beliefs that dreams occur only sporadically and under special conditions. It is not surprising, therefore, that the initial wave of psychophysiological studies of sleep following the discovery of REM sleep was significantly influenced by the belief that REM sleep provides an objective measure of dreaming, and that dreaming occurs only during these periods. These studies commonly reported high recall following arousals from REM sleep (approximately 80%) and a relative mental void in NREM sleep (less than 10% recall). There were also reports of subjects who typically failed to recall dreams even from REM sleep (Goodenough *et al.*, 1959; Goodenough, 1978). However, reports of mental activity following arousals from NREM sleep (Goodenough *et al.*, 1959) persisted. By 1967, there were nine such studies reporting NREM recall values of 23–74% (reviewed in Foulkes, 1967). The apparent discrepancy between the early and later studies regarding the presence of mental activity during NREM sleep can be attributed in part to differences in what investigators accepted as a dream report. The early studies did not provide a definition of what was required for an acceptable dream report, but relied rather on an intuitive and implicit understanding of the nature of the dream. Nevertheless, these studies provided an important insight into the nature of the dreaming process by indicating that in most individuals this process was most evident during REM sleep.

However, a systematic and effective evaluation of mental activity during sleep requires an operational definition of what would be accepted as a dream, and the suggested definitions have been wide-ranging. For example, Berger (1967: p. 16) defined the dream as a 'verbal report describing an occurrence involving multisensory images and sensations, frequently of a bizarre and unreal nature and involving the narrator himself', whereas Foulkes (1978: p. 3) extended this definition to include simply 'thinking'. The acceptance of more fragmentary and less perceptual reports as valid data revealed the presence of a much more extensive mental life during sleep; thus, reports of greater than 50% recall from NREM sleep arousals were not uncommon (Goodenough *et al.*, 1959; Foulkes, 1962; Pivik and Foulkes, 1968; Molinari and Foulkes, 1969; Zimmerman, 1970; Herman *et al.*, 1978). There were, however, qualitative distinctions between REM and NREM reports (Rechtschaffen *et al.*, 1963; Foulkes and Rechtschaffen, 1964; Pivik, 1971; Antrobus, 1983), and these may be characterized as follows:

> Reports obtained in periods of REM activity showed more organismic involvement in affective, visual and muscular dimensions and were more highly elaborated than non-REMP reports. REMP reports showed less correspondence to the waking life of the subjects than did reports from spindle and delta sleep. The relatively frequent occurrence of thinking and memory processes in spindle and delta sleep was an especially striking result (Foulkes, 1962: pp. 24–25).

Although REM reports were generally more complex, vivid, and bizarre, and NREM reports more mundane and less 'dream-like', NREM reports characterized as dreaming can be as common (Goodenough *et al.*, 1965) or more common (Foulkes, 1960, 1962; Rechtschaffen *et al.*, 1963; Bosinelli *et al.*, 1968; Pivik and Foulkes, 1968; Zimmerman, 1968; Pivik, 1971) than NREM thinking reports. However, with one exception, REM and NREM rep ts can be reliably distinguished (Monroe *et al.*, 1965; Bosinelli *et al.*, 1968). The exception relates to mentation elicited following arousals during sleep onset. Sleep onset mentation is similar to REM sleep on variables such as incidence, hallucinatory dramatic quality, and report length (Foulkes and Vogel, 1965; Foulkes *et al.*, 1966; Vogel *et al.*, 1966; Vogel, 1978), as well as perceptual and emotional qualities (Vogel *et al.*, 1972). The finding that dream-like mental activity is not restricted to REM sleep forced a rethinking of concepts such as those equating REM sleep deprivation with dream deprivation (Dement, 1960) or those suggesting that REM sleep dreams are vital to psychological normality during wakefulness (Sampson, 1965, 1966; Vogel *et al.*, 1975).

Recent research has suggested even more drastic revisions of traditional concepts regarding the occurrence and process of dreaming. For example, the presence of large amounts (40–70% of total sleep time) of REM sleep in infants presented an obvious problem for a strict identification of dreaming with this sleep stage (Roffwarg *et al.*, 1966; Pivik, 1983; Louis *et al.*, 1997). The implausibility of infants experiencing such an extensive dream life was recognized, and it forced the question of when in the course of early development the REM sleep dreaming association did become evident. When systematic studies of dreaming in early childhood were conducted (Foulkes, 1982; Foulkes *et al.*, 1990), they revealed that dreams with the formal properties present in adult dreams did not begin to appear until the age of 8 or 9 years, and required the processes used for imagery during waking. The conclusion was drawn that dreaming 'is a symbolic process with strong cognitive prerequisites and with a developmental history much like that of waking symbolic thought [and waking consciousness]' (Foulkes, 1996: p. 619). This parallelism between cognitive processes during waking and sleep is apparent in reports indicating the absence of visual imagery in the dreams of the congenitally blind or those blinded before the time when dreaming with properties similar to those in adults appears, but the presence of such imagery in the dreams of those blinded after this time (Foulkes, 1993; Kerr, 1993).

One implication of the developmental findings reviewed above is that the association between dreaming and REM sleep is not automatic and inevitable. There is a growing literature suggesting not only that dreaming is not confined to a particular sleep stage, but that it may even occur independently of sleep (Foulkes, 1985; Ellman and Antrobus, 1991; Antrobus and Bertini, 1992; Cavallero and Foulkes, 1993; Cicogna *et al.*, 2000). This more pervasive view of the occurrence of dreaming follows if the conditions and properties associated with this experience are distinguished from the larger state contexts in which they may occur. Accordingly, dreaming may be understood as 'the form assumed by consciousness whenever there is residual but somewhat dissociated cognitive/cerebral activation in the relative absence of direction either from the person's environment or from voluntary self-control' (Foulkes, 1997: p. 3). However, a better understanding of the processes defining or contributing to these experiences will require a more specific knowledge of the various brain areas and systems differentially active during dream and non-dream experiences. The use of functional imaging technology in the study of physiological and cognitive processes during sleep (Hong *et al.*, 1995; Maquet *et al.*, 1996; Braun *et al.*, 1997, 1998) promises to provide the kind of psychophysiological specificity necessary for these determinations. Of course, other processes integral to the study of dream experiences are also subject to state-determined influences and variations; for example, learning and memory are

being examined in this regard (Maquet, 2001; Stickgold *et al.*, 2001; Siegel, 2001).

PSYCHOPHYSIOLOGICAL VARIATIONS ASSOCIATED WITH SLEEP DISORDERS

The preceding overview has shown that the marked behavioural and physiological differences between wakefulness and sleep are generally associated with equally marked differences in associated cognitive activity, with waking cognition being generally more organized, rational, and self-directed and sleep cognition more disorganized, irrational, and under little volitional control. Recognizing these state and condition influences on cognitive activity, and appreciating the nature and extent of differences between normal wakefulness and sleep along these dimensions, we may expect the physiological and behavioural correlates defining disorders of sleep to present with unusual and informative psychophysiological relationships. The full range of such relationships remains to be determined since the identification of sleep disorders is an ongoing process. Some appreciation of the breadth and complexity of these disorders can be gained from the number of listings presented in *The International Classification of Sleep Disorders* (1990) — the only systematic updating of sleep disorders since the first diagnostic classification appeared in 1979. This volume identifies 66 disorders, and includes a section describing another 11 disorders which are under consideration for inclusion pending further investigation. The organizational principles used to classify sleep disorders in this publication were generally adopted in the sleep disorders section of the most recent DSM edition (American Psychiatric Association, 1994). Basically, these disorders are differentiated according to whether they are considered primary sleep disorders, that is, those that are 'presumed to arise from endogenous abnormalities in sleep-wake generating or timing mechanisms' (American Psychiatric Association, 1994: p. 551), or whether they result from other conditions — specifically, mental disorders, medical conditions, or substance abuse. The following review will focus on disorders for which the nature of the psychophysiological relationships has been most thoroughly described, namely, those classified among the primary sleep disorders and those associated with mental disorders.

PRIMARY SLEEP DISORDERS

The primary sleep disorders present a wide array of behavioural conditions reflecting the consequences of disturbed sleep-wake control processes. These conditions have been divided into two general categories, namely, those associated with difficulties involving the amount, quality, or timing of sleep (the dyssomnias), and those characterized by disorders of arousal and state transition (the parasomnias).

Dyssomnias

One of the most prevalent of sleep disturbances — primary insomnia — is classified among the dyssomnias. The incidence of this problem in the general population has not been accurately determined. However, of those referred to sleep disorders clinics with complaints of chronic insomnia, 15–25% receive a diagnosis of primary insomnia, placing this second in prevalence only to diagnoses of insomnia related to mental disorders (46%) (American Psychiatric Association, 1994; Hajak, 2000). Although the term 'insomnia' implies an absence of sleep, the nature of the sleep disturbances associated with this disorder commonly involves difficulties in initiating (increased sleep latency) and maintaining sleep (frequent awakenings, and increases in stage 1 and decreases in stages 3 and 4 sleep). One type of primary insomnia, psychophysiological insomnia, is characterized by a combination of somatized tension, resulting in increased physiological arousal, and learned sleep-preventing associations, that is, a conditioning of bad sleep habits. It is therefore commonly referred to as 'learned' or 'behavioural' insomnia (Hauri, 2000). Studies have indicated a high level of dream recall in these subjects, attributed in part to the high frequency of awakenings (Schredl *et al.*, 1998). The dreams of these individuals have been reported to reflect waking life stressors (Schredl *et al.*, 1998), and reports following arousals from fragmented REM sleep have been characterized as vivid and frightening (Lee *et al.*, 1993), suggesting a complementarity of waking and sleep cognition in terms of concerns and affect.

The abutment of wakefulness and sleep states occurs nowhere as unexpectedly and suddenly as it does in patients with 'narcolepsy'. This term was first used in 1880 to describe the pathological occurrence of repetitive, brief episodes of irresistible sleep (Gélineau, 1880). Since that time, the association of these sleep attacks with a distinct set of clinical features has been recognized, and the latter now comprise the diagnostic criteria for this disorder. These features include: excessive daytime sleepiness; cataplexy — the sudden loss of muscle tone, most frequently precipitated by emotional behaviour such as laughter, surprise, or intense excitement; sleep paralysis — a condition of motor inhibition occurring while falling asleep or on awakening during which the individual is fully conscious and aware but unable to initiate any volitional motor activity; and hallucinations — either hypnogogic (occurring while falling asleep) or hypnopompic (occurring while awakening). This syndrome clearly involves a dysfunction of REM sleep mechanisms, as evidenced by the initiation of sleep by full-blown REM periods, or the presence of elements of REM sleep, that is, motor inhibitory processes and hallucinations, during waking-sleep state transitions (reviewed by Guilleminault and Anagnos, 2000). The abrupt and irresistible imposition of elements of REM sleep on the waking subject not only creates potentially hazardous physical situations for the many individuals afflicted with this disorder (prevalence estimated at ~0.05% [Dement *et al.*, 1972, 1973]), but may also introduce psychological disturbances. The latter have been related to the unusual psychophysiological circumstances brought about by the rapid transition from waking consciousness to that associated with REM sleep physiology — a state transition that is associated with striking changes in state-related brain functional relationships (Hong *et al.*, 1995; Braun *et al.*, 1998).

Normally, the transition from wakefulness to sleep is characterized by a gradual relinquishing of waking conscious control that occurs across a succession of introspective stages. Kleitman (1963) considered Vihvelin's (1948) three-stage description as representative of this process and summarized these stages as follows: '(a) a progressive narrowing of the field of consciousness, as a quantitative change in psychological processes; (b) a stage of "pure" hypnagogic hallucinations, as a qualitative change in psychological processes; and (c) a vacillation between wakefulness and sleep, when hypnagogic hallucinations are confused with dreams' (pp. 79–80). Later studies (Foulkes and Vogel, 1965; Vogel *et al.*, 1966) tied the sleep onset-associated psychological changes to EEG and EOG patterns present during this time. Vogel *et al.* (1966), in summarizing the corresponding psychophysiological changes occurring during this state transition, noted that the progression from relaxed wakefulness (characterized by alpha activity and occasional rapid eye movements), through stage 1 sleep and into stage 2 sleep was associated with a progressive loss of control over the course of mental activity, then a loss of awareness of the environment, and finally a loss of reality orientation and the onset of hallucinations. These progressive variations characteristic of normal wakefulness-sleep transitions are bypassed during narcoleptic sleep attacks. During these episodes, the sudden interruption of waking physiological processes by those of REM sleep can create an unusual and disturbing

mental state in which the hallucinatory processes characteristic of REM sleep cognition are joined with the incorporation of elements from the patient's waking environment. The result is a confusing mental experience in which the boundaries between dreaming and reality are blurred to the extent that patients question their sanity (Broughton, 1982; Zarcone and Fuchs, 1976) or may sincerely believe that a hallucinatory experience, such as sexual abuse, was real (Hays, 1992). Many of the dreams of narcoleptics, whether occurring at sleep onset or during nocturnal sleep, are particularly intense, bizarre, and frightening (Ribstein, 1976; Broughton, 1982; Lee *et al.*, 1993; American Psychiatric Association, 1994; Schredl, 1998). In part, these characteristics may reflect the influence of the fragmentation of REM sleep (Montplaisir *et al.*, 1978) or the effects associated with motor inhibitory processes (that is, sleep paralysis) (Ribstein, 1976; Honda, 1988).

Parasomnias

The disorders represented among the parasomnias include behaviours disruptive of sleep that develop during sleep-wake transitions or during sleep. Many of these are behaviours that are normally confined to wakefulness (walking, talking, or urinating) and others, such as sleep terrors and nightmares, reflect intense physiological or behavioural disturbances peculiar to sleep. These disorders may be sleep-stage specific in their expression (sleepwalking and sleep terrors during slow-wave sleep and nightmares during REM sleep) or may occur throughout sleep (sleep talking and enuresis). Several develop early in life and resolve before adulthood (night terrors, sleepwalking, and enuresis), whereas others typically emerge in adults (REM sleep behaviour disorder).

The study of mental activity associated with various parasomnias has provided informative, and at times surprising, insights into brain–behaviour relationships. For example, consistent with findings indicating reduced recall of sleep mentation after awakenings from slow-wave sleep (Pivik and Foulkes, 1968; Pivik, 1971; Armitage, 1980), the recall of mental experiences associated with parasomnias occurring during slow-wave sleep (sleepwalking and sleep terrors) is limited and fragmentary (see Pivik, 2000a and Nielsen and Zadra, 2000 for reviews). This relative absence of recall seems particularly remarkable when associated with the marked physiological arousal and expressiveness that occurs during sleep terrors. During these episodes, subjects appear to be responding to frightful and horrific thoughts and images. Although an identification of these experiences with nightmares may seem warranted, sleep terrors and nightmares have been shown to be clearly differentiated along several dimensions — including time of occurrence — as well as in terms of behavioural and physiological correlates (Nielsen and Zadra, 2000).

The inability to access mental experiences more directly during sleep behaviours such as sleep terrors and nightmares while these experiences are ongoing frustrates our attempts at better understanding of these behaviours. Verbal communication with the sleeping subject could circumvent this barrier, and the spontaneous occurrence of talking during sleep would appear to provide unique access to the sleeping mind. Furthermore, this access would span both REM and NREM sleep since sleep talking — although most prevalent during slow-wave sleep — may occur in all sleep stages (Arkin, 1991). Unfortunately, it has not been possible to initiate and maintain intelligent dialogue with subjects while they are asleep, and attempts to induce subjects to relate ongoing mentation while remaining asleep via posthypnotic suggestions have not been successful (Arkin *et al.*, 1970; Arkin, 1978). Moreover, when reports have been available to assess the relationship between what was said and what was reported, the degree of concordance is quite variable, and often no discernible association is evident (Arkin, 1991).

The unique physiological and psychological attributes that set REM sleep apart from other waking and sleep states provide the basis for an unusual group of parasomnias associated with this state. These can be quite dramatic and exceptionally intense in their expression, and their consequences may include immediate and extended effects that are both mental and physical in nature. Perhaps the most readily recognized among these disorders is the nightmare. The essential characteristics and ramifications of these frightening dream experiences have been succinctly summarized as follows:

> Nightmares typically occur in a lengthy, elaborate dream sequence that is highly anxiety provoking or terrifying. Dream content most often focuses on imminent physical danger to the individual (e.g., pursuit, attack, injury). In other cases, the perceived danger may be more subtle, involving personal failure or embarrassment. Nightmares that occur after traumatic experiences may replicate the original dangerous or threatening situation, but most nightmares do not recount actual events. On awakening, individuals with this disorder can describe the dream sequence and content in detail. Nightmares usually terminate with an awakening that is associated with a rapid return to full alertness and a lingering sense of fear or anxiety. These factors often lead to difficulty returning to sleep. Nightmare Disorder causes significant subjective distress more often than it causes demonstrable social or occupational impairment. However, if nocturnal awakenings are frequent, or if the individual avoids sleeping because of fear of nightmares, the individual may experience excessive sleepiness, poor concentration, depression, anxiety, or irritability that can disrupt daytime functioning. (American Psychiatric Association, 1994: p. 580).

Nightmares are experienced by individuals of all ages, but because of a variety of factors — such as diverse study populations, varying frequency criteria, and different methods of assessment (retrospective self-report vs. daily logs) — reliable estimates of prevalence have been difficult to determine. It is agreed, however, that although this disorder is most common in children (rates of 10–90%), its presence in the adult population remains substantial (8–25%) (Neilsen and Zadra, 2000; Partinen and Hublin, 2000). Despite the prevalence of this form of dream disturbance, the causal factors for these experiences have not been extensively studied and are not well understood. Psychopathology has been implicated, but the findings have not been consistent (reviewed by Nielsen and Zadra, 2000). Possible insights into the mechanisms underlying the nightmare that might come from physiological studies of sleep have been limited because of the paucity of such investigations. Although the general nature of the findings has not been particularly surprising, that is, increased autonomic activation (Fisher *et al.*, 1970, 1973; Nielsen and Zadra, 1997) and signs of increased EEG arousal (Nielsen *et al.*, in press), the degree of autonomic activation was unexpectedly low given the intensity of the associated psychological experiences. As a possible explanation for these findings, it has been suggested that a dissociative process similar to that thought to be effected by behavioural therapies, such as systematic desensitization and flooding, may be invoked. This process is postulated to reduce the level of sympathetic activation that normally occurs in association with the processing of stressful imagery, effectively desomatizing the imagery (Fisher *et al.*, 1970; Perlis and Nielsen, 1993; Nielsen and Zadra, 2000). Although the persistence of such complex functional relationships across sleep-waking states is uncommon, the uncoupling of physiological activation and cognitive activity during sleep is not. For example, the intense levels of electrodermal activity that regularly occur during stages 3 and 4 sleep (Burch, 1965) apparently have little effect on the recall or qualitative aspects of sleep mentation (Hauri and Rechtschaffen, 1963; Pivik, 1971; Tracy and Tracy, 1974). Similarly, it might be expected that because of the common occurrence during REM sleep of penile erections (Fisher *et al.*, 1965; Karacan *et al.*, 1966) and clitoral engorgement

(Cohen and Shapiro, 1970), many REM reports would contain overt sexual content. However, with the exception of lucid dream reports (LaBerge *et al.*, 1983; Laberge, 1985), REM reports with such features are uncommon (Fisher, 1966; Hall and Van de Castle, 1966). Furthermore, even during lucid dreams containing sexual activity, there is some dissociation between content and associated autonomic activity; that is, expected variations have been observed for respiration and skin conductance measures, but not for heart rate (LaBerge, 1985; 1992).

A final example of psychophysiological relationships present during wakefulness that do not persist unchanged during sleep comes from studies examining the correspondence between the visual aspects of dream imagery and the rapid eye movements of REM sleep. The discovery that dreams are associated with a physiological sleep state during which eye movements are prominent suggested the possibility that these eye movements serve the same function during sleep as they do during wakefulness, that is, to track positional changes in visual targets. Attempts to demonstrate this relationship, which came to be known as the scanning hypothesis (Roffwarg *et al.*, 1962), have been largely unsuccessful (reviewed by Rechtschaffen, 1973; Pivik, 1991). However, more positive relationships between eye movement and dream imagery may exist in lucid dreams (LaBerge, 1992), and imaging procedures have indicated that the same cortical areas are involved in the control of eye movements during waking and REM sleep (Hong *et al.*, 1995).

Two other types of REM-associated parasomnias — sleep paralysis and REM sleep behaviour disorder — can be viewed as cases of dissociative behaviours. This perspective is understandable when it is recognized that the REM state consists of the integration of activities from different sensory and motor systems — as evidenced in the disparate physiological activities that define and characterize this state, that is, EEG activation, motor inhibition, eye movements, and autonomic variability — and that these various systems are selectively susceptible to malfunctions that compromise normal sleep-wake state boundaries and result in abnormal amalgamations of sleep and waking behaviours. Sleep paralysis — the experience of motor inhibition during the process of falling asleep or waking up — is an example of such a malfunction. In this case, inhibitory processes involved in the suppression of motor activity during REM sleep occur independently of REM sleep and in conjunction with conscious awareness (Hishikawa and Shimuzu, 1995; Hishikawa, 1976). Sleep paralysis is a cardinal symptom of narcolepsy, but occurs independently of this disorder at least once in the lifetimes of 25–50% of the general population (Spanos *et al.*, 1995; Fukuda *et al.*, 1998; Cheyne *et al.*, 1999; ICSD, 1990). As a chronic complaint, isolated sleep paralysis is reported to have only a 3–6% prevalence rate (ICSD, 1990).

The psychological experiences accompanying these sleep paralysis episodes are distinguished by elements of fear, terror, and panic, often occurring together with sensory hallucinations (Hishikawa, 1976; Hufford, 1982; Buzzi and Cirignotta, 2000). Although these episodes occur at times of state transition during which hallucinatory experiences are common, it is clear that the latter experiences can, and generally do, occur independently of this motor inhibition (Buzzi and Cirignotta, 2000; Nielsen and Zadra, 2000). Even during sleep, arousals made during times of tonic EMG suppression occurring just prior to, but still independently of, REM sleep onset are not associated with either greater recall frequency or reports that are more dream-like (Larson and Foulkes, 1969; Pivik, 1971). Clearly, however, the awareness of motor inhibitory effects during these transitory states can contribute directly to the concurrent cognitive experiences. This is apparent in the reports of the threatening and terrifying feelings that develop in association with the restrictions in movement and respiration that characterize this condition (Hishikawa, 1976; Hufford, 1982). It has been suggested, however, that mental experiences occurring in association with sleep paralysis may be intensified relative to hypnagogic and hypnopompic experiences not accompanied by this motor inhibition (Hufford, 1982; Takeuchi *et al.*, 1994), but the processes involved have not been specified.

Whereas sleep paralysis represents the displacement of REM-associated motor inhibitory processes from REM sleep to sleep-wake transitional states, REM sleep behaviour disorder (RBD) results from the failure of these inhibitory processes to be maintained during REM sleep. This disorder typically develops later in life (after the age of 50 years) and occurs predominantly in men (Mahowald and Schenck, 2000; Olson *et al.*, 2000). The overall prevalence rate for this disorder has been reported to be 0.5% (Ohayon *et al.*, 1997). Recognized as a distinct clinical disorder in 1986 (Schenck *et al.*, 1986), this disorder was included for the first time in the latest edition of the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, 1994).

It is not uncommon for motor inhibition to vary in intensity during REM sleep. Depending on the duration of such reduced inhibition and covariations in other physiological measures, these intervals would normally be interpreted as interruptions in the continuity of REM sleep periods and would be scored as stage 1 sleep or relaxed wakefulness. Certainly, these episodes would go behaviourally unnoticed. What sets these transient reductions in inhibition apart from the loss of inhibition in RBD is the remarkable behaviours that occur at these times in RBD. These outbursts are characterized by excessive motor activity that often results in injuries to individuals or their sleeping partner (Mahowald and Schenck, 2000; Olson *et al.*, 2000). These behaviours are usually related to the associated dream content which commonly deals with themes involving acts of aggression and defence, and may be considered to represent instances where dreams are being acted out. The notion that the motor inhibition during REM sleep may function to prevent the expression of hallucinatory activities finds support in animal studies in which selective subcortical lesions are followed by hallucinatory-like behaviours at times when REM sleep periods are expected to occur (Jouvet and Delorme, 1965; Henley and Morrison, 1974; Morrison *et al.*, 1979). However, the general restriction of these outbursts in RBD to dream behaviour of a violent nature (Nielsen and Zarda, 2000; Mahowald and Schenck, 2000; Olson *et al.*, 2000) and evidence that RBD behaviours can occur despite the presence of full muscle atonia (Schenck *et al.*, 1988; Lapierre and Montplaisir, 1992) precludes the general conclusion that muscle atonia during REM sleep normally functions to prevent the acting out of dreams.

MENTAL DISORDERS

The association between disordered minds and disordered sleep has been recognized for centuries. In the fifth century AD, Caelius Aurelianus noted that 'those who are on the verge of phrenitis or are slipping into the disease show the following signs: continual sleeplessness or troubled sleep with confused dreams' (p. 21). He later advised, 'Then in all cases let the patients sleep — for timely sleep refreshes no less than food' (p. 49) (Drabkin, 1950). Hundreds of years later, in 1892, Hammond, a psychiatrist, stated: 'In my opinion no one cause is so productive of cerebral afflictions as persistent wakefulness' (Hammond, 1892: p. 174). It was to be expected, therefore, that when methods allowing a more extensive and thorough evaluation of sleep became available, they would be applied to the study of sleep in patients with psychiatric disorders. It is of historical interest that the new sleep research became focused almost immediately upon studies of psychiatric patients. To a great extent, this was determined by one of the more remarkable revelations made regarding sleep — namely, the finding

that dreaming was associated with a physiological sleep state that occurred predictably each night. The opening up of this new cognitive territory to systematic study and the influence of earlier speculations linking dreams and psychoses (Wundt, 1897; Jackson, 1958) served to attract many psychiatrists and psychologists to this new discipline, thereby ensuring a cognitive emphasis for these early studies. Among mental disorders, those receiving the greatest attention from sleep researchers have been schizophrenia, and mood and anxiety disorders.

Schizophrenia

The earliest investigations applying the new technology to the study of sleep were conducted in individuals diagnosed with schizophrenia (Dement, 1955; Koresko *et al.*, 1963; Rechtschaffen *et al.*, 1964). Schizophrenia is a very debilitating illness, characterized by a wide range of cognitive and motor symptoms — including the presence of delusions and hallucinations, disorganized speech, and disturbed affect — that occurs in 0.4–2.7% of the population (Benson and Zarcone, 2000). Beliefs of an identity between the processes underlying hallucinations and dreams (Jackson, 1958; Jung, 1960) raised exciting possibilities that studies of sleep in these patients might provide significant insights into the pathophysiology of their disorder. The initial wave of studies failed to find evidence of REM sleep abnormalities (Dement, 1955; Koresko *et al.*, 1963) or indications of REM sleep physiology during waking hallucinations in these patients (Rechtschaffen *et al.*, 1964).

Although subsequent research has identified parameters which distinguish sleep in schizophrenia from that in age-matched comparison groups, such as poor sleep efficiency, shorter latency to the onset of the first REM period (REM latency), and reduced amounts of slow-wave sleep (see Benson and Zarcone, 2000), the research emphasis in this population has been on relationships between REM sleep mechanisms and hallucinations. A significant role in this research has been played by a procedure designed to determine the consequences of the elimination of dreaming upon behaviour. This procedure, originally termed 'dream deprivation', involved awakening subjects at the onset of each REM period and was based on the premise that if REM periods were prevented from occurring, dreaming could not take place. The initial studies applied this technique to non-schizophrenic subjects, and the results were interpreted as indicating that dreaming or REM sleep was essential to waking psychological normality (Dement, 1960; Dement and Fisher, 1963; Sampson, 1965, 1966). Not only has subsequent research failed to support this interpretation of those data (Vogel, 1975), but there are indications that for some clinical groups REM deprivation may have beneficial effects on waking symptomatology (Vogel, 1968; Vogel *et al.*, 1975).

Although the psychological sequelae of REM deprivation did not indicate the existence of a simple transfer function between hallucinatory activity during sleep and wakefulness in schizophrenia, there were other consequences of REM deprivation that offered possible explanations of how such interactions might take place. The most prominent of these were increases in the amount of time spent in REM sleep following deprivation (the REM rebound) and an apparent intensification of physiological activity within REM periods, such as increases in phasic events such as brief muscle twitches and eye movements (Dement, 1960; Dement *et al.*, 1966; Dement *et al.*, 1969). Reports indicating reductions in the amount of REM rebound in actively ill schizophrenics compared to those in remission or to normal subjects raised the possibility that REM-associated behaviours were being expressed during, or 'leaking out' into, wakefulness (Azumi *et al.*, 1967; Zarcone *et al.*, 1968; Gillin *et al.*, 1974; Jus *et al.*, 1977). However, these findings were not consistently observed (see Vogel, 1975), and adverse psychological effects of REM deprivation in such subjects have not been observed (Vogel, 1975; Vogel *et al.*, 1975; Hoyt and Singer, 1978; Benson and Zarcone, 2000). Similarly, extensive examination of variations in indices of REM sleep phasic activity during sleep in these patients, as well as investigations into the relationship of these events to sleep cognition in normal subjects, has not demonstrated the hoped-for identity between these events and hallucinatory activity (Pivik, 1986, 2000b; Benson and Zarcone, 2000).

Relative to the research effort that has been expended in investigating the physiology of sleep in schizophrenia, the research devoted to the study of dream content in these patients has been minimal. It would be quite amazing if the mental experiences of these patients during sleep did not reflect their waking psychopathology, and observations made by Dement (1955) in the first study of these patients after the discovery of REM sleep appeared to confirm this expectation. In that study, he noted the unusual presence of inanimate, stationary objects in over half the reports he obtained. A review of reports limited to the characteristics of dream content in schizophrenia that have been verified, that is, have been replicated at least once (Kramer and Roth, 1979), was limited to 22 studies — only 11 of which were conducted in sleep laboratories. From the entire series of studies, the authors characterized the dreams of schizophrenics as 'more hostile, more affective, and containing more evidence of the schizophrenic thought disorder, i.e., unrealistic, bizarre, and so on, than those of non-schizophrenics' (p. 381). A subsequent review more than two decades later could add only six more papers to this list, and concluded that these 'few additional articles on dreaming in schizophrenia contributed little to our knowledge of the dreams of these patients. The inattention to dreams in schizophrenia may be part of the shift from the psychological to the biological study of psychiatric disorders, and more particularly to the greater interest in mood disorders than in schizophrenia' (Kramer, 2000: p. 513).

Mood Disorders

It is true that mood disorders have been the subject of intense study. This interest appears to be proportional to the prevalence rate of these disorders in the general population and to the prevalence of sleep disturbance among those with this diagnosis. Major depression, the most common of the mood disorders, is present in 5–12% of men and 10–25% of women (Boyd and Weissman, 1981). Furthermore, as indicated in a recent review, this disorder 'has been studied polysomnographically more than any other psychiatric disorder, and the majority of patients have shown objective sleep disturbances' (Benca, 2000, pp. 1142–1143). Common subjective complaints regarding sleep problems in these patients emphasize difficulties in initiating and maintaining sleep and include reports of disturbing dreams (Benca 2000). The sleep disturbances are generally pervasive throughout sleep and are not limited to a specific time of night, sleep stage, or process. They are reflected in measures indicating compromised sleep continuity (increased sleep latency, sleep fragmentation by awakenings, and early morning awakening [Oswald *et al.*, 1963; Zung *et al.*, 1964; Gresham *et al.*, 1965]), decreased slow-wave sleep (Hawkins and Mendels, 1966; Gillin *et al.*, 1979; Kupfer *et al.*, 1985), and REM sleep abnormalities (reduced latency to the first REM period [Kupfer and Foster, 1972; Akiskal *et al.*, 1982; Emslie *et al.*, 1990], prolonged duration of the first REM period [Feinberg *et al.*, 1982; Borbély *et al.*, 1984], and increased eye movement density during REM sleep [Kupfer *et al.*, 1985; Waller *et al.*, 1989]).

The general reliability of these findings, together with insights provided by various treatment procedures that have proven to have antidepressant effects — such as pharmacologic and behavioural

(sleep deprivation) interventions — have led to the development of several hypotheses to account for the observed interactions among depression, sleep, and treatment variables. Prominent among these have been explanations attributing various features of these interactions to the adverse effects of excessive REM sleep in these patients (the REM pressure hypothesis [Vogel, 1975; Vogel *et al.*, 1975]), irregularities in the neurochemical control of REM sleep (the cholinergic-aminergic imbalance hypothesis [Jankowski *et al.*, 1972; McCarley, 1982; Sitaram *et al.*, 1984]), variations in the circadian control of REM sleep, and autonomic and hormonal variables (the phase-advance hypothesis [Wehr and Wirz-Justice, 1981; Wehr *et al.*, 1983]), and a deficiency in homeostatic sleep-inducing processes (the process S deficiency hypothesis [Borbély and Wirz-Justice, 1982]). A recent review that summarized the rationale, strengths, and weaknesses of these hypotheses concluded that, although each offered plausible explanations for some of the recognized sleep and depression relationships, none provided a full accounting for these findings (Benca, 2000).

From his review of 31 studies examining dream characteristics in depressed individuals, Kramer (2000) concluded that 'in depression there is a decrease in the frequency and length of dream reports. The dreams are commonplace, but have content characteristics of interest. There is an increase in death themes in depressed suicidal patients and in bipolar patients before becoming manic.... It is evident that a past focus is not universal in depression, nor indeed was it unique to the depressed state. Affects such as anxiety and hostility are not prominent in their dreams' (p. 514).

Some observations that have been made in these studies are especially relevant to issues of the interactions between cognitive activity during sleep and wakefulness. For example, poorer treatment outcome or greater difficulty in problem resolution has been associated with distinct qualitative dream features, as in disorganized dreams without human characters (Greenberg *et al.*, 1990), masochism (Cartwright and, Wood, 1993), or the extent to which problems are incorporated into the dream (Cartwright, 1991). Such findings not only have prognostic implications, but are consistent with suggestions that dreams in these patients may facilitate waking coping functions (Kramer, 1993).

Anxiety Disorders

That an experience may have profound extended effects on both waking and sleep behaviour is nowhere better exemplified than in subjects with post-traumatic stress disorder. Following the traumatic experience, these subjects typically exhibit a range of anxiety and fear-related symptoms and repeatedly relive or re-experience the event in the form of intrusive images and thoughts during wakefulness, and arousal disturbances and nightmares during sleep (American Psychiatric Association, 1994). In a general population survey, Helzer *et al.* (1987) reported a 1% general prevalence among those surveyed, with an increase to 3.5% if they had been physically attacked or had served in Vietnam.

Sleep-related disturbances are a common, and often the principal, complaint among patients with this disorder (Kramer, 1979; Kramer *et al.*, 1984; Mellman and Davis, 1985). These disturbances are reflected as problems with initiating or maintaining sleep, and sleep disturbance as a result of nightmares occurs in over half of these patients (Horowitz *et al.*, 1980; van der Kolk *et al.*, 1980; Mellman and Davis, 1985). In addition to the obvious REM-related nature of many of these disturbances (Ross *et al.*, 1989), arousals may also develop from NREM sleep (Schlosberg and Benjamin, 1978; van der Kolk *et al.*, 1984; Hefez *et al.*, 1987). However, Uhde (2000) has noted that, despite the absence of a specific REM sleep parameter that was consistently deviant in this disorder, 'it is nevertheless impressive how often *some* type of REM-related abnormality is reported in patients with post-traumatic stress disorder' (p. 1135).

The nature of dreams in patients with this disorder has been a focus of extensive research. Kramer (1979) has argued that disturbing dreams are more defining as characteristics of this disorder than are the other observed sleep disturbances. In summarizing his review of 61 articles that have been published on this topic during the past 34 years, Kramer concluded: 'An adequate characterization of the phenomenology of the disturbing dream remains to be done. It can be confirmed that the dream is disturbing and that the dream events may be outside the realm of current waking experience. The literature does not support the position that the dream is affect-laden or that the dream is easy to recall. It is not known if the dream is any more or less vivid than other dreams' (p. 516).

CONCLUSIONS

This review began with a statement about how understanding what is normal is a prerequisite for understanding what is not. Often, what comes to be recognized as normal borders on the unbelievable. For example, 50 years ago it was inconceivable that hallucinations accompanied by paralysis would occur predictably 4–6 times each night (that is, REM sleep). The nature of some abnormalities also strains the imagination. For example, how could it be that individuals would not only stop breathing while asleep, but also remain unaware this had happened even though it occurred hundreds of time during the night (that is, sleep apnoea)? The cataloguing of normal and abnormal sleep behaviours in this paper, while not exhaustive, does give an indication of the wide range of forms these behaviours may assume, and does provide a glimpse of the complexity of the physiological and psychological processes that accompany these variations.

The generally negative impact of disordered processes on both sleep and waking behaviours — whether emanating primarily from sleep or wakefulness — would seem to force the conclusion that whatever individual or symbiotic functions are served by these states, they are best satisfied with as little between-state interference as possible. However, the dependence of these states on the same biological substrates — although functionally reorganized for some behaviours — makes it inevitable that the barrier separating sleep and wakefulness would be vulnerable to cross-state influences. Usually, these border violations result in minor disturbances in state continuity (sleep fragmentation or decreases in waking attention), but they may also be expressed as dramatic and debilitating full-scale invasions when inappropriate waking behaviours erupt during sleep (as in REM behaviour disorder) or wakefulness is abruptly terminated by the imposition of sleep (as in narcolepsy).

Presumably, if a condition of functional balance were to be achieved between states, it would be reflected in the absence of adverse cross-state influences. However, since achieving such a condition of détente is obviously difficult (but perhaps not impossible; see Carskadon, 1979; Dement and Carskadon, 1982), the biological permission for between-state interactions that exists — even though it may at times work to great behavioural disadvantage — must be considered normal and perhaps functional. It is the task of future research — guided rather than constrained by prevailing theories — to re-examine and revise current concepts and definitions of state and normality, and to provide a more informed understanding of the bases for ordered and disordered behaviours and their relationships to one another.

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XXIV-6

The Neuropsychology of Sleep Disorders

Raymond Cluydts and Edwin Verstraeten

INTRODUCTION

Within the vast area of the life sciences, sleep and sleep disorders have received increasing attention and interest during recent decades from governmental and health authorities, the news media, and the public in general. A big step forward in the recognition of sleep as an important aspect of our health was based on findings revealing the numerous effects of poor or inadequate sleep on daytime functioning and quality of life in general.

Epidemiological studies show (Partinen and Hublin, 2000) that a vast majority of children, adolescents, adults, and the elderly can expect to suffer for either a short or longer period in their lives, from some kind of sleep problem. In addition to primary insomnia, sleep disturbances associated with breathing problems, muscular contractions, or irritability during the night, as well as narcolepsy and circadian rhythm disorders, have been identified. These conditions have selective effects on either sleep stages, such as REM or non-REM sleep, or sleep onset, the continuity of sleep, or sleep architecture. This disturbance or lack of sleep has been associated with serious safety consequences not only for the patient, but also for the public. Daytime sleepiness has been recognized as a major intervening factor. A sleepy brain cannot function normally and attend to ongoing activities. Many disasters witnessed in recent years, such as train, coach, and car crashes, may be related to ignorance of the limits of human performance after sleep loss.

On the individual level, extensive clinical and neuropsychological investigation of the sleep-disordered patient seems necessary to understand fully the impact of sleep disorders on the patient's daily activities. But, next to the process of diagnosis, the results obtained from this neuropsychological examination can provide the clinician with clues for a comprehensive treatment plan and outcome measures of the therapy.

Neuropsychological Findings in Sleep-Wake Disorders

The fact that sleep loss and poor sleep result in daytime impairment of cognitive functioning is inferred not only from real disasters but also mainly from two types of research and clinical findings. First there are research data on the effects of total and partial sleep deprivation and sleep fragmentation; secondly, we can rely on clinical data gathered from patients suffering from sleep disorders.

Decades of research on sleep deprivation show the consistently detrimental effects of sleep shortage on next-day performance. Good reviews on this topic are numerous (Walsh and Lindblom, 1997; Bonnet, 2000; Pilcher and Hufcutt, 1996; Dinges and Kribbs, 1991). Most of these studies were performed to reveal the function(s) of sleep, providing us with key information about areas in the neurocognitive field that are susceptible to these experimental manipulations. These areas include attentional performance (impact of daytime sleepiness), mood changes, and memory function. But can these experimentally induced sleep disturbance be compared to the sleep disturbance experienced by insomniacs? Probably not. At best, this experimentally induced sleep fragmentation and partial sleep deprivation can be compared to the effects of an acute shift-work or jet-lag situation. We do not consider induced sleep deprivation a condition of 'insomnia' but one of 'sleeplessness'. Furthermore, these experiments are conducted in laboratory situations and are very much affected by selection bias (students as volunteers), experimental set-effects (motivation), and testing and instrumentation effects (learning), so that a generalization of these findings to a sleep disorder population is absolutely unwarranted. Our patients are mostly confronted with non-stimulating situations and lack of motivation in everyday life. Nevertheless, the findings in the three areas described above can give us some clues on promising areas to explore in insomniacs. However, we should certainly not restrict our studies to these areas only. Moreover, the finding that, in many studies on partial sleep deprivation in volunteers, few neurocognitive effects were evidenced cannot be simply generalized to the patient population.

From clinical practice, we know that patients suffering from sleep disorders complain not only of poor sleep, but also of significant impairment of their daytime activities. This includes cognitive impairment such as memory deficits (both encoding and retrieval) and limited attention span; some patients even report specific disorders such as visuoperceptual and spatial orientation difficulties. These clinical observations became the focus of some population-based studies in recent years. These studies confirm that patients with insomnia experience more problems with memory, concentration, thinking, and ability to accomplish tasks (Roth and Ancoli-Israel, 1999). These problems not only affect quality of life scales (Zammit *et al.*, 1999) but are also associated with poor occupational performance and increased absenteeism. Leger (1999) estimated the annual economic impact of insomnia in France to be around US$ 2 billion.

A crucial question is whether these neurocognitive phenomena are just one part of a larger 'neurotic' complex that is 'complaint'-driven, or whether a causal relationship between the sleep complaint and the reported daytime neurocognitive impairment can be demonstrated. In the next sections, we will discuss the data available on neurocognitive functioning in patients suffering from following sleep disorders: primary insomnia, sleep apnoea syndrome, and narcolepsy.

PRIMARY INSOMNIA

Insomnia is usually defined according to the *Diagnostic and Statistical Manual of Mental Disorders* (4th edition) (DSM-IV) (American Psychiatric Association, 1994) or the *International Classification of Sleep Disorders* (ICSD) (Revised) (American

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Sleep Disorders Association, 1997). The DSM-IV criteria for 'primary insomnia' are not exactly the same, but overlap largely with the ICSD categories psychophysiological insomnia, sleep stage misperception and idiopathic insomnia. We can assume that in many studies very heterogeneous groups of patients were tested in the morning so that results obtained need to be interpreted with care. Earlier studies performed in the 1970s and 1980s used 'poor sleepers' as research subjects without screening for sleep-related breathing disturbances, movement disorders, or other medical conditions. Therefore, we will discuss only recent studies that used appropriate screening methods. Extensive reviews on the topic can be found in Riedel and Lichstein (2000) and Fulda and Schulz (2001) [see also References]. We will focus on recent studies by Hauri (1997), Edinger *et al.* (1997), and Rosa and Bonnet (2000). Three areas of interest already identified in sleep deprivation studies will be considered: sleepiness, mood, and cognition.

Sleepiness

Three methods for assessing daytime sleepiness are well accepted: the Stanford Sleepiness Scale (SSS) (Hoddes *et al.*, 1973), a 7-point sleepiness rating scale and the Epworth Sleepiness Scale (ESS) (Johns, 1991), which asks patients about the likelihood of falling asleep in eight situations, are subjective measures. The former is a measure of 'state' sleepiness, whereas the latter measures 'trait' aspects of sleepiness.

As an objective test to be performed in a laboratory under strictly standardized conditions, the Multiple Sleep Latency Test (MSLT) (Carkadon *et al.*, 1986) is accepted worldwide as a routine procedure in all sleep disorders centres: it measures sleep onset latency in five 20-min naps with 2-h intervals during the day.

The study of Edinger *et al.*, (1997) is typical: in sharp contrast to the effects of sleep deprivation on daytime napping, insomniacs do not show shortened sleep latencies on the MSLT recorded either in the sleep laboratory or at home. The study of Bonnet and Arand (1995) even found an increased mean sleep onset latency in insomniacs. This finding suggests that insomniacs have difficulty in falling asleep, either at night or during the day; in them, a neurobiological substrate that introduces sleep (sleep drive) seems to be defective and/or the wake drive is too elevated.

Hauri (1997) found higher scores on the SSS in insomniacs, and this finding has been confirmed in other studies that also administered this state measure of sleepiness during the day after a polysomnographic (PSG) recording. Data with the ESS are conflicting and less available, as this scale is much more often used in patient groups presenting with high daytime sleepiness. A floor effect may be the reason for this negative finding in primary insomniacs.

Mood

The Profile of Mood States (POMS) (McNair *et al.*, 1981) is a self-administered rating scale comprising six well-defined dimensions of affect: tension-anxiety, depression, anger, fatigue-inertia, vigour-activity, and confusion-bewilderment. It assesses intensity of a mood state and can cover any time period, but the scale is usually administered in reference to how someone feels at the moment of completion. It is an interesting procedure to be used in insomnia for at least two reasons. Firstly, it allows one to detect an underlying anxiety and/or depressive component. Although primary insomniacs do not fit the criteria for generalized anxiety or major depressive disorder, we see in our consultation primary insomniacs who present with elevated anxiety and depression at non-clinical levels. This was also observed in the study by Bonnet and Arand (1995).

Secondly, as primary insomniacs may confuse feelings of fatigue and sleepiness, the fatigue scale of the POMS can depict this particular state in the absence of sleepiness. Indeed, primary insomniacs complain more about fatigue than sleepiness, as was found by Fichten *et al.* (1995). Maybe we should reconsider more the impact of feelings of fatigue in these patients. In contrast to sleepiness and cognition, there is a clear bidirectionality between mood and sleep. Rosa and Bonnet (2000) found that patients complaining about their sleep had higher tension, depression, anger, and confusion scores on the POMS in comparison to controls, but when the same group of subjects in the study were grouped by poor EEG sleep instead of the complaint itself, the same results were found, indicating dysphoric mood in both situations. Interestingly, the POMS fatigue scale was related only to the poor EEG criterion, and not to the complaint factor.

Cognition

The studies by Hauri (1997) and Edinger *et al.* (1997) will be discussed, as both are methodologically sound and original. Hauri compared not only insomniacs with normal controls, but also the performance of the insomniacs after their best night with that after their poorest of three nights. Edinger compared performance after a PSG recording in the sleep laboratory with performance after an ambulatory recording at the patient's home.

Hauri's study included 26 insomniacs, starting from a screening total of 75. They filled out a sleep diary and the SSS for 1 week, slept in the laboratory on 3–4 occasions, and were tested by a well-validated test battery (Roehrs *et al.*, 1995), tapping many neurocognitive functions. The insomniacs performed worse than matched controls, and, in particular, were more variable on a simple visual reaction time test (initiation time). A complex reaction time test showed less variability but again a slower initiation and total reaction time. Insomniacs also remembered fewer digits in the Wechsler Digit Span Test, but the Digit-Symbol Substitution, Auditory Verbal Learning, and Auditory Vigilance Task were performed at the same level as the healthy controls. Of most importance, no significant differences on the cognitive tasks could be found in comparing performances after the worst and the best nights in the insomniacs.

The Edinger *et al.* (1997) study found no differences in the Simple Reaction Time Test performance between insomniacs and controls, but made an interesting observation in the Continuous Performance Test. This test is a signal detection task in which target letters have to be identified from background letters. Insomniacs recorded in the sleep laboratory performed better at this task than those who were recorded at home; the reverse was found for healthy controls. Moreover, among those who slept at home, the normal sleepers performed better during daytime testing than the insomniacs, whereas after a night in the sleep laboratory the reverse was found. This indicates that after a night in the sleep laboratory, normal sleepers might have been somewhat sleep deprived and perform worse, whereas the worst performance in insomniacs was observed the day after a sleep recording in their usual sleep environment. The sleep laboratory environment may have produced some extra arousal, or there may be a 'reversed first night' effect (Hauri, 1989) in the insomniacs.

In a further study, Rosa and Bonnet (2000) reported that when patients were grouped on the basis of poor EEG nights, a poorer performance on the memory test (number of words recalled) was associated with two nights of poor EEG sleep, but the same phenomenon was observed when the subjects were grouped on the basis of their complaint: subjects with sleep complaints remembered fewer words. All other performance tests (vigilance, motor steadiness, etc.) were not significantly different whatever comparison was made.

Conclusion

It seems that neurocognitive deficits in primary insomniacs are very difficult to demonstrate at this time. Some of problems we encounter in these studies are as follows:

- types of insomnia patients included and characteristics of the insomnia (type, severity, and duration of the sleep problem)
- home versus sleep laboratory PSG recordings
- types of tests/tasks used (real world?)
- effects of age, gender, and education on test performance
- control of confounding variables such as motivation, mood
- robustness of the effect (test-retest).

These issues will be considered in turn.

A selection bias is one possible source of unreliability. The selection criteria in earlier studies were most probably not adequately defined, resulting in very heterogeneous groups of insomnia patients. We can rule out possible medical/psychiatric origins during a screening night, but then we still have different subtypes of primary insomniacs: psychophysiological, idiopathic, and possibly sleep-stage misperception as well. Another possible selection bias is the type of referral. In most studies, patients are solicited by newspaper advertisement, but sometimes they are referred from a sleep disorders centre. In some studies (Rosa and Bonnet, 2000), patients were recruited from both sources. There might have been a tendency towards morbidity in those patients seeking help via a sleep laboratory or their physician. Edinger *et al.* (1997) have posed an interesting question: when studying patients with insomnia, should we study their sleep in the sleep laboratory or at home? Regarding their daytime performance testing, Edinger *et al.* suggest that a sleep laboratory PSG put insomniacs at an advantage and healthy controls at a disadvantage, and that when insomniacs are recorded at home they show the attentional/concentration deficits of which they typically complain. Maybe the daytime performance testing should be administered at home as well to find even more of these daytime complaints, as the trip to the laboratory might induce a certain arousal in these patients.

This brings us to the next point of interest, which is the type of tests used. Some studies use a broad range of clinical psychological tests, mostly multifactorial in nature, whereas other authors prefer tasks developed by cognitive psychologists. Unfortunately, we lack a rationale for the selection of tests or tasks used or a theoretical framework of the neurocognitive functions that are assessed. Given the recent findings on the important role of sleep on memory consolidation (Maquet *et al.*, 2000; Poe *et al.*, 2000), we probably should look in more detail at this phenomenon and at test encoding at night and recall in the morning in these patients. Given the effects of age and gender on sleep, and given the skewed distribution of sleep complaints with respect to these factors (more females and more elderly with complaints), we must not forget that these same factors can also have a tremendous effect on performance testing.

Controlling for confounding factors in these highly complex matters is difficult. Factors such as motivation and mood, which are probably highly influenced by sleep quality, can interfere with daytime performance testing. Therefore, a good definition of the dependent variables and the possible confounders that can be controlled for by analysis of covariance is a necessity. Certainly, simple, univariate analyses lead to simplistic conclusions, given these highly interrelated variables. More studies investigating the robustness of the daytime neurocognitive effects in insomniacs are needed. One good example is the Pilcher *et al.* (1996) study, which shows — albeit in young adults, and not in insomniacs — that there is a stable relationship over time between sleep quality ratings and health, well-being, and daytime sleepiness. Longitudinal studies with insomniacs are rarely found, but therapeutic intervention studies might offer an opportunity here. They obviously include more assessments over time, and if daytime performance outcome measures are included, in addition to mood and other relevant measures, it would be a big step forward if the reversibility of the morbidity at baseline in these patients could be demonstrated.

Patients with primary insomnia do complain about daytime complications comprising the domains of cognition and affect. However, several studies show that it is difficult to demonstrate a clear-cut daytime neurocognitive deficit that is causally related to the sleep problem. Some factors contributing to this have been explained. A subjective sleep complaint itself does not mean a PSG-objectified sleep disturbance *per se*. Only a complaint of 'non-restorative' sleep might be related to an increased number of arousals during sleep.

Thus, there certainly exists a gap in our understanding of primary insomnia, and promising hypotheses have been formulated. Sleep-stage misperception, a disorder in which a complaint of insomnia or excessive sleepiness occurs without objective evidence of sleep disturbance, and psychophysiological insomnia have been related to fast EEG during sleep onset (Perlis *et al.*, 2001). Hyperarousal certainly explains why insomniacs who cannot fall asleep at night have the same difficulty during the daytime multiple sleep latency tests, but hyperarousal itself does not predict poor EEG sleep (Bonnet and Rosa, 2001). In addition to sleep drive, the wake-drive system needs careful consideration in our patients (Bonnet, 1997; Cluydts *et al.*, 2002).

Tension, anxiety, and mild psychopathology in primary insomniacs have repeatedly been demonstrated (Nowell *et al.*, 1997; Buysse *et al.*, 1994; Cluydts *et al.*, 1996), but these phenomena can be the result of years of non-successful searching for help in these patients. Saletu-Zyhlarz *et al.* (1997) showed that generalized anxiety disorder (GAD) patients who complain about poor sleep quality do well in some attention, concentration, and memory tasks, whereas their motor performance decreases, and they related these findings to a state of hypervigilance and hyperarousal. This agrees well with the proposed neurocognitive model of insomnia (Perlis *et al.*, 1999; 2001), which suggests that beta and gamma activity is enhanced during sleep onset in primary insomniacs.

Incorrect beliefs, expectations, and attributes concerning sleep have been identified in insomniacs, and these can be corrected successfully by cognitive-behavioural therapy (Edinger *et al.*, 2001; Morin *et al.*, 1993). Again, are these cognitions the cause or the result of years of suffering? The model of Spielman (1987) might offer some help here, as it describes predisposing, precipitating, and perpetuating factors in the development of insomnia. Within a biopsychosocial perspective, one might consider a 'fragile sleep system' or a hyperaroused nervous system as a predisposing factor that could develop into a sleep complaint in acute stress but could also develop more insidiously. The well-known conditioning processes may then contribute to the perpetuation or exacerbation of the complaint. All these hypotheses need to be further developed and evaluated.

Future research might also redirect our attention to the concept of 'sleep quality' which is so strongly related to daytime well-being and health. A better understanding of the biopsychosocial factors that contribute to the perception of the quality of our sleep might eventually result in the full recognition of the 'non-restorative sleep' syndrome.

SLEEP APNOEA

Obstructive sleep apnoea syndrome (OSAS) is a sleep-related breathing disorder characterized by repetitive episodes of complete cessation of airflow (apnoea) or decreases in airflow (hypopnoea) due to upper airway obstruction. Nocturnal apnoea and hypopnoea result in brief arousals from sleep and are frequently associated with intermittent hypoxaemia. The apnoea-hypopnoea index (AHI)

is defined by the average number of episodes of apnoea plus hypopnoea per hour of sleep. 'Mild', 'moderate', and 'severe' cases of OSAS are defined as AHI of 5–15, 16–30, and >30, respectively (American Academy of Sleep Medicine, 1999). These respiratory disturbances are associated with daytime symptoms, the most important being sleepiness and neuropsychological dysfunctioning. Obstructive sleep apnoea is the most common cause of excessive daytime sleepiness among patients evaluated at sleep disorder centres. The prevalence of this syndrome is about 4% in middle-aged men meeting minimal diagnostic criteria (AHI > 5) (Young *et al.*, 1993), is higher among males than females (Partinen and Telakivi, 1992), and increases in the elderly (Ancoli-Israel *et al.*, 1987).

We begin by discussing excessive daytime sleepiness. Furthermore, the literature on neurocognitive dysfunction is reviewed. This is followed by a discussion of some relevant methodological considerations that are particularly important in hypersomnia research. We also point out pathophysiological mechanisms of the neuropsychological impairments. Next, an overview of sleep apnoea patients' driving performance is provided. Finally, we summarize reported psychosocial symptoms and the effects of treatment in sleep apnoea.

Sleepiness

Excessive daytime sleepiness is the most common complaint in apnoea patients, as evidenced by subjective ratings such as the Stanford Sleepiness Scale (Kribbs *et al.*, 1993; Sforza and Lugaresi, 1995), the POMS (Kribbs *et al.*, 1993), and the ESS (Engleman *et al.*, 1996; Hardinge *et al.*, 1995). Objective measures of sleepiness/alertness also demonstrate clinically significant impairments. The MSLT demonstrates sleep onset latencies in apnoea patients consistently below the normal threshold of 10 min, suggesting mild to moderate sleepiness (Cheshire *et al.*, 1992; Engleman *et al.*, 1994; Kribbs *et al.*, 1993; Lamphere *et al.*, 1989). In severe apnoea patients, the mean sleep onset latency is usually in the pathological range of 5 min or less (Bédard *et al.*, 1991b; Sforza and Lugaresi, 1995). In mild sleep apnoea patients, however, average ESS ratings and average sleep onset latencies on the MSLT are at the high end of the normal range (Engleman *et al.*, 1997; Redline *et al.*, 1997). The Maintenance of Wakefulness Test requires the subject to remain awake while sitting up in bed during four 40-min trials at 2-h intervals throughout the day (Mitler *et al.*, 1982). This procedure provides evidence of significant decreases in sleep onset latency in OSAS patients (Poceta *et al.*, 1992; Sforza and Krieger, 1997). The ability to stay awake may be a more important parameter of daytime performance than the propensity to fall asleep (Roth *et al.*, 1980; Johnson, 1992). The same protocol as in the Maintenance of Wakefulness Test is used in the Oxford Sleep Resistance Test, which is a procedure to evaluate sleepiness from a behavioural perspective by measuring lapses in reaction time responses to a light-emitting diode regularly illuminated for one in every three seconds. This test distinguishes healthy subjects (mean latency to lapsing 39.8 min) from sleep apnoea patients (10.5 min) (Bennett *et al.*, 1997).

In most studies, weak to moderate positive correlations ($r = 0.3–0.5$) are found between severity of sleep apnoea and severity of daytime sleepiness measured by self-report (Johns, 1993; Rosenthal *et al.*, 1997) or by objective assessment (Poceta *et al.*, 1992; Roehrs *et al.*, 1989; Roth *et al.*, 1980). Some studies, though, report no significant correlations between sleep parameters and MSLT scores, thus suggesting hypoxaemia as a major determinant of daytime sleepiness (Bédard *et al.*, 1991a; Cheshire *et al.*, 1992). Notwithstanding this, most findings indicate sleep fragmentation as the primary cause of excessive daytime sleepiness (Day *et al.*, 1999). For example, Colt *et al.* (1991) found that OSAS patients showed similar improvements in daytime sleepiness after two nights of treatment with nasal continuous positive airway pressure, whether or not the researchers induced repetitive nocturnal hypoxaemia. Therefore, they concluded that sleep disruption, rather than hypoxaemia, leads to daytime sleepiness. It should be noted, however, that, in this study, a small sample size was used (seven patients) and that hypoxaemia could be induced for only two nights. Conversely, in a study in which nocturnal oxygen was administered to improve oxygenation without having an effect on sleep fragmentation, no improvement in daytime sleepiness was found (Gold *et al.*, 1986). Moreover, multiple regression research has identified sleep fragmentation as the best predictor of daytime sleepiness in apnoea patients (Roehrs *et al.*, 1989; Verstraeten *et al.*, 1996).

Cognition

Neurocognitive performance reported in the moderate to severe sleep apnoea literature can be subdivided into the areas of attention, executive function, memory and learning, and psychomotor function.

Attention

Sleep apnoea patients typically show vigilance decrements. The so-called Steer Clear, a simulated driving programme, has been used to assess vigilance. It measures simple reaction time performance on a 30-min task that requires the subject to avoid about 780 obstacles presented at varying intervals. Significantly higher obstacle hit rates in severe or older OSAS patients have been demonstrated (Findley *et al.*, 1991; 1995), as well as time-on-task decrements (Findley *et al.*, 1999). However, Ingram *et al.* (1994) found no significant correlation between mild apnoea in community-dwelling older adults and Steer Clear performance. On another laboratory-based Divided Attention Driving Test, 21 severe apnoea patients performed worse than 21 normal controls on all measures, with the largest decrement in tracking error. The performance of half of the patients was worse than any control subject, some even performing worse than alcohol-intoxicated control subjects (George *et al.*, 1996). In addition, community-acquired subjects with mild to moderate sleep-disordered breathing (AHI 10–30) performed worse during the last 2 min on a 10-min visual vigilance test (perceptual sensitivity) than healthy controls (Redline *et al.*, 1997), while severe OSAS patients (mean AHI 65) performed worse on the Continuous Performance Test than chronic obstructive pulmonary disease patients that experience both daytime and nighttime hypoxaemia (Roehrs *et al.*, 1995).

In view of the relationship of working memory to attention (see, e.g., Baddeley, 1992), we discuss working-memory function in this section as far as the 'slave systems' are concerned. The 'visuospatial sketch pad' is assumed to be a slave system capable of holding visuospatial information, while the 'phonological loop' performs a similar function for speech-based information. These systems are coordinated and linked to long-term memory by the 'central executive', being concerned with attentional control rather than storage (see *Executive function* section). It has been shown that moderate to severe apnoea patients exhibit decreased working-memory spans for both verbal (digit span) and visual (Corsi block-tapping) information (Kales *et al.*, 1985; Naëgelé *et al.*, 1995). Severe OSAS patients also performed poorly on the Wechsler Digit-Symbol Substitution (Bédard *et al.*, 1991b), which is a measure of information processing speed, or their AHI was significantly correlated with performance on this test (Cheshire *et al.*, 1992). However, information processing speed in subjects with mild to moderate sleep-disordered breathing did

not differ from controls (AHI < 5) (Kim *et al.*, 1997; Redline *et al.*, 1997).

Executive Function

The concept of 'executive function' refers to a multidimensional construct of (loosely) related higher-order cognitive processes such as planning, cognitive flexibility, response initiation and inhibition, problem solving, goal-directed behaviour, and self-regulation, of which the central executive of working memory is an important subordinate operation. Relatively few controlled studies exist on executive functioning in sleep apnoea. A frequently used neuropsychological task of executive function is the Trail Making Test. In this test, the subject has to connect in proper order 25 circled numbers randomly arranged on a page (part A), and 25 circled numbers and letters by alternating between these numbers and letters (part B). Some authors have found that patients performed worse on the Trail Making Test, part B, assessing cognitive flexibility (Bédard *et al.*, 1991b; Findley *et al.*, 1986), whereas others have not found such deficits (Naëgelé *et al.*, 1995; Greenberg *et al.*, 1987; Kim *et al.*, 1997). As performance on Trail Making, part A, was not included in the former studies, appropriate interpretation may be problematic (see methodological considerations below). Moreover, hypoxaemic patients in the study of Findley *et al.* (1986) showed oxygen desaturation even during the day, thus hampering the evaluation of the specific impact of sleep apnoea. Both moderate to severe (Naëgelé *et al.*, 1995) and mild to moderate (Redline *et al.*, 1997) apnoea patients remembered fewer Wechsler digits backwards, a test which is a measure of the 'central executive' of the working memory. However, Naëgelé *et al.*'s (1995) findings showed that patients also remembered fewer forward digits, while Redline *et al.* (1997) presented no data on forward digits (see methodological considerations below). The latter authors also found no deficits in their mild to moderate OSAS sample in performance on the Trail Making Test, the Wisconsin Card Sorting Test (number of perseverative errors), and a serial digit-subtraction task (Redline *et al.*, 1997). No significant decreases in verbal fluency were found in moderate to severe (Naëgelé *et al.*, 1995) or in mild to moderate OSAS (Kim *et al.*, 1997). In contrast, Bédard *et al.* (1991b) did demonstrate such an impairment. Interestingly, no deficit and no significant association were found between verbal fluency and severity of hypoxaemia in chronic obstructive pulmonary disease patients (Stuss *et al.*, 1997). Regarding planning abilities, apnoeics made more errors on the Wechsler Mazes Test (Bédard *et al.*, 1991b). They also needed a higher number of moves in a three-disc Tower of Toronto (a simplified version of the Tower of Hanoi) experiment, whereas, surprisingly, there was no significant difference between patients and controls on the more difficult four-disc Tower of Toronto (Naëgelé *et al.*, 1995). Furthermore, Naëgelé *et al.* (1995) found that patients needed more time to complete the so-called interference condition of the Stroop Color-Word Test measuring focused attention and inhibition of interfering habitual responses. Unfortunately, no data were provided on the 'easy' test conditions (see methodological considerations below). Scores on a modified Wisconsin Card Sorting Test, another frequently used test of executive function, in 17 moderate to severe OSAS patients, were not impaired as far as the number of categories achieved and total number of errors are concerned. However, these patients made more perseverative errors than the control group matched for age, verbal IQ, and educational level (Naëgelé *et al.*, 1995). When moderate patients (either AHI 10–40 or oxygen saturation level of <85% below 10 min) were compared to severe patients (either AHI > 40 or oxygen saturation level of <85% over 10 min), no significant differences in perseverative errors emerged, a finding which is in agreement with the lack of relationship between severity of hypoxaemia and Wisconsin Card Sorting performance in chronic obstructive pulmonary disease patients (Stuss *et al.* 1997).

Memory and Learning

It has been repeatedly found that moderate to severe apnoea patients show reduced short-term memory of both verbal and visual material (Bédard *et al.*, 1991b; Borak *et al.*, 1996; Kales *et al.*, 1985; Klonoff *et al.*, 1987; Naëgelé *et al.*, 1995) (see also *Attention* section above). In addition, these patients exhibited delayed recall deficits for both verbal and visual information (Bédard *et al.*, 1991b; Naëgelé *et al.*, 1995). In mild to moderate sleep-disordered breathing, however, neither immediate nor delayed verbal recall memory deficits was found (Kim *et al.*, 1997; Redline *et al.*, 1997). Moreover, these patients showed no procedural memory dysfunction, as measured with a 'pursuit rotor' task (Redline *et al.*, 1997).

Psychomotor Function

Manual dexterity was found to be impaired in moderate to severe OSAS patients (Bédard *et al.*, 1991b; Greenberg *et al.*, 1987), but not in mild to moderate patients (Kim *et al.*, 1997). Block *et al.* (1986) also found lowered psychomotor speed in mildly sleep-disordered breathing. Interestingly, a relationship between hypoxaemia and reduced complex perceptual motor and simple motor performance has been repeatedly demonstrated in chronic obstructive pulmonary disease patients (Krop *et al.*, 1973; Grant *et al.*, 1982; Grant *et al.*, 1987). Moreover, these lung patients exhibit a lower finger-tapping speed than OSAS patients (Roehrs *et al.*, 1995).

Before concluding this overview of neurocognitive function, we now return to the available executive function data. In general, it has been claimed that OSAS patients exhibit executive control deficits (see Day *et al.*, 1999; Décary *et al.*, 2000; Engleman and Joffe, 1999, for reviews). It should be noted, however, that research on higher attentional (executive) dysfunction should be guided by sound theoretical neuropsychological models of attention. This is especially important in sleep disorders characterized by excessive daytime sleepiness, since it is well known that sleepiness, as a result of sleep deprivation and/or sleep fragmentation, has significant effects on cognitive function (Bonnet, 2000; Dinges and Kribbs, 1991; Tilley and Brown, 1992), not the least of which is general cognitive slowing. As cognitive slowing underlies the higher executive functions, the effect of this basal slowing in information processing should be controlled for. This can be illustrated using the already mentioned Trail Making Test. Part A of this test is a measure of visual search, psychomotor speed, and visuomotor tracking. Part B, in addition to the same visuomotor activity and visual search processes as in part A, also requires task switching. Because of this additional demand, part B is considered to be a measure of cognitive flexibility. This test is scored in terms of the time in seconds required for part A and part B, respectively. However, it should be kept in mind that performance on part B can never be evaluated without taking into account the performance on task A. In other words, an underlying impaired basic process, such as in part A, can be responsible for a deficit in part B. Overall, this is true for all complex attention tests. Data from other tasks or other task conditions within the same test should always be considered to interpret neuropsychological data appropriately (van Zomeren and Brouwer, 1994). Other typical examples of such tests are the Digit Span Forwards and Backwards (Lehto, 1996) and the Stroop Color-Word Task (Henik, 1996). As the pervasive effects of sleepiness on cognitive function are not fully taken into account in the sleep apnoea literature, the findings of executive deficits should be regarded as tentative.

Aetiological Factors of Cognitive Dysfunction

The pathophysiological mechanisms that cause neurocognitive deficits in sleep apnoea have been argued to be sleep fragmentation and nocturnal hypoxaemia. The relative contribution of these aetiological factors is still a contentious issue (e.g., Verstraeten *et al.*, 1996). The investigation of the specific impact of these causal factors is complicated by the fact that sleep fragmentation is highly correlated with nocturnal respiratory disturbances. In any case, most studies suggest that both sleep fragmentation and nocturnal hypoxaemia contribute to the neurocognitive impairments (see Engleman and Joffe, 1999, for a detailed review). Some researchers have suggested differential effects of alertness impairment and nocturnal hypoxaemia (Bédard *et al.*, 1991b). According to their interpretations, reductions in psychomotor and executive function seem to be related to the extent of hypoxaemia, whereas attention and memory dysfunction appear to be associated with daytime sleepiness. As there is no agreement on this, further research is needed. One strategy to assess the impact of the two hypothesized pathophysiological mechanisms is to examine to what extent treatment leads to improvement in neuropsychological function (see below).

Driving Performance

For an excellent review on the relationship between sleepiness and motor and working accidents, the reader is referred to the December 1995 Supplement of the *Journal of Sleep Research* (Åkerstedt, 1995). It has been repeatedly shown that sleepiness may account for a considerable portion of automobile accidents (e.g., Horne and Reyner, 1995). To investigate the relationship of motor vehicle accidents to sleep-disordered breathing, Young *et al.* (1997) performed a population-based study in which 913 employed adults were involved and objective governmental records of accidents were used. Men with AHI of >5, compared to those without sleep-disordered breathing, were significantly more likely to have at least one accident in 5 years (odds ratios = 3.4 for habitual snorers, 4.2 for AHI 5–15, and 3.4 for AHI of >15). Men and women combined with AHI of >15 were more likely to have multiple motor vehicle accidents in 5 years (odds ratio = 7.3). Laboratory-based driving simulators were employed to assess driving ability in OSAS. For instance, the 20-min Divided Attention Driving Test requires division of attention between tracking and visual search. Severe apnoea patients performed worse than age-matched normal controls in mean tracking error (George *et al.*, 1996). The MSLT and AHI explained less than 25% of the variance in tracking error. Self-reported accident frequency does not appear to be significantly related to the severity of sleep-disordered breathing (Stoohs *et al.*, 1994). These findings indicate the difficulty of identifying individual OSAS patients at increased risk of accidents by PSG or behavioural testing.

Psychosocial Impact

The most frequently, though not always reported, psychological problem in the sleep apnoea literature is depression, as assessed with personality inventories or psychiatric screening questionnaires. It should be noted, however, that depressive symptoms on, for instance, the Zung self-rating depression scale and symptoms of sleep apnoea may overlap (e.g., fatigue, sleep disruption, impaired task performance, indecisiveness, decreased libido, and constipation from inactivity), indicating that such scales have not been adequately validated for use in sleep-disordered patients (Lee, 1990). Therefore, it is recommended in medical disorders to use assessment instruments that exclude many somatic items. For instance, the Freiburger Personality Inventory, a questionnaire measuring 12 personality traits, has only two out of 138 items confounded with daytime sleepiness. Using this inventory, it was found that even severe OSAS patients' average values were within the normal range of all scales (Cassel, 1993). In another interesting study, severe OSAS patients waiting for surgical treatment were compared to patients awaiting coronary artery bypass surgery (Klonoff *et al.*, 1987). Both patient groups showed similar subclinical elevations on the Minnesota Multiphasic Personality Inventory scales of depression, hysteria, and hypochondriasis. Moreover, these elevated levels of emotional distress before surgery in both groups were significantly ameliorated 3 months after successful surgical intervention for the respective diseases. Overall, these findings are in line with the consensus nowadays that the psychological problems of OSAS patients are not unique to sleep apnoea, but are rather a characteristic response to any chronic medical disorder (Roth *et al.*, 1995), and that psychological well-being is normalized after treatment (Engleman *et al.*, 1994; Platon and Sierra, 1992).

Treatment Effects

Nasal continuous positive airway pressure (CPAP) has become the non-surgical treatment of choice for obstructive sleep apnoea. Overall, CPAP treatment has shown, either by self-report (Engleman *et al.*, 1996) or by randomized placebo-controlled crossover design (Engleman *et al.*, 1994), that daytime function improves significantly. CPAP therapy reduces effectively both subjective (Hardinge *et al.*, 1995; Kribbs *et al.*, 1993) and objective daytime sleepiness (Sforza and Lugaresi, 1995; Kribbs *et al.*, 1993; Poceta *et al.*, 1992). Driving performance on a realistic simulator (Haraldsson *et al.*, 1995), on Steer Clear (Engleman *et al.*, 1994), or on the Divided Attention Driving Test (George *et al.*, 1997) recovers significantly after therapy. In addition, research shows that CPAP therapy reverses most neurocognitive deficits (Bédard *et al.*, 1993; Engleman *et al.*, 1994; Naëgelé *et al.*, 1998). However, Naëgelé *et al.* (1998) found no improvements in verbal and visual short-term memory. Furthermore, Bédard *et al.* (1993) demonstrated persistent deficits in planning abilities and manual dexterity after CPAP treatment. As these functions were related to hypoxaemia in their study, these researchers have suggested irreversible anoxic frontal brain damage. However, poor treatment compliance (Berthon-Jones *et al.*, 1996; Hoy *et al.*, 1999; Reeves-Hoche *et al.*, 1994: average duration of use ranging from 3.2 to 4.7 h/night) may explain the residual deficits, all the more so because some daytime somnolence, although greatly improved, persisted compared to healthy controls. Finally, as already noted, enhancements in psychological well-being were consistently found after nasal CPAP therapy (Engleman *et al.*, 1994; Platon and Sierra, 1992).

NARCOLEPSY

The narcoleptic tetrad was well described by Gélineau (1880) in a classic paper: excessive daytime sleepiness with sleep attacks, cataplexy, sleep paralysis, and hypnagogic hallucinations. The onset of adolescence is often the beginning of this neurological condition, which is more prevalent in males than females. Regarding cognitive dysfunction, these patients often complain of memory disturbances (especially encoding). Lhermitte (1930) gave a neuropsychological description of the amnesic phenomena, and Broughton *et al.* (1981), in a series of studies, repeatedly encountered subjective memory complaints in narcoleptics.

More recent studies, such as that of Beusterien *et al.* (1999), with large patient groups ($n = 558$) also found that, in addition to other psychosocial and intrapersonal problems, narcoleptics have difficulty in maintaining concentration and attention, a problem which

can be reversed by appropriate treatment. Brain-imaging studies (Bassetti *et al.*, 1997; Heyde *et al.*, 2001) did not provide evidence of structural anatomical anomalies. As the emphasis in narcolepsy research moved towards the hypocretin/orexin deficiency (Siegel, 1999; Nishino *et al.*, 2000) (hypocretinergic neurons are located in the lateral hypothalamus and are believed to control monoaminergic and cholinergic activity during sleep), interest has rekindled in possible neurocognitive deficits associated with this disorder. Initially, these deficits were thought to be related to a suspected monoaminergic and cholinergic involvement. Can the subjective attention and memory complaints of these patients be objectified by formal neuropsychological testing?

Aguirre *et al.* (1985) compared 10 untreated narcoleptics with matched controls on a battery of verbal and non-verbal memory tests and could not detect a memory deficit. They concluded that it is these patients' drowsiness that induces a subjective memory problem, as the short duration of the tests and the test session itself made it possible for the patients to counteract sleepiness. Henry *et al.* (1988) found a reduced vigilance and diminished memory encoding with longer response latencies in a classic Sternberg task in four of their eight patients when they were not treated. (The Sternberg task is a recognition memory-scanning task in which subjects need to make a reaction time response to indicate whether or not a test digit is a member of a previously memorized list of digits. The reaction time increases with the size of the set to be memorized.) Under treatment, these deficits almost fully recovered. Rogers and Rosenberg (1990) recorded on-task EEG in 30 narcoleptics and 30 healthy controls during the Wechsler Memory Scale and Digit Substitution tests, Rey's Auditory Verbal Learning and Complex Figure test, and the Profile of Mood States. No significant differences on the different measures of attention/concentration or memory tests could be detected. The continuous EEG recordings did show changes in alertness that were not accompanied by poor test performance. In a 'temporal isolation' study in which six untreated narcoleptics were allowed to follow their free-running circadian rhythm for a part of the study (Pollak *et al.*, 1992), only very minor differences in neuropsychological test performance were found. Again, the impairment of an accuracy measure in a serial search task was related to occasional lapses in attention, according to the authors. The only evidence of a very specific perceptual encoding deficit that underlies poor memory and complex reaction time performance comes from Henry *et al.* (1993), who compared 10 unmedicated narcoleptic patients with controls.

It is a common observation that most studies on cognitive processes in narcolepsy include a very small number of patients and use short duration tests that are often too engaging and stimulating. Often a theoretical rationale for the tests selected is missing and, finally, no comparison group comprising other hypersomnia, but non-narcoleptic, patients is introduced in the study design, so that it is difficult to detect narcolepsy-specific cognitive deficits.

Schulz and Wilde-Frenz (1995) used a monotonous task, the Critical Flicker Fusion Test, during a period of 10 h and found that the 10 narcoleptic patients were unable to maintain a steady performance level throughout the experiment. Finally, Hood and Bruck (1996) used an experimental protocol in which narcoleptic patients were tested in a low- and in a high-arousal condition. During the high-arousal condition, narcoleptics performed at the same level as healthy controls in the task under automatic control. On the contrary, complex tasks such as word fluency, semantic reasoning, and the paced auditory serial addition task were highly dependent on fluctuations in arousal.

From the evidence available, it seems correct to conclude that cognitive impairment in narcoleptic patients is a function of the daytime sleepiness they suffer from. These episodes of sleepiness have a temporal character, making it difficult to demonstrate cognitive deficits without an appropriate experimental protocol.

GENERAL CONCLUSION

Summarizing the currently available evidence on the existence of neuropsychological impairment as a consequence of disturbed sleep, we find few hard data on significant daytime cognitive deficits in chronic primary insomnia. Memory and attention problems often occur, but they can also be interpreted as symptoms of a more general malaise in these patients. They worry excessively about their sleep, and the possible consequences of poor sleep, and behavioural treatment seems to be their first choice. Studies on both pharmacological and behavioural interventions should include these cognitive outcome measures to find out whether the above described morbidity can be reversed.

Regarding sleep apnoea syndrome, the size and range of cognitive deficits increase with the severity of sleep-disordered breathing. Most of the community-based studies with only mild sleep-disordered breathing have documented no or only weak relationships between sleep apnoea and neuropsychological function. In contrast, studies using clinic patients have found neuropsychological impairments. These impairments may exist only in patients with more severe sleep-disordered breathing and/or in conjunction with significant daytime sleepiness. Narcoleptic patients do present with specific memory and attention problems, but these seem to be related to the attacks of daytime sleepiness they suffer from.

We have chosen to discuss possible daytime dysfunctions following some selective sleep disorders, thereby inevitably making a selection. Other sleep disorders such as idiopathic hypersomnia, and shift-work and jet-lag-related sleep disturbances are also suspected to result in cognitive deficits, as has been shown by Cho (2001). The mechanisms involved, including prolonged higher cortisol levels affecting sensible brain structures such as the hippocampus, should also be investigated and explored in chronic primary insomnia.

Further research is certainly needed to address the many methodological considerations we discussed in the previous paragraphs. Fine-grained analyses to divide cognitive abilities into their subcomponents in well-defined groups of insomnia, sleep apnoea, idiopathic hypersomnia, and narcoleptic patients are urgently needed.

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XXIV-7

Sleep Disorders — Functional Neuroanatomy

Pierre Maquet

INTRODUCTION

Sleep is probably the last complex and integrated behaviour of which the functions remain poorly understood. Nevertheless, since the last half of the 20th century, an impressive body of knowledge has been accumulated on sleep in mammals, especially cats and rodents. Much is now known on the mechanisms that maintain wakefulness, non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep, down to cellular and molecular levels.

In parallel, studies on patients and normal human subjects suggest that similar mechanisms generate sleep and sustain wakefulness in humans. However, we are still far from a comprehensive understanding of human sleep and its disorders at the fine-grained level of description reached in animal studies. Therefore, at present, it remains difficult to interpret the human pathology of sleep in terms of the underlying neuronal disturbances. Some exceptions, however, are mentioned in this chapter. For instance, recent breakthrough in molecular biology shed some light on the pathophysiology of narcolepsy in animals and, hopefully, in humans.

Sleep regulation does not involve only the generation of wakefulness and sleep periods. It also includes the interaction of sleep processes with the internal circadian rhythms and the external synchronizers, especially light. These aspects of sleep regulation are also important to consider because of the related human pathology.

This chapter provides an overview on the generation of sleep and wakefulness. It is not meant to review these mechanisms in the greatest detail but to put emphasis on the mechanisms that might be disturbed in sleep disorders. It is divided into two parts: (a) the regulation of circadian cycles and (b) the generation of wakefulness, non-REM sleep and REM sleep.

GENERALITIES ABOUT SLEEP

Sleep is not a homogeneous process. It is composed of two main sleep states: non-REM sleep and REM sleep. These two types of sleep differ in many aspects, such as their circadian distribution, their pattern of cellular activity and their physiological regulation. Non-REM sleep is usually recognized by the appearance of sleep spindles and K-complexes, the hallmarks of the light non-REM sleep (stage 2 sleep). The deepest stage of non-REM sleep, called slow-wave sleep (SWS), is defined by the presence of large-amplitude, low-frequency EEG waves. The power density of these slow waves is maximal at sleep onset and decreases exponentially during the night (Borbely, 1982).

Rapid eye movement sleep, also called paradoxical sleep (PS), is characterized by low amplitude, relatively fast rhythms on EEG recordings, ocular saccades and muscular atonia interspersed by muscular twitches. REM sleep typically occurs by periods of about 20 min, recurring every 90 min. In contrast to non-REM sleep, the duration of REM sleep episodes increases as the night progresses.

REGULATION OF CIRCADIAN RHYTHMS

Sleep/waking cycles, like many other physiological and behavioural parameters, occur with circadian periodicity. In mammals, a large body of data proves that the suprachiasmatic nucleus (SCN) of the hypothalamus is the site which controls these circadian rhythms, especially sleep/wakefulness cycles.

First, in animals, lesions of the SCN abolish the rest/activity rhythmicity and alter sleep/waking patterns (Ibuka and Kawamura, 1975; Mouret *et al.*, 1978). Second, circadian rhythms are reinstated by SCN transplants, the restored rhythms being derived from the donor SCN (Ralph and Lehman, 1991). Third, individual SCN cells maintain for long periods a near 24-h periodicity in their firing rates (Welsh *et al.*, 1995). This observation suggests that the periodicity of the SCN activity does not emerge from local networks but reflects the genuine pacemaker properties of the SCN cells. This hypothesis was confirmed at the molecular level. The circadian rhythms are generated by autoregulatory feedback loops of transcription and translation of a set of clock genes and their gene products (for review, see King and Takahashi, 2000; Wager-Smith and Kay, 2000). Manipulation of these clocks genes can lead to mutant individuals with intrinsic circadian rhythms different than the wild type (King and Takahashi, 2000; Vitaterna *et al.*, 1994).

In addition, the clock of the SCN does not tick only on its own. It is entrained to the night/day cycle by light. Anatomically, SCN is in position both to receive light signal and synchronize several physiological rhythms. The SCN receives light signal from the retina, mainly through the retino-hypothalamic tract (Dai *et al.*, 1998; Morin, 1994). It projects primarily to hypothalamic nuclei and, to a smaller extent, to limbic, thalamic and mesencephalic structures (van Esseveldt *et al.*, 2000). The SCN can influence sleep/waking cycles via the secretion of melatonin, using a specific polysynaptic pathway (paraventricular nucleus, intermedio-lateral column of the spinal cord, superior cervical ganglion, and pineal gland) (see Moore, 1996).

Biological rhythms in humans seem to be generated by similar molecular and cellular mechanisms, which thus have important implications in sleep or circadian disorders. SCN is described in humans and, as in other mammals, it receives fibres from the retina and projects extensively to other hypothalamic nuclei (Dai *et al.*, 1998). The disturbances of biological rhythms in demented patients have been attributed to structural modifications of the SCN (Swaab *et al.*, 1985). As in other mammals, circadian rhythms in humans can be entrained by light (Lewy and Sack, 1996; Shanahan and Czeisler, 2000) and by melatonin (Cagnacci, 1997; Lewy and Sack, 1996). Blind people are known to suffer from sleep disorders due to free-running circadian rhythms (Nakagawa *et al.*, 1992), with a circadian period rather longer than normal subjects (Sack *et al.*, 2000). Melatonin may help these patients to entrain circadian rhythms and improve their condition (Sack *et al.*, 2000). Finally, polymorphisms have been identified in several human clock genes.

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A link was reported between such a polymorphism and human diurnal preferences (Katzenberg *et al.*, 1999). Likewise, probands of a familial advance sleep-phase syndrome have been shown to have an intrinsic circadian rhythm of 23 h (Jones *et al.*, 1999), 1 h less than normal (Czeisler *et al.*, 1999). This trait segregates as an autosomal dominant with high penetrance. These examples provide a clear evidence of the direct effect of genotype on the phenotype of human biological rhythms.

GENERATION OF WAKEFULNESS, NON-REM-SLEEP AND REM SLEEP

The Hypothalamic Control of Sleep and Wakefulness

Wakefulness and sleep are generated continuously, in an ordered succession, through the action of interacting structures of the brainstem and the diencephalon. In particular, the anterior hypothalamus and the posterior hypothalamus play an important role in the generation of sleep and wakefulness, respectively. Two neuronal populations seem critical in this respect, the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus and the tuberomammillary nucleus (TMN) in the posterior hypothalamus.

Lesions of the anterior preoptic area of the anterior hypothalamus (POAH) and adjacent basal forebrain (BF) cause prolonged insomnia in mammals (for review, see Szymusiak, 1995; for the most recent study using lesions restricted to the VLPO only, see Lu *et al.*, 2000). In contrast, local stimulation of this region induces sleep (McGinty *et al.*, 1994; Sterman and Clemente, 1962). In this area, electrophysiological studies have detected neurons with firing rates two or three times higher during sleep than during wakefulness (Szymusiak *et al.*, 1998). It was also shown that the number of Fos-immunoreactive cells in VLPO increases after sleep and is proportional to the duration of sleep episode during the preceding hour (Sherin *et al.*, 1996). These data indicate that VLPO plays an instrumental role in the generation of sleep. VLPO receives a sparse projection from the SCN, thereby allowing the modulation of sleep by circadian rhythms (Novak and Nunez, 2000). VLPO neurons in turn project to the tuberomammillary nucleus of the posterior hypothalamus (Sherin *et al.*, 1998) and to other wakefulness-sustaining structures such as the locus coeruleus (LC) (Steininger *et al.*, 2001).

By contrast, the posterior hypothalamus seems to sustain arousal. Lesions of the posterior hypothalamus (Von Economo, 1926), as its inactivation (Lin *et al.*, 1988; 1989; 1990; 1994), decrease wakefulness and favour sleep. Within the posterior hypothalamus, a population of histaminergic neurons in the tuberomammillary nucleus have been particularly involved in wakefulness (Lin *et al.*, 1988; 1989; 1990; 1994). It is also a common clinical observation that antihistaminic drugs induce somnolence (Meltzer, 1990). Histaminergic neurons project to a wealth of structures (such as cortex, POAH, thalamus, and mesopontine tegmentum) thereby controlling the arousal state of the whole brain (Lin *et al.*, 1996).

It seems that feedback loops exist between the anterior and the posterior hypothalamus and regulate the succession of sleep and wakefulness periods. The GABAergic VLPO neurons probably have an inhibitory effect on TMN cells (Sherin *et al.*, 1998) but are themselves inhibited by wakefulness-sustaining neurotransmitters such as 5HT and NA (Gallopin *et al.*, 2000). Moreover, enhancement of histaminergic neurotransmission in the POAH decreases sleep and favours wakefulness periods (Lin *et al.*, 1994).

Finally, a small hypothalamic neuronal population was recently the subject of great experimental scrutiny. Located in the lateral posterior hypothalamus, they project throughout the brain and, importantly, to arousal-related structures (LC, raphe nuclei, and thalamus) (Peyron *et al.*, 1998). They secrete two newly described peptides, the hypocretins 1 and 2 (de Lecea *et al.*, 1998). Their effect is essentially neuroexcitatory and is mediated by two different receptors (hypocretin receptors 1 and 2), which are differently expressed in the cerebrum (Kilduff and Peyron, 2000). The presumptive functions of hypocretins concern eating behaviour and sleep (Sutcliffe and de Lecea, 2000). Local administration of hypocretin 1 into the LC suppresses REM sleep and increases wakefulness in a dose-dependent manner (Bourgin *et al.*, 2000) while perfusion of hypocretin 2 receptor antisense in the pontine reticular formation increases REM sleep (Thakkar *et al.*, 1999). These discoveries bear directly on one major sleep disorder, narcolepsy. In narcoleptic dogs, a mutation of the hypocretin 2 receptor gene leads to a truncated receptor protein (Lin *et al.*, 1999). Furthermore, systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep/waking cycles in these dogs (John *et al.*, 2000). Likewise, knockout mice in which the hypocretin gene was inactivated suffered from cataplectic attacks, a hallmark of human narcolepsy (Chemelli *et al.*, 1999). Finally, perfusion of hypocretin 2 receptor antisense in the pontine reticular formation induces cataplectic attacks in rats (Thakkar *et al.*, 1999). It is unlikely that human narcolepsy is due, as in the dog, to a mutation of a single gene, because epidemiological studies do not point to a simple genetic transmission (Mignot, 1998). However, it was recently found that hypocretin concentration in the cerebrospinal fluid was undetectable in seven out of nine narcoleptic patients (Nishino *et al.*, 2000). Moreover, post-mortem examination of the narcoleptic hypothalamus revealed a decreased number of hypocretin cells (Thannickal *et al.*, 2000). It remains to be shown whether human narcolepsy is caused by a defect in hypocretin production, secretion or signalling. These pathological mechanisms could be due to genetic, viral or autoimmune causes (van den Pol, 2000).

HOW IS WAKEFULNESS MAINTAINED?

Besides hypothalamic sites, the generation of wakefulness depends on the mesencephalic reticular formation, and on the modulation of cerebral activity by the aminergic neurotransmission: acetylcholine (mesopontine reticular formation and basal forebrain), serotonin (raphe nuclei) and noradrenaline (locus coeruleus) (for review, see Steriade and McCarley, 1990).

The mesencephalic reticular formation, as well as the cholinergic neurons of the mesopontine tegmentum, maintain wakefulness by tonically bombarding the thalamic nuclei, thereby supporting a tonic firing in thalamocortical neurons and, consequently, a cortical arousal (Datta and Siwek, 1997; Datta *et al.*, 2001; Steriade and McCarley, 1990).

The cholinergic magnocellular neurons of the basal forebrain project directly to the whole cortex and are thought to be critical for the cortical arousal. Ibotenic acid lesions of the BF reduce acetylcholinesterase cortical staining and result in an ipsilateral increase in slow waves (Buzsaki *et al.*, 1988). In contrast, electrical and chemical BF stimulation elicits a shift of the spike discharge pattern of cortical neurons from phasic to tonic and consequently leads to EEG activation (Metherate *et al.*, 1992). By the same token, the unit activity of cortically projecting BF neurons (most of which are cholinergic) increases their discharge rate during EEG activation (Detari and Vanderwolf, 1987).

Serotonergic cells of the raphe nuclei and noradrenergic neurons of the locus coeruleus can also influence the firing pattern of thalamic cells, maintaining a tonic firing in thalamocortical neurons. They also project to widespread areas of the cortex, thereby influencing the activity of large neuronal population in the telencephalon.

THALAMOCORTICAL LOOPS AND THE GENERATION OF NON-REM SLEEP

Current theories of non-REM sleep generation put a strong emphasis on the activity patterns in the thalamocortical loops. Several rhythms and activities coalesce during non-REM sleep: spindles, delta rhythm, slow rhythm and K-complexes. All these rhythms occur through complex interactions between the reticular thalamic cells, the relay thalamic neurons and cortical networks. For didactic reasons, they are described as separate processes but, in practice, these oscillations coexist (spindles and slow rhythm, delta and slow rhythm [Steriade and Amzica, 1998]) or smoothly alternate (spindles and delta rhythm [Merica and Fortune, 1997]).

In animals, it is known that thalamic neurons become hyperpolarized because the activating brainstem structures described above decrease progressively during their firing rate at sleep onset. Due to this disfacilitation mechanism, thalamic neurons change their firing mode from tonic to phasic (Steriade and McCarley, 1990). First, due to their intrinsic membrane properties, GABAergic cells of the reticular thalamic nucleus burst in the spindle frequency range and entrain thalamocortical neurons in spindle oscillation (Steriade and McCarley, 1990; Steriade *et al.*, 1993b). As sleep deepens, thalamic neurons get more hyperpolarized and a clock-like delta rhythm appears in thalamocortical cells, due to their intrinsic membrane properties (Steriade *et al.*, 1993a). This delta rhythm arises from the interplay between a low threshold calcium current (It) and a hyperpolarization-activated K^+ current (Ih) (Steriade *et al.*, 1993a). Thalamic delta rhythm is conveyed to the cortex, which further reorganizes it and incorporates it into a cortically-induced, slow rhythm (Steriade and Amzica, 1998).

Neuroimaging data support the role of thalamic nuclei in non-REM sleep generation. A significant regression was found between thalamic blood flow and the power density within the spindle frequency band (Hofle *et al.*, 1997). Likewise, significant changes in thalamic blood flow and glucose metabolism is the most consistent feature reported in human non-REM sleep (Maquet, 2000). The observation of patients with thalamic lesions also confirms the role of thalamic nuclei in non-REM sleep. Thalamotomized Parkinson patients show significant changes in their pattern of spindles (Roth *et al.*, 2000). Likewise, fatal insomnia, a prion disease characterized by a severe loss of sleep, is related to thalamic lesions and a decrease in thalamic metabolism (Cortelli *et al.*, 1997).

The slow rhythm relies on the properties of the cortical networks and the alternate silent periods of hyperpolarization with activity periods (Steriade *et al.*, 1993a; Steriade *et al.*, 1993c). The slow rhythm was recently identified in humans (Achermann and Borbely, 1997). Moreover, K-complexes, a hallmark of human stage 2 sleep, are viewed as an expression of the slow rhythm (Amzica and Steriade, 1997; Amzica and Steriade, 1998). The long-lasting silent periods appear to be due to a cascade of disfacilitation (Contreras *et al.*, 1996). Activity periods depend on aminoacidergic neurotransmission (Steriade *et al.*, 1993a). The participation of intracortical inhibitory neurons (Steriade *et al.*, 1993a), as well as cortico-cortical connection fibres (Amzica and Steriade, 1995a; Amzica and Steriade, 1995b), has also been emphasized. Recent data suggest that the slow rhythm is actively generated in the cortex. The depolarization state is related to recurrent excitatory interactions between pyramidal cells, regulated by inhibitory networks. The hyperpolarization state is characterized by an after-hyperpolarization in pyramidal cells and the withdrawal of synaptic barrages (Sanchez-Vives and McCormick, 2000).

REM SLEEP: BRAINSTEM MECHANISMS AND LIMBIC/PARALIMBIC ACTIVATION

Two aspects of REM sleep are worth describing: its generation by brainstem nuclei and the characteristic activation of limbic and paralimbic structures.

The widely held theory on REM sleep generation relies on the balance between, on the one hand, the activity of cholinergic REM sleep-on neurons of the mesopontine tegmentum (laterodorsal tegmenti [LDT], pedunculo-pontine nucleus [PPT], and peri-locus coeruleus alpha [periLCa]) and, on the other hand, the noradrenergic cells of the LC and serotonergic neurons of the raphe nuclei (Hobson *et al.*, 1975). The reciprocal interaction model is based on the observation that, during REM sleep, raphe nuclei and LC cells remain silent while, at the same time, mesopontine cholinergic cells increase their firing rate (Steriade and McCarley, 1990). The inhibition of LC and raphe nuclei cells could be due to a direct effect of cholinergic cells, but other mechanisms are possible, such as the inhibition of LC by GABAergic or glycinergic periaqueductal grey (PAG) neurons (Gervasoni *et al.*, 2000; Rampon *et al.*, 1999). Recently, it was also shown that the midbrain structures responsible for REM sleep generation were themselves modulated by higher structures, such as the central nucleus of the amygdala (Morrison *et al.*, 1999).

The activation of cholinergic mesopontine neurons leads to the three characteristics of REM sleep: the cortical activation, the muscular atonia and rapid eye movements. Mesopontine neurons project monosynaptically to the thalamic nuclei. The thalamic activation is then conveyed to the cortex. The ensuing cortical activation would explain the small-voltage, relatively high-frequency EEG activity characteristic of REM sleep (Steriade and McCarley, 1990).

The characteristic muscular atonia observed in REM sleep also depends originally on cholinergic mesopontine neurons (Lai and Siegel, 1999). Cells in or near the periLCα project and stimulate the bulbar reticular formation, directly or via a polysynaptic pathway through the pontine reticular formation. Bulbar reticular formation in turn projects and inhibits motoneurons in the spinal cord, through glycinergic neurotransmission, leading to a tonic decrease in muscle tone. In animals, lesions in the pontine reticular formation lead to REM sleep without atonia. A similar condition, the REM sleep behaviour disorder, was recently described in humans (Schenck *et al.*, 1986). It is characterized by movements of the limbs or body associated with dream mentation and at least one of the following criteria: dreams that appear acted out or sleep behaviour that disturbs sleep continuity (American Sleep Disorder Association, 1997). This condition is related to extrapyramidal disorders (Parkinson's disease, multiple system atrophy). In some of these patients, symptoms have been related to the marked neuronal loss in brainstem nuclei, such as the locus coeruleus, that usually inhibit cholinergic mediated atonia during REM sleep (Turner *et al.*, 2000).

Finally, ocular saccades in REM sleep also depend on cholinergic mesopontine neurons, especially the peribrachial region (Datta, 1999). In animals, saccades are related to ponto-geniculo-occipital (PGO) waves, prominent transient activities that are readily (although not exclusively) recorded in the pons, the lateral geniculate nucleus and the occipital cortex (Datta, 1999). These PGO waves have not been directly recorded in humans, but many arguments suggest that they exist in humans (Peigneux *et al.*, 2000).

The other striking aspect of REM sleep is the predominant activation of limbic and paralimbic structures. Although described in animals for some time (Lydic *et al.*, 1991; Ramm and Frost, 1986), this aspect of REM sleep was recently emphasized in humans by functional neuroimaging studies (Maquet, 2000). Activation in both amygdala and hippocampal formation was reported. Moreover, amygdalo-cortical functional relationships specific to REM

sleep were observed. These results suggest that brain function during REM sleep is mainly driven by an interplay between the limbic areas and posterior (temporal and occipital) cortices. The relation between this cortico-limbic interplay and the dreaming activity, although sensible, remains to be formally assessed (Maquet, 2000).

CONCLUSIONS

Sleep is not a state of complete cerebral quiescence. The patterns of brain activity certainly are different in sleep than during wakefulness but do not correspond to the absence of brain activity. The understanding of sleep mechanisms is crucial since they probably underlie some important — or even vital (Rechtschaffen *et al.*, 1989) — function.

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XXIV-8

Functional Neuroimaging in Sleep and Sleep Disorders

Pierre Maquet

INTRODUCTION

Functional neuroimaging characterizes the human brain function in time and space, using various techniques such as electroencephalography (EEG), positron emission tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI) or near infrared spectroscopy (NIRS). In this chapter, we review the studies of human sleep using PET, SPECT or fMRI, all techniques which allow us to investigate the whole cerebral volume. In the following sections, we consider successively studies performed in normal subjects and in several sleep disorders.

FUNCTIONAL NEUROIMAGING OF NORMAL HUMAN SLEEP

The regional organization of brain activity is completely different in sleep than in wakefulness and reflects cellular processes similar to the ones described in sleeping animals.

Functional neuroimaging by PET has recently yielded original data on the functional neuroanatomy of human sleep. A more comprehensive discussion of these results, which is beyond the scope of the present chapter, can be found in a recent review paper (Maquet, 2000). These recent data describe the very reproducible functional neuroanatomy in sleep. The core characteristics of this 'canonical' sleep may be summarized as follows. In slow-wave sleep (SWS), the most deactivated areas are located in the dorsal pons and mesencephalon, cerebellum, thalami, basal ganglia, basal forebrain/hypothalamus, prefrontal cortex, anterior cingulate cortex, precuneus and the mesial aspect of the temporal lobe. Briefly, these findings are in keeping with the generation of non-rapid eye movement (REM) sleep in mammals, whereby the decreased firing in brainstem structures causes hyperpolarization of the thalamic neurons and triggers a cascade of processes responsible for the generation of various non-REM sleep rhythms (spindles, theta, and slow rhythm; see Chapter XXIV-7). The human data showed for the first time that the pattern of cortical deactivation was not homogeneous but predominated in various associative cortices, particularly the dorso-lateral and orbital prefrontal cortex. There seems to be a functional link between these cortical regions and sleep processes. Indeed, these areas are known to be involved in mood regulation and in various cognitive functions (such as planning or probability matching) that help to adapt individual behaviour and are known to deteriorate after a short deprivation of sleep (Harrison and Horne, 1998, 1999; Horne, 1988, 1993; Pilcher and Huffcutt, 1996).

During REM sleep, significant activations were found in the pontine tegmentum, thalamic nuclei, limbic areas:amygdaloid complexes (Maquet *et al.*, 1996; Nofzinger *et al.*, 1997), hippocampal formation (Braun *et al.*, 1997; Nofzinger *et al.*, 1997), and anterior cingulate cortex (Braun *et al.*, 1997; Maquet *et al.*, 1996; Nofzinger *et al.*, 1997). The posterior cortices in temporo-occipital areas were also activated (Braun *et al.*, 1997) and their functional interactions are different in REM sleep than in wakefulness (Braun *et al.*, 1998). In contrast, the dorso-lateral prefrontal cortex and parietal cortex, as well as the posterior cingulate cortex and precuneus, were the least active brain regions (Braun *et al.*, 1997; Maquet *et al.*, 1996). Once again, these regional distributions of brain activity are in good accordance with the knowledge already acquired on sleep in animals (see Chapter XXIV-7). REM sleep is generated by neuronal populations of the meso-pontine reticular formation that monosynaptically activate the thalamic nuclei and in turn the cortex. Although early animals studies had mentioned the high limbic activity during REM sleep (Lydic *et al.*, 1991), functional neuroimaging in humans highlighted the contrast between this activation of limbic, paralimbic and posterior cortical areas, and the relative quiescence of associative frontal and parietal cortices. This particular pattern of activation has been thought to account for the main characteristics of human dreaming activity (Hobson *et al.*, 1998; Maquet, 2000; Maquet *et al.*, 1996). The perceptual aspects of dreams may be related to the activation of the posterior (occipital and temporal) cortices and the emotional features to the activation of the amygdalar complexes and related mesio-temporal areas. In contrast, the lack of insight, the loss of time perception, and the amnesia on awakening may be related to the relative hypoactivation of the prefrontal cortex.

Sleep as a Period Favourable to Brain Plasticity

In the adult brain, it is believed that sleep periods participate in memory trace consolidation (Hennevin *et al.*, 1995; Smith, 1995). If this were the case, the regional brain activity in sleep would not be fixed and stereotyped but would depend on previous waking experience. It was recently shown that waking experience indeed influences regional brain activity during subsequent sleep (Maquet *et al.*, 2000). During REM sleep, several brain areas, activated during the execution of a serial reaction time (SRT) task during wakefulness, are significantly more active in subjects previously trained on the task than in non-trained subjects. The functional connectivity of these brain regions was also examined (Laureys *et al.*, unpublished data). One of the reactivated areas, the left premotor cortex, was functionally more tightly correlated with the posterior parietal cortex and the supplementary motor area (SMA) during post-training REM sleep than in 'typical' REM sleep. It was hypothesized that this increase of functional connectivity during post-training REM sleep reflects some processing of the memory traces recently acquired during wakefulness and embodied in the parietal-premotor-SMA network. It remains to be shown that these processes lead to memory trace consolidation.

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The Brain's Reactivity to External Stimulation in Sleep

During sleep, the brain remains able to process external stimuli (see, for instance, Perrin *et al.*, 1999). During non-REM sleep, as during wakefulness, several areas continue to be activated by external auditory stimulation: the thalamic nuclei, the auditory cortices and the caudate nucleus (Portas *et al.*, 2000). However, in contrast to wakefulness, no activation is observed in the left parietal, or in the prefrontal and cingulate cortices. Furthermore, in sleeping normal subjects, the activation related to the subject's own name, as compared to beeps, involved the amygdala and the prefrontal cortex (Portas *et al.*, 2000). This demonstrated the persistence during sleep of specific responses to meaningful or emotionally loaded stimuli.

Sleep Drugs and Regional Brain Function

Functional neuroimaging can also be used to explore the effect of a therapeutic agent on brain function. For instance, during REM sleep, zolpidem administration decreased regional blood flow in the anterior cingulate cortex during REM sleep, and in the prefrontal cortex and the insula during non-REM sleep. The results indicate that some differences in regional cerebral blood flow (rCBF) from wakefulness to sleep are modulated by zolpidem (Finelli *et al.*, 2000).

SLEEP DISORDERS

Sleep is deteriorated in a wide range of conditions, ranging from environmental situations (such as jet lag, shift work, and noisy environment) to medical diseases (such as endocrine disorders, chronic pain, brain lesions, sleep apnoea) and psychiatric disorders (such as anxiety and depression). Only a handful of these situations have been explored by functional neuroimaging. Our relative ignorance of the pathophysiology of most sleep disorders and the technical difficulties related to sleep studies probably explain why neuroimaging techniques have not been widely used in the exploration of human sleep disorders and certainly less than in other neurological conditions such as epilepsy or degenerative diseases. In this chapter, we will cover only a few disorders of sleep. We exclude single-case reports, however interesting they may be in their own right (see, for instance, the report on Kleine-Levine syndrome by Lu *et al.*, 2000). Depression is extensively dealt with elsewhere in this book and will be only briefly mentioned here in the context of sleep and sleep deprivation studies.

Narcolepsy

To our knowledge, the voxelwise functional neuroanatomy of the waking state, REM sleep or SWS is not yet described in narcoleptic patients. Nor have been the brain areas related to the other cardinal symptoms of diseases — cataplectic attacks and hypnapompic/hypnagogic hallucinations or sleep paralyses — characterized.

Early observations using ^{133}Xe inhalation showed that during wakefulness, brainstem and cerebellar blood flow was lower in narcoleptic patients than in normal subjects (Meyer *et al.*, 1980). During sleep (3 out of 13 subjects in REM sleep), the cerebral blood flow increased in all regions, and particularly in the temporo-parietal regions. This pattern was putatively attributed to dreaming activity. More recently, a HMPAO-SPECT study compared the waking state to REM sleep (Asenbaum *et al.*, 1995). The global uptake was similar in both conditions. Analysis by regions of interest indicated again an activation of parietal regions. These parietal activations are not in agreement with the parietal deactivation observed by PET studies during normal REM sleep (Maquet, 2000). Further observations are required, using present technology of the acquisition and analysis of functional neuroimaging data, to characterize better the functional organization of the narcoleptic brain during wakefulness and sleep.

Some ligand studies have been reported. Because acetylcholine is an important neurotransmitter in the generation of REM sleep, it was thought that some disturbance in the cholinergic system might underlie narcolepsy. Unfortunately, no change in muscarinic cholinergic receptors could be observed in narcoleptic patients (Sudo *et al.*, 1998). Likewise, post-mortem examination of the brain of narcoleptic patients indicated an increased dopamine D2 binding (Aldrich *et al.*, 1992). This finding was not confirmed by *in vivo* measurements by PET or SPECT, three studies out of four not showing any change in dopamine receptor-binding potential in narcoleptic patients, as compared to controls (Hublin *et al.*, 1994; MacFarlane *et al.*, 1997; Rinne *et al.*, 1996; Rinne *et al.*, 1995). In one study, there was a significant increase in the uptake of ^{11}C-raclopride, a specific D2-receptor ligand, in the putamen of narcoleptic subjects older than 31 years (Khan *et al.*, 1994). Finally, although the binding of IBZM, another D2 receptor ligand, was similar in narcoleptic patients and normal controls, treatment by stimulants and antidepressants for 3 months significantly changed the ligand uptake in four out of five patients. This suggested that the post-mortem findings were more related to treatment than to the disorder itself (Staedt *et al.*, 1996).

The effects of stimulant drugs on cerebral function in narcoleptic patients was assessed in two fMRI studies. The first one tested the effect of modafinil, a sleep-preventing drug (Ellis *et al.*, 1999). First, it was observed that the activation related to visual or auditory stimulation was dependent on the time of day. Normal subjects show higher levels of activation at 10 am than at 3 pm. In a group of 12 narcoleptic patients, the reverse pattern was observed. The administration of modafinil did not modify the average level of activation in either normals or narcoleptics ($n = 8$). However, in both populations, the post-drug activation level was inversely proportional to the pre-drug activation level. This finding is not easily interpreted, but it suggests that modafinil can modulate the brain activation to external stimuli.

Finally, an fMRI study assessed the effects of amphetamines in a small sample of patients with narcoleptic syndrome ($n = 2$, Howard *et al.*, 1996). As compared to three normal control subjects, the activation related to auditory and visual stimulation was decreased in extent after amphetamine administration in normal subjects. The reverse pattern was observed in patients. Once again, these findings are difficult to interpret, and larger samples should be studied before any generalization can be made.

Following the advances made recently in the understanding of narcolepsy (see Chapter XXIV-7) and the discovery of the implication of the hypocretin system in this disorder, it is expected that new neuroimaging studies will soon appear that provide a better description of regional brain function in the various aspects of the disease, such as excessive daytime somnolence, cataplexy and hypnapompic/hypnagogic hallucinations.

Periodic Leg Movements

Periodic leg movements during sleep (PLMS) are characterized by repetitive flexion of the extremities. PLMS can occur in isolation or together with other sleep disorders (narcolepsy, sleep apnoea syndrome or REM sleep behaviour disorder [RBD]). In any case, PLMS disturbs sleep, being responsible for repeated brief arousals. A disinhibition of the descending inhibitory pathways, involving the dopaminergic, adrenergic and opiate systems, is thought to cause PLMS (Wetter and Pollmacher, 1997). Among other therapeutic possibilities, dopaminergic drugs were shown to relieve PLMS (Montplaisir *et al.*, 1991). Only one group has tested the hypothesis of a decreased dopaminergic activity in PLMS patients. In a

series of reports, they mention a decreased IBZM striatal uptake, indicating a lower D2 receptor occupancy in PLMS patients (Staedt *et al.*, 1993; Staedt *et al.*, 1995a; Staedt *et al.*, 1995b). Treating patients with dopamine replacement therapy increased the IBZM binding and improved the sleep quality in these patients (Staedt *et al.*, 1995b).

REM Sleep Behaviour Disorder (RBD)

This condition, which has been described only recently (Schenck *et al.*, 1986), is characterized by movements of the limbs or body associated with dream mentation and at least one of the following criteria: dreams that appear to be acted out or sleep behaviour that disturbs sleep continuity (American Sleep Disorders Association, 1997). Patients with REM sleep behaviour disorder are likely to develop extrapyramidal disorders (Schenck *et al.*, 1996) as multiple system atrophy (Plazzi *et al.*, 1997), i.e., degenerative disorders related to alteration in the central dopaminergic system.

With SPECT and IPT (a ligand of striatal presynaptic dopamine transporter), it was recently shown that IPT binding in RBD patients ($n = 5$) is lower than in normal controls but higher than in Parkinson patients ($n = 14$; Eisensehr *et al.*, 2000). These results suggest that the number of presynaptic dopamine transporters is decreased in both Parkinson and RBD patients. It remains to be shown whether this alteration plays a causal role in the pathophysiology of RBD. Although there is evidence that some Parkinson patients do show excessive nocturnal movements (Trenkwalder, 1998), it is intriguing that not all Parkinson patients develop full-blown RBD. This suggests that modifications of other systems of neurotransmission are probably necessary for RBD to occur.

Depression: Studies of Sleep and Sleep Deprivation

There are good reasons to study brain function during sleep in depressed patients. Sleep disturbances are an integral feature of depressive disorders, ranging from hypersomnia to marked difficulty in maintaining sleep. The architecture of sleep itself is modified and characterized by a reduced SWS, an early onset of the first episode of REM sleep, and increased phasic REM sleep (Thase, 1998).

Sleep deprivation had rapid beneficial effects on about 60% of depressed patients (Wirz-Justice and Van den Hoofdakker, 1999). Responders to sleep deprivation are usually patients with high behavioural activation and low levels of tiredness (Bouhuys *et al.*, 1995; Szuba *et al.*, 1991). The hypothesis has been put forward of an increased arousal in depressed patients. Functional neuroimaging lends support to this hypothesis. Beta activity was proposed as a marker of arousal during sleep (Nofzinger *et al.*, 2000). Beta power is negatively correlated with subjective sleep quality, in both normal and depressed individuals. Depressed patients tend to have increased beta activity during the night as compared to normal controls. Finally, beta power is correlated with the glucose metabolism of ventro-medial prefrontal cortex, one of the most deactivated regions during consolidated SWS (see above).

Brain activity during sleep differs in several aspects from normal human sleep. Early studies indicated that cerebral metabolism during non-REM sleep in depressed patients is higher than in normal subjects (Ho *et al.*, 1996). The greatest increases was observed in the posterior cingulate, the amygdala, the hippocampus, and the occipital and temporal cortex (Ho *et al.*, 1996). During REM sleep, more recent data showed that the anterior paralimbic areas (anterior cingulate cortex, right insula, right parahippocampal gyrus) were less active, as compared to wakefulness, in depressed patients than in normal subjects (Nofzinger *et al.*, 1999).

Sleep deprivation has profound effects on brain metabolism, in both normals and depressed subjects. After 24 h of total sleep deprivation in normal subjects, cerebral glucose metabolism decreases by 8% (Thomas *et al.*, 2000). This decline involves the brain as a whole but is particularly prominent in the thalamic nuclei and associative cortices of the frontal and parietal lobes. No brain area increases its glucose metabolic rates after 24 h of continuous wakefulness. In depressed patients who respond to sleep deprivation, the baseline brain activity (measured in the awake patient) is higher in the anterior cingulate cortex (Wu *et al.*, 1992) or nearby mesial frontal cortex (Ebert *et al.*, 1994; Wu *et al.*, 1999) than in non-responders. This feature normalizes after sleep deprivation. The same pattern was observed in elderly depressed patients, the normalization of anterior cingulate metabolism persisting even after recovery sleep (Smith *et al.*, 1999).

These data suggest a close link between the mood alteration and the activity in limbic and paralimbic structures. In particular, it is as if the anterior cingulate cortex were hyperactive in depressed patients during wakefulness, masking any further increase in REM sleep. Sleep deprivation would decrease both the activity in the anterior cingulate cortex and depression ratings. Further studies are needed to understand the causes and consequences of these mesial frontal metabolic disturbances.

CONCLUSIONS

Functional neuroimaging provides unprecedented possibilities to explore the brain function during sleep. In the near future, it should help to clarify the functions of sleep and the mechanisms underlying sleep disorders.

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XXIV-9

Genetics of Sleep and Sleep Disorders

Paul Linkowski

INTRODUCTION

The relationship of genes to behaviour has always been a major topic of research and interest in the neurosciences. In the study of sleep, the traditional way to determine whether distinct phenotypes have a familial component was to study family pedigrees or to investigate sleep in monozygotic (MZ) and dizygotic (DZ) twins, a classical method in human and psychological research. In animals, the inheritance of sleep traits was previously studied by Valatx *et al.* (1972) in cross-breeding mice strains. The developments of molecular genetics and gene technology favoured a renewed interest in the behavioural neurobiology of sleep, leading to an increasing number of papers on the genetics of sleep and the molecular aspects of sleep disorders.

Current scientific investigations address various phenotypic dimensions, including the genetic components of rapid-eye-movement (REM) sleep and delta sleep, and the circadian components of human sleep.

In the present paper we will review some of the classical genetic studies of sleep and summarize important recent results.

GENETICS OF SLEEP IN NORMAL MAN: TWIN STUDIES

The importance of the investigation of the genetic aspects of sleep was demonstrated in some early studies that focused mainly on the investigation of twin pairs in man. In 1937, Geyer (quoted in Vogel, 1958) reported that concordance for a number of sleep characteristics was much better in MZ than in DZ twins. Other pioneering efforts were made by Vogel (1958), who was the first to study the sleep EEG in MZ twins, and by Zung and Wilson (1967), who performed computations of the sleep EEG of three pairs of MZ and three pairs of DZ twins. They reported that the all-night-sleep EEG and REM patterns of MZ twins were similar, whereas those of DZ twins were dissimilar and variable.

It should be noted, however, that some further studies relied mainly on questionnaire investigations of sleep habits (duration of sleep, subjective reports of sleep timing and quality) in MZ and DZ twins (Partinen *et al.*, 1983; Heath *et al.*, 1990). These studies were conducted mainly in adult twins, and, in general, correlations were higher for MZ than for DZ twins, with a significant synchronizing effect of cohabitational status on sleep length (Partinen *et al.*, 1983). Heath *et al.* (1990) found that genetic influences accounted for at least 33% of the variance in sleep quality and sleep disturbance, and 40% of the variance in sleep pattern. A substantial genetic effect was also reported for some parasomnias such as sleepwalking and nocturnal enuresis (Kales *et al.*, 1980).

The hereditary components of sleep were also suggested by some polygraphic twin studies of newborns (Gould *et al.*, 1978), of a limited number of adolescent twin pairs, and of single nights of identical and fraternal twins. In the study of Webb and Campbell (1983), measures of awakening and REM sleep amounts were found to be significantly correlated in identical twins. In a study of seven twin pairs, Hori (1986) suggested that sleep spindles were determined by a genetic trait.

The inheritance of properties of the wake EEG has also been a focus of interest in recent years, since the pioneering observations of Vogel (1958). Stassen *et al.* (1987) reviewed 22 twin studies and noted that MZ co-twins were generally more alike than DZ co-twins, indicating the presence of genetic influences on the spontaneous EEG. In a more recent study, Van Beijsterveldt *et al.* (1996) measured four frequency bands (delta, theta, alpha, and beta) and reported that the average heritability of brain functioning, as indexed by rhythmic brain electrical activity, is one of the most heritable characteristics in humans.

Finally, a large number of animal studies also indicate unequivocally that certain aspects of sleep might be genetically determined.

These studies have mainly suggested a genetic determination of sleep length in rodents (Valatx *et al.*, 1972; Friedman, 1974; Franken *et al.*, 1999) as well as a significant genetic effect on REM sleep in mice (Valatx *et al.*, 1980; Valatx, 1984).

Taken together, most studies that addressed the issue of genetic aspects of sleep in normal men have relied on the twin method, which allows the determination of heritability estimates and is frequently used to partition variance of phenotypic quantitative traits into environmental and genetic components.

The twin design has also been frequently used to evaluate the heritability of other primary sleep disorders such as sleepiness, nightmares and sleepwalking. This will be reviewed later in the chapter. We will first present the results of the Brussels twin study, which was conducted between 1986 and 1990 (see Table XXIV-9.1) (Linkowski *et al.*, 1989; 1991).

In this study of two samples of 26 pairs of male twins, we have found that stages 2, 3, $3 + 4$ and 4 were significantly determined by genetic components. This was also the case for REM density. No genetic effect was found for total sleep period, period of sleep, total sleep time, sleep onset latency and REM latency. Waking was found to be significantly determined by genetic factors in one of the two samples.

Therefore, our results substantiate previous reports suggesting that some components of human sleep might be genetically determined (Partinen *et al.*, 1983; Webb and Campbell, 1983). In our study, stages 2, 4 and delta sleep (stages $3 + 4$) unequivocally show a strong genetic component: the same is true, but is less pronounced for REM density measures. It is of interest that the stages with the strongest genetic component show the best relative stability from night to night (Merica and Gaillard, 1985). This is also supported by previous reports of substantial within-subject, night-to-night correlation of stage 4 (Webb and Agnew, 1968) and a greater inter- than intraindividual variation of stage 4 (Merica and Gaillard, 1985).

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Table XXIV-9.1 Selected heritability estimates of markers of sleep-wake homeostasis

Parameter	Authors	No. of pairs	Gender	Age range (years)	Heritability 2 ($r_{MZ}-r_{DZ}$)
Minimum of stages 2 + 4	Linkowski *et al.*, 1989	14 MZ 12 DZ	M	16–35	0.50
REM density	Linkowski *et al.*, 1991	11 MZ	M	20–36	0.95
Minimum of stages 3 + 4		15 DZ			0.52
REM density	Linkowski *et al.*, 1998	9 MZ	M	16–35	0.64
Minimum of stages 3 + 4 (with catheter)		8 DZ			0.90
Minimum of stages 3 + 4 (with catheter)	Linkowski *et al.*, unpublished	11 MZ 11 DZ	M	16–35	0.68

More recently, Franken *et al.* (2001) have reported that the increase of slow-wave sleep need after sleep deprivation is under strong genetic control in the mouse. Our data also show that regulation of REM stage variability shows substantial environmental determination rather than evidence of significant genetic effect, in opposition to what is observed in animals (Valatx, 1984).

GENETIC ASPECTS OF SLEEP DISORDERS

For most of the disorders of sleep, twin or family studies support a genetic component in a majority of sleep disorders such as sleepwalking nightmares, hypersomnia, restless legs syndrome (RLS), insomnia, obstructive sleep apnoea and narcolepsy. With reference to the last disease, the gene responsible for dog narcolepsy has recently been cloned (Lin *et al.*, 1999), also implicating the dysfunction of orexins (hypocretins), neuropeptides active in energy homeostasis and arousal (Thannickal *et al.*, 2000; van den Pol *et al.*, 2001).

FAMILY ASPECTS OF INSOMNIA

A family history of insomnia is a well-known symptom among patients with a complaint of chronic insomnia (Hauri and Olmstead, 1980; Bastien and Morin, 2000). In the study of Bastien and Morin (2000), 35% of the patients consulting for insomnia had a positive family history of sleep disturbances. Insomnia was the most common type of sleep disturbance identified (76%), and the mother was the most frequently afflicted family member.

The reported sleep disturbances among family members were more prevalent when the onset of insomnia was before 40 years of age than when it was later in life. These findings are supported by other reports such as that of Dashevsky and Kramer (1998), which shows that 42% of patients with psychiatric disturbances have a positive family history of insomnia.

Another major contribution to the genetic studies of insomnia came from the studies that explored the clinical and molecular genetics of a new syndrome: fatal familial insomnia (FFI) (Lugaresi *et al.*, 1986; Cortelli *et al.*, 1999). FFI is a rare autosomal dominant disease clinically characterized by sleep loss that inevitably proves fatal, disordered sleep-wake cycle, dysautonomia (increased perspiration and salivation, impotence, constipation, tachycardia, systemic hypertension and fever progressively worsening), and motor signs (ataxia, dysarthria, astasia-abasia and myoclonic jerks). At autopsy, both in the thalamus and in the cortex, the limbic structures (the orbito-frontal cortex, the cingulate gyrus and the mediodorsal and ventral thalamic nuclei) are those most consistently and severely involved.

Genetically, the disease is characterized by a missense mutation at codon 178 coupled with methionine at the polymorphic codon 129 of the prion protein gene (Goldfarb *et al.*, 1992; Medori *et al.*, 1992). The selective atrophy of the limbic thalamus that characterizes FFI might be due to the binding of toxic prion proteins to specific receptors on thalamolimbic neurons, leading to this rare but well-characterized inherited prion disease in humans.

SLEEPINESS

Carmelli *et al.* (2001) have explored the responses to the eight-item Epworth Sleepiness Scale obtained from 1560 World War II male veteran twin pairs to determine the extent to which genetic influences are involved in self-reported daytime sleepiness in the elderly. The heritability of responses to the Epworth Sleepiness Scale was estimated to be 38% while environmental influences not shared by twin brothers accounted for the remaining variance in daytime sleepiness. The authors interpreted the heritability of the responses to the Epworth Sleepiness Scale as related to a genetic susceptibility to disordered breathing during night sleep.

NIGHTMARES, SLEEPWALKING AND SLEEPTALKING

The twin design was also applied to quantify the genetic influences affecting the liability to nightmares, sleepwalking, night terrors and sleeptalking.

Nightmares are frightening dreams that usually awaken the sleeper from REM sleep. They are usually long and complicated, exhibiting a progressive content that becomes increasingly frightening toward the end (Broughton, 1994).

Sleepwalking belongs to the group of parasomnias characterized by a series of complex behaviours that are initiated during slow-wave sleep and result in walking during sleep.

Talking, screaming, striking out or walking during the nightmare rarely occurs, distinguishing the nightmare from sleep terror and REM sleep behaviour disorder (ASDA, 1990). No firm evidence for a familial basis of nightmares was available until the study of Hublin *et al.* (1999), who investigated 1298 MZ and 2419 DZ twin pairs by questionnaire. They showed a persistent genetic effect on the disposition to nightmares in both childhood and adulthood. Of the phenotypic variance, the estimated proportion of genetic effect in childhood was 44% in males and 45% in females. In adults the values were 36% in males and 38% in females. Nightmare frequency and psychiatric disorders were linearly associated.

The same authors explored the prevalence a genetic predisposition to sleepwalking in the same population of twins and reported a substantial genetic effect in both childhood and adulthood (Hublin

et al., 1997). The proportion of total phenotypic variance attributed to genetic influences was 80% in men and 36% in women. The same values were 60% in men and 57% in children for sleepwalking.

RESTLESS LEGS SYNDROME (RLS)

RLS is a common sleep disorder, present in about 6% of the adult population. It is characterized by motor restlessness and painful sensations during sleep in the legs. RLS has been reported to be familial: 30–64% of RLS patients have positive family history (Lazzarini *et al.*, 1999). It has been shown that familial RLS follows an autosomal dominant mode of inheritance with variable expressivity (Becker *et al.*, 1993). It is also important to note that genetic anticipation (increased severity with age and an earlier age of onset of the illness in successive generations) has been suggested for RLS indicating a possible trinucleotide repeat expansion in the molecular mechanisms of the disease (Lazzarini *et al.*, 1999).

NARCOLEPSY

Narcolepsy is a disabling sleep disorder which typically arises during adolescence and early adulthood, and affects 0.02–0.18% of the general population (Mignot, 1998). This syndrome is characterized by excessive daytime sleepiness and pathological manifestations of REM sleep. These include cataplexy (a sudden loss of muscle tone generally caused by a strong emotion), sleep paralysis (muscle atonia when falling asleep or at awakening) and hypnagogic hallucinations, a complex state that occurs between waking and sleep. The full tetrad of excessive daytime sleepiness, cataplexy, sleep paralysis and hypnagogic hallucinations is present in only 15% of the patients (Aldrich, 1998).

Familial narcolepsy is rare (sporadic cases are more frequent) and the risk to first-degree relatives is estimated at 1–2%; only 8–10% of narcoleptic patients identify another member of the family as having the narcolepsy-cataplexy syndrome. Furthermore, a risk of 2% is 10 times higher than what is observed in the general population, suggesting the existence of predisposing genetic factors (Mignot, 1998).

The importance of environmental factors is supported by the fact that up to 30% of MZ twin factors are concordant for narcolepsy. This led some authors to suggest that undefined environmental factors act on a susceptible genetic background to initiate the disease. Several studies have reported a strong association between certain HLA haplotypes on human chromosome 6 and human narcolepsy; HLA DQB1*0602 and DQA1*0102 are found in 90% of narcoleptics, but in only 30% of the general population, suggesting that autoimmunity plays a role in the disorder (Kadotani *et al.*, 1998).

The major breakthrough, however, occurred recently, shedding new light on the physiopathological and molecular basis of the disease. In 1998, two peptides were identified in the hypothalamus, and were termed hypocretin-1 (HCRT-1) and hypocretin-2 (HCRT-2), reflecting their hypothalamic origin. They project to monoaminergic and cholinergic components of the ascending reticular activating system (Kilduff and Peyron, 2000). The sleepiness in narcolepsy appears to reflect lack of hypocretin excitatory inputs on histaminergic, dopaminergic and cholinergic components of the ascending reticular activating system, which normally promote thalamo-cortical arousal (Silber and Rye, 2001).

Other recent molecular and post-mortem studies further support the role of hypocretins, indicating a mutation in a case of early-onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains (Peyron *et al.*, 2001). These studies are paralleled by similar studies in mice (Hara *et al.*, 2001) and canine narcolepsy (Hungs *et al.*, 2001). HLA and hypocretin findings can be integrated into a concept of immunologically mediated destruction of hypocretin-containing cells in human narcolepsy (Mignot *et al.*, 2001). The genetic contribution of HLA to narcolepsy might also be estimated by the use of the lambda statistic (Risch, 1987), which computes the relationship between increased risk in relatives and population prevalence. The results indicate that complex HLA-DR and -DQ interactions contribute to the genetic predisposition to human narcolepsy but that additional susceptibility loci are also probably involved.

HCRT-1 was undetectable in the CSF of seven of nine patients with narcolepsy (Nishino *et al.*, 2001). In an autopsy study, hypocretin neurons were damaged in 95% of narcoleptic brains (Thannickal *et al.*, 2000). Finally, Dalal *et al.* (2001) confirmed low to absent HCRT-1 concentrations in the CSF of narcoleptic patients and demonstrated that serum HCRT-1 was normal in narcolepsy.

These results add further fascinating details to the neurochemical aspects of sleep and offer a new potential for the discovery of pharmacological tools targeting hypocretin receptors in order to cure the disorders of excessive sleep.

CONCLUSION

Current research suggests that both normal sleep and various sleep disorders either have a genetic basis or are influenced by genetically determined physiological or environmental predispositions. Identifying the genes that influence the various components of sleep may ultimately contribute to a better understanding of the processes that control normal sleep, and also to the understanding of sleep disorders, and might add new pathways to the development of interventions to alleviate these disabling conditions.

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XXIV-10

Gender Issues Related to Sleep

Joyce Walsleben

Sleep is an active cyclic process that involves both physiological and behavioural changes. There are two major states of sleep: rapid-eye-movement (REM) sleep, commonly known as 'dream sleep', and non-REM (NREM) sleep. In the adult, these states cycle every 70–90 min across the night, usually beginning with NREM sleep. The state of NREM is divided into four stages: stages 1–4, which denote the 'depth' of sleep, based upon one's ability to respond to stimulation. Stage 1 is thought to be transitional and the lightest. Stage 2 fills approximately 50% of the night and is frequently felt to be the first definite stage of sleep. The deepest stages are 3 and 4, called delta sleep, or slow-wave sleep (SWS). SWS is felt to be the most restorative stage. The depiction of these states and stages of sleep in a histogram shows the structure or architecture of one's sleep at any given time point.

Originally, scoring of sleep stages was performed visually only by comparison to standard guidelines which evaluated the EEG frequency and amplitude (Rechtschaffen and Kales, 1968) of the electroencephalogram (EEG), electro-oculogram (EOG) and electromyogram (EMG). For instance, delta sleep is represented by an EEG frequency of 0.5–4 Hz and an amplitude $>75\,\mu V$, with steady EOG and decreased EMG, and it is further delineated into stages 3 or 4 by the percentage of a 30-s epoch which displays this frequency and amplitude. More recently, with the advent of computer scoring and spectral analysis, more definitive changes of EEG power can be documented, allowing subtle changes across and between stages of sleep to be noted.

Two main biological drives regulate the timing and amount of sleep. They are the circadian drive, or 24-h sleep/wake cycle, and the homeostatic drive, which may represent the accumulation of sleep factors or toxins produced during wake. These drives change as we age, altering the normal flow of sleep across our 24-h 'day'.

We begin to form the various stages of sleep around the fourth month of life. Sleep architecture continues to evolve throughout childhood and adolescence, and subtle differences of architecture between sexes may exist at times during this period. Sleep then reaches a fairly stable period until mid-life when gender differences begin to be more noticeable and suggest a female advantage. Sleep architecture continues to change subtly with ageing, sometimes affected by health and lifestyle.

Besides the biological changes that occur in sleep across age and gender, sleep disorders may develop, and certain of these have a higher prevalence or impact in one sex than the other. Furthermore, females' sleep is often affected by significant hormonal influences that may cause day-to-day alterations from menarche through menopause. Some women are more sensitive to these influences than others. Finally, psychosocial issues, particularly stressful lifestyle, affect one's ability to sleep well and cannot be ignored in a discussion of gender differences in sleep. Females, perhaps secondary to hormonal influences, appear to be more affected by stress than men. In addition, the psychosocial demands of mothering and caregiving frequently have a negative impact on woman's sleep.

SLEEP CHANGES ACROSS AGE AND SEX

A seminal work examining sleep in normal subjects was reported by Williams *et al.* (1974), who showed sleep architectural changes across ages and between genders (Table XXIV-10.1)

Table XXIV-10.1 Comparison of gender differences in sleep characteristics (Williams *et al.*, 1974)

Age (years)	Females	Males
3–5	Increased SWS, decreased stage 2	Increased TIB, SPT, TST
6–9	Increased SWS	
10–12	Increased SWS, no. of stage changes	
13–15	Same, begin to see decreased TIB, TST and TSP	
16–19	Same, decreased REM	
20–29	Increased stage 2	Increased awakenings
30–39	No differences	
40–49	Increased TST, SE	Increased WASO, decreased SE
50–59	Increased SWS, REM	
60–69	Increased SWS (50% have it)	Increased wake, only 10% have SWS
70–79	Increased stage shifts, SWS, REM	Increased TIB

SWS = slow-wave sleep, TST = total sleep time, TSP = total sleep period, TIB = time in bed, REM = rapid-eye-movement sleep, WASO = wake after sleep onset.

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Non-complaining sleepers, categorized by decade and gender, were studied with full laboratory-based polysomnography. While not all variables that could influence sleep were controlled for at this early time in the science, this work did attempt to control for the influence of the menstrual cycle by studying only adult women in their follicular phase. Sleep differences between males and females were seen to begin around 9–10 months of age, with female babies sleeping longer. Around the age of 3 years, males showed greater time in bed (TIB), longer total sleep time (TST), longer REM cycle length and higher percentage of stage 2 sleep. Females demonstrated a trend toward increased SWS that continued throughout most of their lives. Teenage females tended to show less TIB, and in the later teens females slept less than males. In their twenties, females demonstrated more TST than men and continued to do so throughout life. Additionally, they showed more stage 2 sleep. Males began to show increased levels of arousal and decreased SWS toward the end of the second decade. The decline of SWS continued throughout their life, but it did not begin in females until the fourth or fifth decade. Males continued to show increased levels of arousal and decline in REM sleep during their fourth decade. Women began to show less SWS in their fifties and began to exhibit patterns like those of men in their thirties. Sleep became fragmented in women during the perimenopausal years with increased arousal. During the sixth decade, larger differences were seen in the amount of SWS; only 10% of males showed SWS while 50% of females did 50. In their seventh decade, women showed more stage changes than men did, perhaps simply because they continued to show all stages of sleep. By seventy, few men showed appreciable amounts of SWS. In addition, they tended to show shorter REM cycles. The REM cycles of younger adults showed increasing length across the night such that the last REM cycle of the night was the longest. This pattern changed in the elderly, with REM cycles becoming more even in length across the night.

While numerous smaller studies have confirmed these findings using visually scored sleep staging, many have resulted in controversy, particularly regarding the sleep of infants. Some studies of infants have shown no gender-related differences (Parmalee *et al.*, 1961, Hoppenbrowers *et al.*, 1989). Others have shown increased variability in males (Moore and Ucko, 1957) or females (Cornwell, 1992). The environment is one influence that could alter findings. To examine infant sleep and the influence of environment, Bach *et al.* (2000) examined the relationship between gender-related sleep differences with a thermal challenge. Healthy preterm neonates were studied (21 males/17 females) during three consecutive morning sleep sessions while sleeping in their closed, convectively heated incubators. The first morning served as a screen at thermoneutral conditions. The second session provided baseline data at thermoneutral conditions. The experimental condition, day 3, monitored sleep at cool conditions ($<1.5^{\circ}$C from baseline). Sleep continuity and architecture were altered in both males and females. Active sleep (AS) increased at the expense of quiet sleep (QS). Males exhibited a larger variability in their sleep than females, sleeping less, waking more frequently and showing more AS. Since QS is believed to be highly controlled by developing brain centres, the investigators suggested that stable QS might mark the more advanced development of the female infants. The investigators also proposed that the increased variability seen in male infant sleep might be similar to that seen in older males. Furthermore, they suggested the need to consider the role of environmental factors in the variability noted in infants' sleep.

Few differences have been noted among young adults by visual scoring. However, Dijk *et al.* (1989) studied gender differences in young adults aged 19–28 years (13 males/15 females), using computer-quantified spectral analysis of EEG. He noted no change between sexes in the distribution of sleep stages but saw an increase of delta power in the EEG (0.25–11 Hz) of women during NREM sleep and higher power densities in women during REM sleep that was consistent across the night. The changes were independent of those seen after sleep deprivation or increased homeostatic drive for sleep, which should have dissipated across the night. The authors suggest that these differences may reflect gender differences in skull size or thickness, which has previously been shown to be negatively correlated to EEG amplitude (Pfefferbaum and Rosenbloom, 1987) rather than physiological differences between the sexes. Unfortunately, nine of the females in this study used oral contraceptives, which may have acted to blunt the temperature changes that affected sleep.

Also using visually scored staging and period-analysis algorithms, Armitage (1995) studied young men and women (mean age 25 years, 11 men/11 women) to examine EEG frequencies during sleep. She evaluated EEG in five conventional frequency categories: delta (0.5–4 Hz), seen in SWS; theta (4–8 Hz), predominantly seen other stages of sleep; alpha (8–12 Hz), found in quiet wakefulness; sigma (12–16 Hz), seen in spindle activity of stage 2; and beta (16–32 Hz), seen in lighter stages of sleep and wake. No striking gender-related differences were noted. However, when the global delta power of NREM was examined, females again showed significantly more delta across the sleep period. Unfortunately, all the women were taking oral or injectable contraceptives, a factor that may have altered the findings. As we will see, this may have an important impact on the findings.

To examine the effects of ageing on the EEG, Ehlers and Kupfer (1997) studied young adults aged 20–40 years, using visually scored sleep staging and spectral analysis of EEG characteristics. No gender differences in SWS or delta wave activity were seen among subjects in their twenties. Both sexes had a decline in the spectral power of spindles with age. No significant change of a visually scored sleep variable was noted for women across the groups. However, significant reductions of delta activity and percent SWS were noted for men in their thirties. Additionally, males showed significant reductions of REM time, and REM density, activity and intensity, with an increase of stage 2 sleep. These authors suggested this might indicate that men and women age differently over the second and third decade.

To avoid confounds of the laboratory environment, numerous studies have evaluated sleep in the subject's home. Kobayashi *et al.* (1998) studied 18 older subjects (8 males/10 females, mean age 60.6 and 61.7 years, respectively) during two 36-h sleep/wake periods. Full polysomnography was obtained in the home. They noted that men napped more frequently, had lower sleep efficiency, more stage 1 sleep, less stage 3/4 sleep, less REM sleep, and more state changes than females. However, no mention was made of concomitant sleep disorders, such as apnoea, that could be expected to produce similar patterns.

In another small study, Fukuda *et al.* (1999) evaluated sleep in older subjects (8 males/8 females, aged 54–72), also using full polysomnography in their homes. Visually scored sleep staging and spectral analysis of two frequency bands (0.5–2 HZ and 2–4 Hz) were done. No significant gender differences were noted in the following visually scored sleep parameters (percent wake, stages 1 and 2 sleep, REM sleep and REM latency, TIB, TST, sleep efficiency (SE), and awakenings). However, females had a higher percentage of SWS (stages 3 and 4). With spectral analysis, the investigators report females had larger amounts of delta power across the night than men. Furthermore, the females maintained clearer periodicity of delta power across the night. The authors suggest that the SWS generator is better conserved in middle-aged and elderly females than in males of the same age.

In another home-monitored study, Reyner and Horne (1995) studied 400 adults (211 female/189 males) aged 20–70 years for 15 consecutive nights, using sleep logs and actigraphy. Actigraphy provides objective confirmation of sleep length and timing as well as sleep continuity. Subjects were divided into three age groups: young, 20–34 years; middle-aged, 35–49; and older, 50–70. Home

environmental factors were not controlled in this field study. Overall, there appeared to be minimal effects of ageing in both the subjective and objective parameters. However, women, particularly the older ones, tended to go to bed earlier than men and stay in bed longer, but they reported worse sleep with frequent awakenings, a report which was confirmed by actigraphy. They also reported longer sleep latency (time needed to fall asleep). There was also a significant difference in rising time between middle-aged men and women, with men rising earlier. Both men and women awoke earlier with age. Young men slept longer than both their middle-aged and older counterparts and middle-aged women slept longer than middle-aged men. Women in all age groups had significantly more awakenings than men. Many of these awakenings were noted to be environmental (children's needs).

CIRCADIAN RHYTHMICITY

As previously noted, sleep is regulated in part by our circadian cycle, with input from the pacemaker located in the suprachiasmatic nuclei, as well as from exogenous factors such as food, light and ambient temperature. Body temperature also has a circadian rhythm, oscillating between a nadir at night and a maximum in the afternoon, and is thought to influence sleep onset and offset. There has been interest as to whether the circadian rhythm of temperature and sleep is disrupted in ovulatory women who have a menstrual cycle, with increased body temperatures in the luteal phase, or in women taking oral contraceptives.

To examine this question, Baker *et al.* (2001) studied eight normally cycling women, eight women taking birth-control pills, and eight men (all aged 21–22). All subjects had a screen/adaptation night in the laboratory. Women were alternately studied in their mid-follicular and mid-luteal phases over a 3-month period. Polysomnography and continuous core body temperature were measured along with levels of estradiol and progesterone in the women. Both subjective and objective sleep measures were compared. Rectal temperatures of the normally cycling women were elevated, as expected in the luteal phase of the menstrual cycle compared to that in the follicular cycle, and they had blunted nocturnal drops of temperature compared to men, but not compared to the follicular phase. Both groups of women attained temperature minima earlier than men, suggesting a gender effect. The temperatures of the women taking exogenous hormones were similar to that of the luteal phase in normally cycling women. Therefore, hormonal supplementation may render these women poor subjects for sleep evaluations since the hormones alter the temperature phase. There was no significant difference in the sleep architecture (macrostructure) of men and women except that more SWS was seen in the luteal phase of normally cycling women. Those women on exogenous hormones demonstrated less SWS than normally cycling women.

Buysee *et al.* (1993) studied 17 healthy, 20–30-year-old men ($n = 9$) and women ($n = 8$) and 18 healthy 80-year-olds (11 men/7 women) during a 36-h constant routine paradigm to evaluate temporal patterns of unintended sleep episodes. The constant routine followed a 24-h adaptation period of normal routines. During the constant routine, balanced nutrition was supplied every hour. Core body temperatures were tracked on line, and cortisol and melatonin levels were sampled over the entire period. All subjects had continuous EEG with measurement of mood and sleepiness every hour. Women showed stronger rhythmic trends for sleep than men, and the older women in this study showed higher temperature amplitudes than the younger women. All temperature measurements of the younger women were taken during their follicular menstrual phase. However, three of the younger women were taking oral contraceptives, and this could have served to blunt the temperature curves for younger women.

To examine further the relationship between age-related changes in sleep and the circadian timing system, Campbell and Murphy (1998) studied 60 subjects (32 females/28 males) aged 40–84. For some analyses, the group was divided by age: a group of 40–60 middle-aged subjects, and two older groups of 65–81. One of the older groups was a subset that had experienced sleep difficulties for at least 1 year prior to the study. Polysomnography and measures of core body temperature were performed at an adaptation and baseline night. There was no difference between the sleep quality of non-complaining older subjects and that of those middle-aged; however, there was an advance of minimum body temperature in older subjects. Complaining older subjects had decreased TST, SE, and REM with increased wake after sleep onset (WASO) compared to non-complaining older subjects. There was no difference in the rhythm of body temperature between complaining and non-complaining older subjects. With age controlled for, women showed significantly higher temperature amplitude than men. In addition, there was a significant age effect on amplitude in women, but not men. According to the authors, these findings argue that older women maintain a stronger circadian rhythmicity than older men, as measured by temperature curve amplitude, and that age-related sleep disturbances may have multiple causes.

Parry *et al.* (1997) studied the nocturnal patterns of melatonin in women with premenstrual dysphoric disorder (PMDD) and controls, and noted a delay in the secretion of melatonin and a decrease in melatonin secretion during the luteal menstrual phase, as compared to the follicular phase in women with PMDD. The authors suggest that the circadian pacemaker of women with PMDD is more responsive to the hormonal changes of the menstrual cycle. Furthermore, they postulate that this sensitivity may render these women more subject to mood, cognitive and sleep disturbances as a result of endogenous and exogenous stimuli.

HORMONAL INFLUENCES: MENSTRUATION, PREGNANCY, LACTATION, AND MENOPAUSE

Limited data suggest that secretions of gonadal hormones influence/modulate sleep physiology in animals, and both the physiology and disorders of sleep in humans. Neuroendocrine function is influenced at both the pre- and postsynaptic levels. However, most animal studies have been carried out in males of species where the direct influence of exogenous hormones may differ from that endogenously produced. Animal studies show that neurosteroids (oestrogen, progesterone and testosterone) play a pivotal role in the mediation of stress and the effect on brain functions. In humans, studies examining the role of these hormones have been small-scale, and few studies have taken even menstrual cycling into consideration in any systematic way. Nevertheless, both animal and human studies indicate neurosteroidal effects at both the genomic and nongenomic levels. Furthermore, effects have been noted in brain regions related to the control of sleep and wake (medial preoptic nuclei, medial hypothalamus) and limbic areas such as the hippocampus and amgydala.

For instance, oestrogen has been shown to increase the turnover of norepinephrine in the brainstem, hypothalamus, nucleus accumbens and locus coeruleus, as well as to alter expression of c-fos proteins in A2 adrenergic neurons. These effects act to decrease REM sleep, but the action appears to depend on the phase of the circadian cycle. Temperature phase may be altered, suggesting that oestrogen may weaken the coupling between the core body temperature and the sleep/wake cycle, or otherwise modulate circadian rhythms (Dijk, 1989).

Oestrogen acts as an excitatory stimulus by increasing the production and receptor concentrations of neurotransmitters such as serotonin, norepinephrine and dopamine, as well as the B-endorphins, and increasing the availability of glutamate, which

stimulates the excitatory *N*-methyl-D-asparate system (NMDA) (Arpels, 1996). In theory, increased levels of oestrogen occurring as a consequence of oestrogen hormone replacement therapy (HRT) could increase activity and irritability and lessen sleep. However, studies evaluating HRT in post-menopausal women demonstrate increased sleep times; that is, less fragmentation and enhanced REM sleep compared to baseline and placebo controls (Leproult *et al.*, 1998).

Progesterone and its metabolites act directly on the $GABA_A$ receptor and, through a complex mechanism, this action results in either a hyperpolarization and decrease in neuronal excitability (allopregnanolone and pregnanolone) or an antagonistic reaction that limits GABA-induced chloride transport (pregnenolone sulphate) (Mellon, 1994). The sedating action of progesterone is comparable to that of the benzodiazepines and is dose-dependent (Lancel *et al.*, 1997). Progesterone has been shown to decrease sleep latency, decrease wakefulness and increase EEG spindle activity in both REM and NREM sleep, in both animal and human studies. Furthermore, the progesterone antagonist, mifepristone (RU-486), has been shown to produce an increase of wake time, longer sleep latencies and decreased REM and SWS when administered to healthy men (Wiedemann *et al.*, 1998). Progesterone also acts as an anti-oestrogen and downregulates the oestrogen receptors.

Antonijevic *et al.* (1999) note the likelihood that ovarian steroids, such as estradiol and progesterone, may modulate GABA receptors, as well as the adrenergic system, thus effecting changes in sleep between the sexes. Women secrete more cortisol early in the night and show a pulsatile pattern of release of growth hormone (gH) across the night as compared to men, who typically show a single peak at the start of sleep.

Researchers have also detected changes in cellular immune function across the menstrual cycle that appear to be associated with fluctuations of progesterone. Moldofsky *et al.* (1995) have shown that there is less natural killer (NK) cell activity during the luteal phase, when progesterone is high. During the follicular phase, the NK responses are the same as in men. This suggests that progesterone may modify immune function and may affect women's risk of lupus and rheumatoid diseases that also alter their ability to sleep.

Human sleep changes during the perimenopausal years may therefore reflect the impact of fluctuating and ultimately decreasing levels of oestrogen and progesterone. Additionally, sleep across the menstrual cycle, for those women sensitive to subtle hormonal changes, can be problematic. Numerous studies and surveys of subjective complaints of sleep disturbance demonstrate large numbers of women with occasional, menstrual-related sleep difficulty (National Sleep Foundation, 1998). However, few objective studies confirm these complaints. This may be partly because few studies have accurately measured hormonal levels, many dividing the menstrual cycle into a variety of phases that could not be matched for meta-analysis, and most studies having few subjects. Furthermore, the two well-controlled studies that follow illustrate the subtlety of sleep changes across the menstrual cycle in healthy, non-complaining women.

Ito *et al.* (1995) studied seven women aged 18–19 who spent three consecutive days in each of five successive weeks in the laboratory. The investigators studied the plasma melatonin circadian pattern under controlled conditions with measurements every hour for 24 h on the third day of each week. They divided the menstrual cycle into four phases. Plasma melatonin was raised in the late luteal phase, and the rise time was delayed on the first of the three days in the laboratory. Additionally, SWS was increased in the follicular phase as was TIB and TST, suggesting that the menstrual cycle did affect melatonin and sleep/wake rhythm.

In a study of nine menstruating women free of oral contraceptives, Driver *et al.* (1996) evaluated 138 sleep episodes spaced every other night throughout a 36-day period. Menstrual status was measured with biphasic temperature rhythms, to capture pre/postovulatory phases, urinary luteal hormone (LH), and mid-luteal plasma levels of oestrogen and progesterone. Lighting, activity, and alcohol and caffeine intake were controlled. Subjective and objective measures of sleep showed no significant change over the menstrual cycle, with the exception of an increase of spindle (14.25–15 Hz) activity that had large variations across the cycle, reaching a high in the luteal phase in parallel with body temperature.

Animal work also suggests that the androgens (testosterone) play a role in the sexual dimorphism of REM sleep during a critical period of brain development around the perinatal time (Manber and Armitage, 1998). The administration of exogenous testosterone to adult animals has been shown to have little effect on sleep. However, it is felt that in humans it is the lessening of progesterone, as well as the change in the ratio of oestrogen to testosterone, that may influence the sleep changes around the perimenopause.

Pregnancy

Significant changes in sleep occur during pregnancy, in part due to hormonal changes and changes in the woman's girth. Profound sleepiness occurs during the first trimester secondary to elevated levels of progesterone. Progesterone also affects smooth muscle and the resultant urinary frequency disturbs sleep. Restless legs and pain begin to disrupt sleep during the second trimester. In the third trimester, women are uncomfortable due to the gravid uterus.

Using quantitative EEG, Brunner *et al.* (1994) studied nine healthy pregnant women, on two consecutive nights during each trimester. WASO was elevated across the pregnancy. No other significant differences were noted by visual scoring, but the quantitative techniques revealed significant changes across trimesters. There was an increase in WASO from trimester 2 to trimester 3, a decrease in REM sleep from trimester 1 to trimester 2, and a progressive reduction in NREM power density of spindle bands of $>14.25–15$ Hz across the pregnancy.

A second study of this type confirmed a loss of SWS as the pregnancy progressed. Schoor *et al.* (1998) studied four pregnant women and four non-pregnant controls with polysomnography in each trimester. There were no differences between groups on comparing time until sleep onset, latency to stage 2 and latency to REM. There were no differences in cardiopulmonary indices. However, there was a decrease in SWS even during the first trimester, and alpha intrusion into SWS was noted in the pregnant women.

Not only does pregnancy affect sleep but also sleep problems continue into the post-partum phase for many women. Lee and Zaffke (1999) followed 24 primiparous and 18 multiparous women to evaluate perceived levels of fatigue and energy before, during and after pregnancy. Studies were carried out in the subjects' homes during the follicular and luteal phase of menstruation prior to conception that served as baseline. Studies were repeated during each trimester and again at months 1 and 3 of the post-partum period. The measures included polysomnography, and scales such as the Lee Fatigue Scale, the POMS and the Dupuy General Well-Being and Vitality subscale. Serum chemistries included iron (Hg, HCT, ferritin, B_{12} and folic acid); progesterone; and thyroid serum T3, serum T4, T3 resin uptake, free T4 index, and thyroid antimicrosomal antibody titre. Complaints of fatigue during the first trimester were related to younger age and lower levels of iron, haemoglobin and ferritin. Haemoglobin levels remained low normal throughout the pregnancy while ferritin levels dipped more slowly across trimesters. During the third trimester, fatigue was related to decreased TST. Post-partum, fatigue was related to decreased sleep, ferritin and haemoglobin. Even at 3 months post-partum, these women still felt less energy than their baseline levels. The

perception of energy appeared to be influenced by parity, with multiparas having less energy throughout pregnancy. This suggests that pregnancy takes a significant toll on the sleep patterns and energy levels of women. Poor sleep may incline women to profound mood changes and depression during the post-partum period. Some believe the 'baby blues' may be a consequence of prolonged sleep deprivation.

Additionally, during pregnancy, changes in respiration and airway mechanics occur. Snoring and apnoea are not uncommon. To evaluate the extent of this problem, Loube *et al.* (1996) carried out a large survey of pregnant women, determining snoring status and comparing infant outcomes to examine the impact of self-reported snoring on infant health. A total of 350 pregnant women and 110 age-matched women as controls answered a survey about snoring and daytime sleepiness. Those with reported daytime sleepiness and snoring were given full sleep evaluations. Fourteen percent of pregnant women snored versus 4% of the non-pregnant. Eleven women reported anecdotal apnoea. Of these, only four were recorded. Two of them met the criteria for mild apnoea; one had positional apnoea and the other only snored. There were no significant differences between infants from either group.

In contrast, Franklin *et al.* (2000) noted in a study of 500 Swedish women that habitual snoring was a sign of pregnancy-induced high blood pressure (preeclampsia), and was associated with lower birth weights and lower Apgar scores. The study also noted that women started to snore before any sign of hypertension appeared, and that snoring was related to sleep apnoea. While it may seem reasonable to suppose that the increased abdominal girth of pregnancy (with or without excessive weight gain) underlies the increase in apnoea, there is little evidence to support this. A more likely explanation is that the frequent nasal congestion experienced by pregnant women predisposes to upper airway collapse due to the large negative pressure in the airway needed to overcome the nasal obstruction.

Perimenopause and Menopause

Many researchers and clinicians have noted an increase in complaints of fragmented sleep secondary to the hot flashes of menopause. Women complain of difficulty in falling asleep and experience disrupted sleep, as hot flashes occur frequently at night. The hot flash typically is characterized by peripheral vasodilatation, profuse sweating, and subsequent chilling, and is thought to be a disorder of thermoregulation. Alterations in central catecholamines occur; specifically, levels of plasma 3-methoxy-4-hydroxyphenylgylcol (MHPG) are higher in women who experience hot flashes (Woodward and Freedman, 1994). In a sense, the hot flash is a general alerting mechanism which interrupts sleep and requires time to settle before sleep can comfortably recur, resulting in sleep loss and daytime consequences such as loss of concentration and memory. The addition of oestrogen and oestrogen HRT reduce the hot flash and have been seen to increase SWS in post-menopausal women (Leproult *et al.*, 1998). Furthermore, oestrogen HRT has been shown to reduce the respiratory disturbances more common in post-menopausal women (Pickett *et al.*, 1989).

Menopause is preceded by many years of hormonal changes. This period, called the perimenopause, can stretch for 5–10 years before the onset of the menopause. Perimenopausal women frequently note mood changes and sleep disruption during this time. Baker *et al.* (1997) confirmed these complaints in a study of 15 perimenopausal and 13 premenopausal women. Perimenopausal women had higher anxiety levels and lower vigour than premenopausal women, as measured by the State-Trait Anxiety Inventory (STAI) and the POMS. There was a significant correlation between the STAI scores and sleep arousals, and it was clear that sleep disruption had a mediating role in mood changes.

In another interesting paper, Shaver and Paulsen (1993) reported on subjective and objective studies of 135 healthy women aged 37–59. Women were grouped as premenopausal and menopausal, each with and without 'poor' sleep. The authors noted that women with objectively documented poor sleep had higher psychological distress rather than active menopausal symptoms. This suggests that the sleep disruption in the menopausal years may not be totally due to hormonal changes. This is also an age of life-altering changes that can produce stress. Children are leaving, parents are requiring more care, marriages are being renegotiated and women may be redefining their roles. These stresses add to a hormonally volatile time in women's lives.

DREAMING

Gender differences in dream content begin to emerge during adolescence. Males show more aggression than females, who dream about relationships and pleasant emotions. This difference continues into adulthood, depending upon the socialization developed (Hall *et al.*, 1982). Working women's dreams, especially those of women in traditionally male professions, become more male-like than do those of the homemaker. This suggests that dreams have a role in reflecting experiences of the waking state (Lortie-Lussier *et al.*, 1985).

PREVALENT SLEEP DISORDERS

Insomnia

Far more women than men complain of insomnia; that is, difficulty in falling or staying asleep (Table XXIV-10.2). This increase may be related to the increase of depression in women or at least the reporting of somatic symptoms related to sleep difficulties, which is increased among depressed women both acutely (Angst and Dobler-Mikola, 1984) and chronically (Kornstein *et al.*, 2000). This association has been noted in epidemiological studies (Bliwise *et al.*, 1992). The causation of symptoms is still unclear, that is, whether sleep difficulties precede mood symptoms or not (Browman *et al.*, 1996). Furthermore, women may be subject to the entrainment of poor sleep habits first developed during years of parenting.

The increase of reports of insomnia among women is especially marked during the perimenopausal years when hormone levels are rapidly shifting, and this disorder continues to be prominent as women age. Maggi *et al.* (1998) evaluated by questionnaire 2398 (867 males/1531 females) community-living older (>65 years) subjects. The prevalence of insomnia was 54% in women compared to 36% in men. There was an increased odds ratio for insomnia (1.69) and depression (1.93) in women, even when results were controlled for potential risk factors such as health status and smoking and alcohol habits. Night waking was the most common complaint.

The causes of insomnia are usually multi-factorial and include the major categories of physical changes such as poor health; hormonal status; physiological changes such as jet lag; and psychological, psychiatric, and pharmacological influences. Because sleep loss has a profound effect on quality of life and daytime functioning, recognition and treatment of insomnia are important.

Treatments should be designed to alleviate the cause when possible. In cases of depression, adequate treatment of the depression usually heralds improvement in sleep, as long as poor sleep habits have not begun. In this event, short-term use of hypnotics may be appropriate while better sleep habits and behavioural changes are being reinforced. Ultimately, long-term improvement is best achieved with behavioural techniques (Morin *et al.*, 1999).

Fibromyalgia

Frequently, pain syndromes, more common in women, can negatively influence sleep. In a study of 11 women with fibromyalgia

Table XXIV-10.2 Sleep disorders prevalent in women compared to men Diagnostic Classification Committee, 1990)

Females	ICSD code	ICSD code	Males
Psychobiological insomnia		780.53	Obstructive sleep apnoea
Sleep state misperception	307.49	780.54	Hypersomnia
Restless legs syndrome	780.56	780.51	Central alveolar hypoventilation
Adjustment sleep disorder	307.41	780.55	Delayed sleep phase
Hypnotic dependent syndrome	780.52	307.46	Sleep terrors
Nightmares	307.49	307.3	Rhythmic movement disorders
Familial sleep paralysis	780.56	307.47	Somniloquy
Headaches	346	780.59	REM behaviour disorder
Fibromyalgia	729	788.36	Sleep enuresis
Nocturnal sleep-related eating disorders	780.52	780.59	Sudden unexplained nocturnal death syndrome
		786.09	Primary snoring
		798	SIDS

and 11 matched controls, Shaver *et al.* (1997) studied subjective reports as well as physiological indicators of stress. The study showed that sleep in the early part of the night is more fragmented, with increased arousal but no prominent EEG change in subjects with fibromyalgia. The 11 women with fibromyalgia reported worse sleep and higher psychological stress, as measured with SCL-90, on the somatization, obsessive-compulsive and anxiety subscales, although the scores were still within the normal range, despite showing no changes on measures of sympathetic activity, such as morning levels of cortisol and catecholamines from urine spills. The authors speculate that a disturbance of nocturnal growth hormone secretion may be related to these complaints.

Restless Legs Syndrome

Restless legs syndrome (RLS) is a common sleep disorder affecting more women than men, and it may affect the ability to fall asleep (Lavigne and Montplassir, 1994). RLS is characterized by the irresistible need to move one's limbs (particularly the legs). Feelings can be described as 'creepy crawling' or 'bubbling in the veins'. The constant need to move disrupts sleep. Unfortunately, the circadian rhythm of RLS worsens the symptoms in the evening. Many sufferers also demonstrate periodic leg movements (PLM) during sleep, further disrupting the continuity of sleep. Symptoms generally increase with age but occur frequently during pregnancy as well.

Recent research suggests the association of RLS with low brain ferritin levels (Early and Connors, 1999). Increasing serum levels of iron may be therapeutic. Furthermore, dopaminergic drugs and antispasmotic medications have also proven useful in treating this disorder.

Sleep Apnoea

Another common sleep disorder, the prevalence of which differs between men and women, is obstructive sleep apnoea. Apnoea, or hypopnoea, refers to the cessation of airflow during sleep, usually as a result of complete (apnoea) or incomplete (hypopnoea) closure of the airway. The closure is brief, lasting $\geq$10 s, but may be repeated every minute across the night. Sleep in this condition is disrupted by brief arousals at the end of each respiratory event. Furthermore, depending on the extent of apnoea and the cardiopulmonary health of the subject, significant oxygen desaturations may occur.

The estimated prevalence of sleep-disordered breathing, which is defined as an apnoea-hypopnoea score over 5/h sleep, is 24% in men and 9% in adult women. Four percent of males and 2% of females have the apnoea-hypopnoea syndrome, which includes daytime sleepiness (Young *et al.*, 1993).

The symptoms of obstructive apnoea in both men and women include dramatic snoring (although many women do not know whether they snore), gasping during sleep, restlessness and daytime sleepiness. Unfortunately, despite giving the classic symptoms of apnoea to their health-care provider, many women go undiagnosed. It is not clear whether this results from the bias of the health-care worker or the concurrent reporting of psychologically oriented symptoms, such as insomnia, depression and anxiety, which attract more attention in women (Young *et al.*, 1996). In fact women with sleep apnoea report far more symptoms of depression and anxiety, as measured with the SCL-90, than men regardless of the severity of their apnoea. Furthermore, complaints of depression and anxiety were higher in women with severe apnoea than women with mild apnoea (Pillar and Lavie, 1998).

Much speculation has occurred as to why there is a sexual dimorphism in this disorder. Popovic and White (1998) studied 12 pre- and 12 post-menopausal women. They found an increase in pharyngeal dilator muscle activity, probably related to levels of progesterone in awake females, such that the genioglossis tone is increased in the luteal menstrual phase, as well as in those post-menopausal women taking oestrogen HRT. However, during sleep, Thurnheer *et al.* (2000) noted no such difference. This group studied two age groups (18–35 years and 40–70 years) of both men and women with nocturnal polysomnography, measuring airway resistance during sleep, and saw no differences in airway tone between genders and across age. They did note an increase in total respiratory resistance at sleep onset in men compared to women. The authors suggested that the waking increase in upper airway resistance previously seen in women reflected the narrower size of their airway.

Obesity tends to be a major factor in the development of obstructive apnoea. However, for equal degrees of obesity, a man would probably have more sleep apnoea than a woman, perhaps because of the placement of male fat in the mid-torso area. However, women who are severely overweight are at risk at any age. They may not have apnoea from airway obstruction, but they may suffer from hypoventilation, a decreased effort to breathe, particularly in REM, allowing significant hypoxia to develop (O'Connor *et al.*, 2000). As previously mentioned, women can also develop a temporary form of sleep apnoea during pregnancy.

Treatment of apnoea is essential to good health. Recent data suggest that apnoea is an independent risk factor for hypertension and cardiovascular morbidity in the general public (Peppard *et al.*, 2000). Treatments range from weight loss and positional therapy to mechanical devices such as CPAP, a pressure of air presented to the airway which acts as a pneumatic splint to keep the airway

open, and dental devices which increase muscle tone and space in the pharyngeal airway.

CONCLUSION

Subtle differences in sleep architecture exist between males and females, particularly as they age. Most of these differences appear to be influenced by the gonadal hormones oestrogen and progesterone in females, and testosterone around the perinatal period in males. Oestrogen seems to increase REM sleep in humans and improve sleep continuity in menopausal women. Progesterone is known to be sedating and similar to the benzodiazepines in that it decreases REM sleep and increases spindling and stage 2 sleep in humans. Testosterone in males appears to influence REM sleep, and the ratio of testosterone to oestrogen in females appears to influence irritability and sleep fragmentation. Females' cyclic hormonal nature significantly influences sleep architecture across their reproductive lifetime.

Furthermore, certain sleep disorders appear to 'favour' one sex over the other. Women have a risk of depression twice that of men. Anxiety and depression increase the risk of insomnia in this group. Other sleep disorders such as apnoea appear to be twice as common in men. Significant respiratory disturbance during sleep lessens REM sleep and fragments NREM sleep, resulting in excessive daytime sleepiness.

It is sensible to take this information into consideration when attempting to evaluate or improve sleep in both men and women.

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XXIV-11

Sleep Disorders — Therapeutic Armamentarium

Malcolm Lader

INTRODUCTION

We spend a third of our lives asleep and yet sleep remains an enigma. The numerous and complex disorders of sleep have been dealt with in several previous chapters. The most common complaint is that of insufficient and unsatisfying sleep. Problems with excessive sleep are much less common. Abnormal sleep is also noted on occasion, usually manifested as aberrant behaviour such as night terrors or sleepwalking.

A range of medications is available to treat insomnia, ranging from folk remedies such as valerian to the most recently introduced compound, zaleplon. Many medications possess sedative and sleep-inducing properties as a side effect, such as many tricyclic antidepressants and the first-generation antihistamines. Fewer remedies exist to treat excessive or qualitatively abnormal sleep, but progress is being made.

The purpose of this chapter is to review the available remedies, concentrating on the newest introductions that are of both theoretical interest and practical utility. The emphasis will be on the remedies for insufficient sleep — the hypnotic drugs — as these are among the most-widely prescribed medications, particularly by primary care practitioners (Simon and VonKorff, 1997).

SLEEP HYGIENE

Before dealing with hypnotic medication, it is useful to outline the stratagems that people use to induce and maintain sleep (Table XXIV-11.1). A regular bedtime routine is conducive to sleep, and many people follow a time-hallowed sequence of events, almost a ritual, before they retire at night. Regular hours for going to bed and for rising in the morning help consolidate sleep, and this routine should be maintained at the weekends and during vacations as well. Many people read before going to sleep, but arousing or disturbing material should be avoided. Rehearsing the next day's agenda or worrying about finances, family, etc. is unhelpful.

Exercise late at night is used by many people to hasten the onset of sleep although it is probably the routine that is most important. However, the optimum is moderate exercise in the afternoon or early evening, as anyone taking a vigorous sport-oriented vacation can testify. By contrast, heavy, exhausting exercise may be unhelpful (Horne, 1981).

The bedroom should be quiet, so that sound-attenuation may be needed if it abuts on a noisy street or lies under the flight path to an all-night airport. The room should not be too hot nor too cold. Many people take a milky drink at bedtime and there are many on the market. Tea and coffee may induce insomnia (Stradling, 1993). Coffee was once dubbed an 'antihypnotic' (Miller, 1722). Tolerance to the effects of the active principles, caffeine and, to a lesser extent, theobromine, tends to get lost as the individual ages.

Table XXIV-11.1 Elements of sleep hygiene

A. Regular bedtime routine
B. Standard hours of retiring and getting up
C. Avoid worry
D. Exercise in late afternoon/early, but not late, evening
E. Quiet, warm, comfortable bedroom
F. Avoid caffeine-containing drinks and too much alcohol

Table XXIV-11.2 Behavioural techniques

A. Stimulus control
B. Sleep restriction
C. Sleep hygiene education
D. Paradoxical intention
E. Attention-focusing procedures: thought-stopping, imagery training, meditation, yoga
F. Relaxation: progressive muscular relaxation, autogenic training, biofeedback

Alcohol is also used by many people, the traditional 'nightcap'. However, too much may disturb sleep due to its diuretic effect and to rebound insomnia later on.

RELAXATION AND NON-PHARMACOLOGICAL TECHNIQUES

Some people find formal relaxation techniques very helpful, especially if meditation techniques have been learnt for daytime use (Table XXIV-11.2). Nevertheless, meditation and the induction of sleep are distinct physiological and psychological processes, and it is the muscular relaxation that is most relevant. Simple relaxation exercises in which the person relaxes muscle groups progressively from toes to legs, trunk, arms, neck and head are appropriate.

More elaborate behavioural techniques have been advocated (Bootzin and Perlis, 1992; Sloan and Shapiro, 1993). Although not widely available, a variety of techniques have been developed. Morin and his colleagues evaluated 59 treatment studies involving over 2000 patients. These were predominantly women, and the mean age was 44. Most suffered from chronic primary insomnia, on average, of 11 years' duration. Most subjects received sleep-focused interventions with an average treatment time of 5 h. Follow-up lasted a mean of 6 months.

Of four outcome variables assessed, two in the meta-analysis — sleep-onset latency and time awake after sleep onset — were significantly improved following behavioural interventions. The absolute number of awakenings and total sleep time

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were not significantly improved. The average effect size for sleep-onset latency was 0.88, signifying that patients with insomnia were better off after treatment than 81% of untreated control subjects. The average effect size for time awake after sleep onset was lower at 0.65, indicating that patients with insomnia were sleeping better than 74% of the untreated sample. Stimulus control and sleep restriction were the most effective therapy procedures given alone, whereas education about sleep hygiene was not effective when used alone. These improvements were well maintained at follow-up assessments.

What are these apparently useful therapies? *Stimulus control* comprises a set of instructions designed to help the insomniac avoid sleep-incompatible behaviours and to regulate sleep-wake schedules (Bootzin *et al.*, 1991). Such manipulations include instructions to go to bed only when sleepy; that the bedroom is to be used only for sleep and sex, and not to work or watch television; that whenever unable to sleep for 15–20 min, patients are to get up and go into another room, and return only when feeling sleepy again; that patients are to rise every morning at the same preset time regardless of quality and quantity of sleep the previous night; that there is to be no daytime napping.

Sleep restriction consists of curtailing the amount of time the insomniac is allowed in bed to the actual duration of sleep (Spielman *et al.*, 1987). Thus, the insomniac who reports sleeping only 5/8 h in bed is allowed to stay in bed for only 5 h. Once 90% sleep efficiency is achieved, the allotted time is increased by 15–20 min for the next week. If sleep efficiency is below 80%, the time window is decreased by 15–20 min. Between 80% and 90% efficiency, the time in bed is kept constant. Constant monitoring is carried out by means of a sleep diary, and appropriate adjustments are made.

Sleep hygiene education is essentially informing insomniacs about the factors that influence sleep, as set out above. It also involves exploring individual sleep expectations and setting realistic targets. A regular routine, avoidance of napping, and advice concerning substance use and medication are the components of sleep hygiene that are easiest to modify.

Paradoxical intention is somewhat akin to the implosion therapies used in phobias. It consists of persuading the insomniac to confront the feared behaviour, i.e., failing to fall or stay asleep. Often patients become 'insomnophobic', worrying about staying awake and thereby instituting a vicious circle of anxiety, insomnia and further insomnia. The theory is that if the patient stops trying to fall asleep but tries to stay awake instead, sleep will supervene more easily. However, rather like implosion treatment, paradoxical intention treatment has been disappointing.

Attention-focusing procedures use techniques such as thought-stopping, imagery training and meditation to focus the mind away from concerns about staying awake. *Relaxation* therapies focus on the body rather than cognition and comprise techniques such as progressive muscular relaxation, autogenic training and biofeedback.

Most studies evaluating non-pharmacological therapies rely on sleep diary records. This is not necessarily a limitation, as insomnia is a subjective complaint and coordinations with polysomnographic data are disappointing. Where objective techniques have been used as outcome criteria, some efficacy is still seen but less than that relating to subjective reports.

In conclusion, although psychological treatments are time-consuming and should be provided by trained personnel, benefits do accrue with some of the therapies and tend to persist. The long-term drawbacks of drug therapies do not apply. Behavioural and cognitive methods, rather than analytically based psychotherapies, are preferred. Sleep hygiene education is not enough without more specific techniques.

Table XXIV-11.3 Some benzodiazepines and similar hypnotics

Benzodiazepine	Elimination half-life (h)
Long-acting	
Nitrazepam	25–35
Flunitrazepam	10–20
Flurazepam	40–100
Quazepam	40–100
Intermediate-acting	
Temazepam	10–15
Lormetazepam	8–15
Short-acting	
Triazolam	3–5
Non-benzodiazepine	
Zopiclone	4–6
Zolpidem	2–4
Zaleplon	0.8–1.5

HYPNOTIC DRUGS

A wide range of drugs is used to treat insomnia (Table XXIV-11.3). They can be divided into two groups: a) those whose primary purpose is to induce, consolidate or prolong sleep; b) those where side effects of sedation are exploited. In the former group, the benzodiazepines still dominate the market despite misgivings concerning adverse effects, tolerance and dependence (Lader, 1994). Long experience of usage and cheapness still commend them to many practitioners. The properties of the ideal hypnotic include rapid onset of action, dependable onset or prolongation of sleep, minimal residual daytime sedation, safety in overdosage, lack of rebound dependence and abuse potential, and flexible dosage formulation. Safety in the elderly is particularly desirable, as they are heavy users of hypnotics.

Several effects of benzodiazepine hypnotics are clearly dose-related. Efficacy is increased at higher doses, as are side effects on psychomotor and cognitive function. Severe adverse reactions such as aggressive outbursts and episodes of amnesia also increase in frequency as the dosage increases: this is apparent with triazolam and flunitrazepam. Discontinuation effects, such as rebound and dependence, are also dose-dependent. Finally, abuse generally involves very high doses of hypnotic benzodiazepines.

BENZODIAZEPINE HYPNOTICS

Many of these compounds are available throughout the world, the range varying from country to country. The commonest ones encountered are temazepam, flurazepam, triazolam, nitrazepam and flunitrazepam. The last two are not available in the USA. These drugs can be divided for convenience into long-, intermediate- and short-acting, depending on their duration of action in normal dose. This in turn, mostly reflects the mean elimination half-life.

LONG-ACTING COMPOUNDS

As sleep is a night-time phenomenon, averaging 8 out of the 24 h, a long-acting hypnotic raises questions of desirability and logicality. Inevitably drug effects will persist the next day and accumulation is very likely. The development of tolerance will lessen such residual effects, but it is impossible to predict whether any particular individual will remain affected. Furthermore, objective effects may occur with subjective effects so the patient may be impaired unwittingly. The advantage of a long-acting hypnotic

benzodiazepine is that an anxiolytic effect will be present during the day. Thus, if the insomnia is a component symptom of an anxiety disorder, the latter will also receive symptomatic treatment. However, there are less contentious drug treatments for anxiety disorders (see Chapter XIX-13).

Nitrazepam was the first benzodiazepine marketed as a hypnotic. Its half-life ranges between 25 and 35 h (Breimer *et al.*, 1977). Residual effects are quite marked the next day, even after doses of 5 mg. Accumulation is likely, but, generally speaking, it is well tolerated and has been widely used in Europe.

Flunitrazepam is a similar compound with a duration of action of at least 24 h, reflecting an elimination half-life of 10–20 h (Boxenbaum *et al.*, 1978). The recommended dosage is 0.5–1 mg, but adverse events such as amnesia have attended the use of higher doses. Flunitrazepam is associated with two types of notoriety. It has a very rapid onset of action and powerful effects. It has been used as a 'date-rape drug', administered clandestinely in alcoholic drinks to unsuspecting women (and men). Usually, the victim is amnesic after the period during which the sexual assaults took place. The formulation has been changed in many countries to obviate surreptitious administration. The second problem is illicit abuse 'on the street'. In this respect, it has given rise to the greatest problem worldwide of all the benzodiazepines, even in the USA, where it is not licensed. It is taken orally, intranasally and by injection. Its scheduling has been strengthened because of its particular menace.

Flurazepam (and the closely related *quazepam*) (Ankier and Goa, 1988) are long-acting drugs with a half-life of 40–100 h. This reflects its metabolism to the long-acting *N*-dealkylflurazepam (Greenblatt, 1991). Residual daytime sedation is marked, particularly at the upper, marketed dose of 30 mg. Accumulation is also marked (Mamelak *et al.*, 1989).

Loprazolam is best regarded as a long-acting compound, as its absorption can be erratic and slow (Clark *et al.*, 1986).

INTERMEDIATE-ACTING COMPOUNDS

Temazepam is the most-widely used of these compounds. It has a half-life of about 10–15 h and is devoid of active metabolites (Greenblatt, 1991). It is generally well tolerated, and residual effects are not usually a problem even in the elderly. A wide dose range is available (7.5–30 mg). Temazepam was originally marketed in the UK in liquid-filled capsules which were extensively abused by addicts. Reformulation as a gel was unsuccessful; therefore, the drug is available in the UK only as a tablet. Its scheduling has also been strengthened.

Lormetazepam is somewhat shorter-acting but is otherwise similar to temazepam (Pierce *et al.*, 1984).

Lorazepam is also licensed as a hypnotic in many countries at higher doses than those recommended for anxiolytic effects.

SHORT-ACTING COMPOUNDS

The major member of this group is *triazolam*. This hypnotic has been the focus of much controversy since the late 1970s. It is rapidly absorbed and has a half-life of 3–5 h (Greenblatt, 1991). Residual day-time sedation is not usually a problem unless the recommended dose of 0.25 mg is exceeded. The other problem at higher dosages is an increased incidence of anterograde amnesia, and aggressive and suicidal behaviour. This resulted in much concern in the early 1990s. The UK Licensing Authority took the hardest line, withdrawing the drug from the market. It was never reinstated. In other countries, notably the USA, triazolam has remained available but with cautions regarding monitoring and recommendations that the appropriate dose be 0.25 mg in adults and 0.125 mg in the elderly.

Midazolam is generally available as an intravenous anaesthetic induction agent. In a few countries it is formulated for use orally as an hypnotic.

UNWANTED EFFECTS

These have been catalogued many times (e.g., Lader, 1994). As emphasized earlier, many adverse effects with hypnotic medication are strongly dose-related (Roth and Roehrs, 1992). The main problems are as follows:

1. There is residual sedation, which may be associated with psychomotor and cognitive impairment. Practical activities, such as driving, may be quite markedly affected so that the accident rate of benzodiazepine hypnotic users is substantially elevated (Ray *et al.*, 1992).
2. Sedation is easily detectable following administration of most hypnotics and is often associated with muscle relaxation. In its most severe form, the sedation is manifested as confusion and the muscle relaxation as ataxia. Both can present major difficulties in the elderly, who often wake at night to urinate. For this reason, some geriatricians eschew the use of benzodiazepines in the very elderly.
3. Memory, particularly for current events, can be impaired by benzodiazepines such as lorazepam. Another problem is amnesic episodes, often accompanied by nocturnal wanderings.
4. Discontinuation syndromes have been well-documented although their clinical significance is difficult to establish. Rebound insomnia and dependence syndromes can occur, the former more commonly with short-acting compounds such as triazolam, and the latter with long-acting drugs.
5. Abuse is often overlooked but can be iatrogenic with escalation of dose or as a feature of illicit use. Benzodiazepines are generally among the most important drugs of abuse, but usually in the context of polydrug abuse or alcohol problems. The combination with alcohol is particularly dangerous, with major psychological impairments, amnesia and aggressive behaviour.

OVERVIEW

The benzodiazepines continue to be the most-widely prescribed of hypnotics, having supplanted the barbiturates because of their relative safety in overdosage. They are effective drugs, at least in the short term before tolerance sets in. Residual sedation is a problem with many of these compounds but is often overlooked although the insomniac patient feels exhausted the next day. Adverse behavioural events, such as amnesia and aggression, have led to much concern. The major problems of rebound, dependence and abuse have curtailed the recommended duration of usage of these drugs. Nevertheless, long-term administration is the rule rather than the exception. Dosage is an important consideration but minimal dosage is not usually adhered to. The advent of more selective compounds has opened up the debate about the risk/benefit ratio of the various benzodiazepine hypnotics.

BENZODIAZEPINE-LIKE HYPNOTICS

Three compounds currently available as hypnotics are chemically non-benzodiazepine although pharmacologically similar; however, they are more selective. They will be reviewed in some detail.

Zopiclone

Zopiclone has been available in most countries for over a decade. The major exception is the USA, where, apparently, the company that developed the drug had no facilities for registration and marketing at the crucial time. Zopiclone can be regarded as the first of the more selective benzodiazepine-type hypnotics

Chemically, zopiclone is a cyclopyrrolone compound that potentiates the inhibitory effects of GABA at $GABA_A$ receptors. However, it differs in some ways from a typical benzodiazepine in the nature of its binding. For example, tolerance is not seen in the $GABA_A$ receptors of mice, in contrast to benzodiazepine effects (Serra *et al.*, 1996).

The other point of interest is its relatively short elimination half-life. Zopiclone is rapidly and extensively absorbed after oral administration, with a bioavailability of about 75% after the standard dose of 7.5 mg. Protein binding is low, but concentrations in the saliva can be quite high, resulting in an unpleasant, bitter taste. It is metabolized in the liver by cytochrome P450 enzymes to essentially inactive metabolites. The elimination half-life is generally accepted to be about 5 h, but it is prolonged in the elderly, particularly those aged over 75 (Gaillot *et al.*, 1987). Half-strength formulations are usually available for the elderly and for patients with impaired liver function. The main drug interaction of clinical relevance is that carbamazepine increases the plasma concentrations of zopiclone.

The hypnotic efficacy of zopiclone has been evaluated in numerous studies comparing it with placebo and with standard hypnotics, mostly benzodiazepines (for detailed reviews, see Goa and Heel, 1986; Noble *et al.*, 1998). Zopiclone shows effects on sleep induction superior to placebo and at least equivalent to short- and intermediate-acting benzodiazepines. It does not generally prolong total sleep time; therefore, some trials have shown long-acting benzodiazepines to be superior to zopiclone in this respect (e.g., Ponciano *et al.*, 1990). In general, zopiclone 7.5 mg is equivalent to 30 mg flurazepam or 5 mg nitrazepam with respect to sleep onset (e.g., Anderson, 1987). A large-scale study in primary care involving 1507 patients showed zopiclone, but not flunitrazepam 1 mg, to be significantly superior to placebo with respect to various measures of sleep quality (Hajak *et al.*, 1994). This study also included a triazolam arm: zopiclone, but not triazolam, separated from placebo. However, in general, zopiclone 7.5 mg is equivalent to or better than triazolam in the high dose of 0.5 mg (e.g., Autret *et al.*, 1987).

Zopiclone has variable effects on sleep architecture, but, in general, changes are minor (Parrino and Terzano, 1996). Tolerance to polysomnographic effects of zopiclone is usually minor, although few long-term studies have been carried out (Fleming *et al.*, 1988). An early review of short-term clinical studies lasting up to 8 weeks concluded that the hypnotic effects of zopiclone were maintained over this period for both younger and older insomniacs (Brun, 1988).

Hypnotics are generally licensed for short-term use, and a substantial proportion of users are middle-aged and elderly. Studies in elderly subjects have shown zopiclone 5 mg to be superior to placebo (Elie *et al.*, 1990), as was 7.5 mg (Klimm *et al.*, 1987). However, nitrazepam 5 mg was superior to zopiclone 7.5 mg with respect to onset latency on day 12 (Klimm *et al.*, 1987), and flunitrazepam 1 mg was superior to zopiclone 5 mg for difficulty in falling asleep (Dehlin *et al.*, 1995). Zopiclone has also shown useful efficacy in facilitating withdrawal from benzodiazepine hypnotics (Shapiro *et al.*, 1995).

Zopiclone, like the other newer hypnotics, is less likely to produce psychomotor and cognitive impairment than benzodiazepines such as nitrazepam or temazepam (Agnoli *et al.*, 1989; Ngen and Hassan, 1990). In particular, although immediate impairments can be detected following zopiclone, effects the next day are generally minor or undetectable. Subjective effects such as difficulty in waking, impaired well-being and reduced morning concentration are minimal providing a period of 5–8 h has elapsed since ingestion (Broadhurst and Cushnaghan, 1987).

Zopiclone is generally well tolerated, the commonest complaint being a bitter aftertaste (Allain *et al.*, 1991). Elderly patients tolerate the drug well, particularly in the lower doses of 3.75 and 5 mg.

Rebound following abrupt withdrawal of zopiclone is much less of a problem than with benzodiazepines such as triazolam (Bianchi and Musch, 1990). Dependence and withdrawal also seem to be far less common (Lader, 1998). However, some cases of dependence have been reported, often a transfer from a pre-existing benzodiazepine dependence. Occasional instances of street abuse have also been documented.

The usual dosage for short-term usage in patients with primary insomnia is 7.5 mg, with lower doses in the elderly. Zopiclone is now established (outside the USA) as a commonly used, well-tolerated drug with particular effects on shortening delay to sleep onset. Residual effects are generally not troublesome.

Zolpidem

The second of these new selective benzodiazepine-like hypnotic drugs is zolpidem (Holm and Goa, 2000). It has been marketed successfully for several years in many countries of the world including the USA (Langtry and Benfield, 1990). It is chemically an imidazopyridine agent and acts as a selective agonist on subtypes of benzodiazepine receptors (Sanger and Depoortere, 1998). This is believed to confer some advantage with respect to adverse effects, as compared with non-selective agonists such as the benzodiazepines.

Zolpidem has a very short half-life (Fraisse *et al.*, 1996), averaging about 2.5 h. The drug is rapidly absorbed; it has a bioavailability of around 70% and is highly protein-bound. It is largely metabolized by P450 isoenzymes, predominantly, CYP3A4, to inactive metabolites. Elimination is somewhat reduced in the elderly so that lower doses, such as 5 mg, are recommended. The pharmacokinetics are linear over the dose range 5–20 mg. The area under the curve is increased in individuals with liver or renal impairment.

The numerous clinical trials comparing zolpidem with placebo and with active comparators, mainly benzodiazepines, have been reviewed in detail by Holm and Goa (2000). The usual dosage was 10 mg in younger adults and 5 mg in the elderly. Most were primary insomniacs with sleep-onset latency beyond 30 min, and/or a total sleep time less than 6 h and/or more than three nocturnal awakenings. As is now standard, the primary outcome variables were derived from questionnaires, visual analogue scales and sleep diaries. Some polysomnographic data are also available.

Several placebo-controlled inpatient trials with acute and chronic insomnia have established the efficacy of zolpidem, mainly with respect to shortening of sleep-onset latency (Scharf *et al.*, 1991; Dockhorn *et al.*, 1996; Lahmeyer *et al.*, 1997). It was also efficacious in patients receiving concomitant selective serotonin reuptake inhibitors (SSRI) therapy (Asnis *et al.*, 1999). Zolpidem generally has equivalent efficacy to benzodiazepines. For example, the effects of 10-mg doses were similar to nitrazepam 5 mg with respect to sleep latency, number of awakenings and total sleep time (Kazamatsuri *et al.*, 1993), but it was preferred in another trial (Kudo *et al.*, 1993). Conversely, patients receiving flurazepam 30 mg reported better sleep than patients given zolpidem 10 or 20 mg (Fleming *et al.*, 1995). Comparisons with intermediate- and short-acting benzodiazepines have generally shown equivalence (e.g., Rosenberg and Ahlstrom, 1994). Studies with triazolam have used both 0.25 and 0.5 mg. In one study, zolpidem 10 mg was more effective than triazolam 0.5 mg in prolonging sleep duration (Monti *et al.*, 1994).

Zolpidem 10 mg had equivalent efficacy to zopiclone 7.5 mg in one large-scale study (see Holm and Goa, 2000: Table IV, p. 877).

In view of increasing concern about the efficacy and safety of hypnotics in the elderly, comparative efficacy data are available from some well-controlled studies in elderly insomniacs. In one placebo-controlled study, zolpidem 5 mg was equivalent to triazolam 0.125 mg and temazepam 15 mg (Leppik *et al.*, 1997). Zolpidem 5 mg is the preferred dose in the elderly (Roger *et al.*, 1993).

Zolpidem does not significantly affect sleep architecture in younger or older subjects (e.g., Blois *et al.*, 1993; Scharf *et al.*, 1991). Indeed, zolpidem tends to increase slow-wave sleep rather than diminish it. Tolerance to the effects of zolpidem was not detected in early studies (Langtry and Benfield, 1990). Later studies have tended to confirm this.

Because of concerns about long-term nightly administration of hypnotics, attempts have been made to prescribe zolpidem on an intermittent or 'as-needed basis'. Studies have shown efficacy for zolpidem in both regimens (Cluydts *et al.*, 1995).

Numerous studies have been conducted on the psychomotor and cognitive effects of zolpidem, both immediate and present the next day. Some impairments are usually detectable immediately following 10-mg doses, and they are definitely present, particularly with respect to memory at higher-than-recommended doses. As predictable from the short elimination half-life, deficits the next day tend to be minimal (Unden and Schechter, 1996). Clinical tolerability is good, as found in clinical trials and postmarketing studies (Allain and Monti, 1997). As with most of these agents interacting with the $GABA_A$ systems, the commonest adverse effects are nausea and dizziness, malaise, headache and nightmares. Reports of amnesic episodes, which have so bedevilled the use of triazolam, are rare with zolpidem.

Rebound discontinuation is uncommonly reported with zolpidem treatment (Soldatos *et al.*, 1999). Dependence and abuse are also quite rare (Lader, 1994), and probably less common than with benzodiazepines. Nonetheless, careful monitoring continues to be a wise procedure.

The recommended dosage in adults is 10 mg–5 mg in the elderly. At these doses, zolpidem is an effective inducer of sleep with little effect on sleep patterns. It is well tolerated with few residual effects. Rebound and withdrawal are not a problem. Intermittent and/or 'as-needed' administration seems feasible and widens the prescriber's choice of stratagems.

Zaleplon

Zaleplon has been recently licensed in several countries as a short-acting hypnotic. It has easily the shortest half-life of licensed hypnotics — only 1 h. This opens up new avenues for therapy, which are explored later in this chapter.

Chemically, zaleplon is quite complex; it is a pyrazolopyrimidine derivative. It has agonist activity at a subtype of the benzodiazepine receptor in the $GABA_A$–chloride-ionophore complex. This selectivity, as with zopiclone and zolpidem, may confer some clinical advantages (Hurst and Noble, 1999). It is rapidly absorbed after oral administration of the usual doses of 10 mg, with 5 mg in the elderly. Its bioavailability is about 30%. Peak plasma levels are achieved within 1 h (Greenblatt *et al.*, 1998), and the elimination half-life is about the same. Kinetics are linear within the usual dosage range (Beer *et al.*, 1994). The major metabolites, 5-oxo-zaleplon and 5-oxo-desethyl zaleplon, appear to be inactive. Pharmacokinetic parameters are only minimally altered in the elderly, but lower dosages are still recommended.

The efficacy of zaleplon was established by standardized questionnaire responses, backed up by polysomnographic data. Subjective sleep latency with 5, 10 and 20 mg of zaleplon was significantly lower than with placebo 1 week into a 4-week trial in 574 patients with insomnia (Elie *et al.*, 1998). This effect continued throughout the trial for the two high doses, but not for zolpidem 10 mg used as the active control. Similar data were obtained in another comparison of zaleplon, zolpidem, and placebo, with 20 mg of zaleplon being superior to 10 mg of zolpidem (Fry *et al.*, 1998). However, in general, zaleplon and zolpidem are equipotent with respect to effects on subjective sleep latency.

A polysomnographic comparison of zaleplon 5 or 10 mg, triazolam 0.25 mg, or placebo for 14 nights found the 10-mg, but not the 5-mg, dose to shorten sleep-onset latency (Walsh *et al.*, 1998). The effects of triazolam were significant for the first two nights only. Zaleplon leaves the sleep architecture unaffected.

A study in 422 elderly insomniac patients compared zaleplon 5 and 10 mg with placebo (Hedner *et al.*, 1998). Both doses significantly reduced subjective sleep latency during both the first and second weeks of treatment. Sleep quality was also improved.

Zaleplon 10 mg was associated with a small but significant reduction in time to return to sleep after a middle-of-the-night wakening (cited in Hurst and Noble, 1999). Flurazepam 30 mg did not show this effect but did prolong residual sleep duration.

In a phase 1 study in normal volunteers, zaleplon 10 mg and 20 mg was compared with 2 mg of lorazepam with respect to psychomotor and memory function (Allen *et al.*, 1993). The effects of zaleplon were ephemeral and also not very marked, compared with those of lorazepam.

A series of studies has examined next-day psychomotor, memory and driving ability in both normal volunteers and insomniac patients. In contrast to various benzodiazepines used as active controls, zaleplon caused no impairments in comparison to placebo (e.g., Walsh *et al.*, 1998; Ware *et al.*, 1998). Lack of residual effects occurs even when zaleplon is given 4 h prior to testing (Vermeeren *et al.*, 1998). This was in contrast to zopiclone 7.5 mg.

Zaleplon is well tolerated in general, adverse effects usually being no greater than following placebo. Headache is the commonest adverse effect, but this is a notoriously difficult complaint to assess. Subjective residual effects the next day are minimal or undetectable, even at 20-mg doses. Daytime anxiety is not increased (Elie *et al.*, 1998).

Discontinuation of zaleplon is not associated with any notable rebound or withdrawal effects. A study in subjects with histories of social drug use suggested that higher doses (25, 50 and 75 mg) significantly increased drug-liking ratings above those for placebo (Rush *et al.*, 1999). Triazolam 0.5 and 0.75 mg had similar effects.

In summary, the available published data shows that zaleplon shortens subjective sleep latency at doses of 10 mg (5 mg in the elderly). It does not alter sleep architecture. It is not associated with rebound or dependence, but abuse liability at high doses cannot be ruled out.

Because of its very brief duration of action, alternative therapeutic strategies can be adopted and are reviewed later.

IMPLICATIONS OF SUBTYPE-SELECTIVE BENZODIAZEPINE RECEPTOR LIGAND HYPNOTICS

Recent work on subtypes of benzodiazepine receptors has resulted in the discovery of numerous subtypes of receptor depending on a rather complex subunit composition (Griebel *et al.*, 2000). The commonest are designated $GABA_{A1a}$, $GABA_{A2a}$, $GABA_{A3a}$ and $GABA_{A5a}$. Benzodiazepines in general are non-selective agonists at all these receptors. Zopiclone has not yet been evaluated in detail for its selectivity but may bind somewhat atypically to a variety of subtypes.

Zolpidem and zaleplon, among the hypnotics, are selective for the $GABA_{A1a}$ subtype. A second consideration is the degree of agonism ('intrinsic efficacy') at the receptor. Zolpidem has high activity at the 1a subtype. When it does bind to the other subtypes, it does so

with low efficacy. Zaleplon is also a full agonist at the 1a subtype but also has high intrinsic activity at the high concentrations needed for binding to the other subtypes.

What benefits might the selective binding confer? The cognitive effects of zolpidem and zaleplon appear to be less than for equivalent hypnotic doses of benzodiazepines. Some animal evidence supports this notion, with the acquisition of conditional fear being disrupted at high doses only (Sanger *et al.*, 1986).

Discriminative stimulus studies also suggest that selectivity on subtypes of receptors plays a part. Zolpidem and zaleplon only partly substitute for chlordiazepoxide as a discriminative cue, and only at high sedative doses (Sanger *et al.*, 1999). Tolerance to depressant effects in animals, such as impaired locomotion, is also minimal with the selective compounds as compared with non-selective benzodiazepines (Sanger and Zivkovic, 1992). Similarly, discontinuation effects are not detectable following withdrawal of zolpidem or zaleplon (e.g., Von Voigtlander and Lewis, 1991).

The animal data outlined above are consistent with clinical findings that zolpidem and zaleplon are associated with less psychomotor and cognitive impairment, less tolerance and fewer discontinuation effects than doses of benzodiazepines equipotent for hypnotic effects. The position of zopiclone is unclear. The implication is that compounds selected for development as an hypnotic (and as an anxiolytic) should display some selectivity at the 1a subtype. The consequences of selectivity at other benzodiazepine receptor subtypes remain unexplored.

COMPOUNDS USED AS HYPNOTICS EXPLOITING SECONDARY PROPERTIES

Lorazepam is licensed both as an anxiolytic and as an hypnotic. It is quite useful as a general tranquillizer and sedative, and is even advocated for use in psychotic agitation.

Diazepam is long-acting in view of its own duration of action and that of its very long-acting metabolite, *N*-desmethyldiazepam. However, it also has a marked redistribution phase, with sedative effects lasting a few hours. These can be exploited for use as an hypnotic where daytime anxiolysis is also required.

Barbiturates are still available in some countries but are restricted to use in insomniac patients long habituated to them. They should never be used ab initio.

Clomethiazole is a powerful sedative, which is sometimes used as an hypnotic, particularly in the elderly. However, it has most of the drawbacks of the barbiturates — paradoxical excitement, confusion, dependence, and marked interaction with alcohol.

Chloral and its derivatives are cheap, obsolescent hypnotics, with a dependence liability and a high incidence of gastrointestinal upset.

Antihistamines, such as diphenhydramine and promethazine, are on sale to the public for occasional insomnia. Their prolonged duration of action may cause residual effects the next day. Tolerance is often quite marked. Although dependence does not supervene, polydrug addicts use these drugs as adjuncts to opioids.

Other drugs can be dosed in such a way that their side effects of sedation and torpor can be used to induce sleep. The classic examples are the *tricyclic antidepressants*, trimipramine, amitriptyline and dothiepin (dosulepin) in particular being given at night to help patients sleep. This is quite appropriate when insomnia is secondary to depression.

Antipsychotic drugs are sometimes used in the same way but carry the risk of extrapyramidal syndromes such as tardive dyskinesia.

Melatonin has its advocates as a 'natural hypnotic'. It is the major hormone produced by the pineal gland and is mainly secreted during the hours of darkness (Brown, 1995). Its production falls off with age, so that the elderly often secrete very little. Some preliminary trials have shown encouraging results in the elderly, mainly with respect to quality of sleep. Various formulations of melatonin are being developed, as are synthetic analogues. Melatonin has also been used, often effectively, as a phase-shift agent in sleep disorders associated with jet lag, shift work, periodic sleep disorder in the blind and brain-damaged children. Further trials are required in all these indications, but, meanwhile, melatonin preparations of variable pharmaceutical purity are available in health-food shops and pharmacies in some countries.

HYPERSOMNIA

The commonest disorder under this rubric is narcolepsy. The treatment of narcolepsy can be divided into the management of the daytime sleepiness and that of the cataplexy. Stimulant drugs such as amphetamine and methylphenidate lessen the incidence of daytime drowsiness but are attended by the usual problem of tolerance, dependence, and rebound hypersomnia and depression on withdrawal. The minimal effective dosage must be sought assiduously; for example, methylphenidate 5–20 mg, three times a day. Part of the problem is the difficulty patients have in assessing their own difficulties, and a careful observer is invaluable in establishing the extent of any therapeutic effect. As tolerance develops, the stimulant can be withdrawn to allow a 'drug holiday' before reinstitution of the drug at a lower dose.

The treatment of cataplexy is more satisfactory. Antidepressants, both tricyclics and monoamine-oxidase inhibitors (MAOIs), suppress episodes of rapid-eye-movement sleep and are effective in lessening cataplectic attacks. Clomipramine seems to be currently favoured in the usual antidepressant dosage of 25–50 mg three times a day. The usual unwanted effects are sometimes troublesome. MAOIs have also been used but are less acceptable because of their dietary and drug interactions. They must *never* be combined with clomipramine. *Modafinil* has been recently licensed for the treatment of narcolepsy. The dose is 200–400 mg^{-1} per day, but lower in the elderly. It should be used cautiously in patients with liver impairment or hypertension. The main side effects are those of central nervous system stimulation, such as insomnia, euphoria, and anxiety.

PARASOMNIAS

These comprise a wide variety of behavioural disturbances occurring at various stages of sleep. Diazepam can be used to reduce slow-wave sleep in somnambulists and children with night terrors, and to control excessive nightmares. Otherwise, drug treatments are relatively ineffective.

CONCLUSIONS

A whole range of compounds is available for the treatment of sleep disorders, particularly insomnia of various types. However, the treatment of insomnia largely revolves around drugs that act on benzodiazepine receptors. Recent developments have produced hypnotics which are more selective than the older compounds such as the barbiturates and the widely used benzodiazepines. Whether this selectivity in biochemical terms is translated into clinical advantages, such as reduced impairment of cognitive and psychomotor function and lowered dependence potential, remains to be proved in practice.

The advent of very short-acting compounds, such as zolpidem and zaleplon, has allowed different strategies for the usage of these drugs. Thus, they can be given after the patient has gone to bed and found sleep elusive. In this way, patients recover control of

their drug taking and can revert to 'as-required' medication instead of taking the medication 'prophylactically' every night on the presumption that insomnia will occur. The next step will be the use of these very short-acting compounds for middle-of-the-night wakening, when, say, a period of 4 h remains before the sufferer has to rise. Whether or not this changes prescribing practice noticeably will depend on such factors as the conservatism of the medical profession and the concern that patients have that they will have a full night's sleep.

Meanwhile, the use of hypnotics remains a controversial subject, and the development of new compounds and of new strategies to improve the benefit/risk ratio of these compounds remains an important issue.

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XXV

Psychobiology of Impulse-Control Disorders Not Otherwise Specified (NOS)

Stefano Pallanti, Nicoló Baldini Rossi, Jennifer Friedberg and Eric Hollander

Impulsivity can be defined as the failure to resist an impulse, drive or temptation that is harmful to oneself or others (Hollander *et al.*, in press). Impulsivity is a measurable aspect of behaviour, manifesting as impatience (including the inability to delay rewards), carelessness, risk-taking, sensation- and pleasure-seeking, an underestimated sense of harm, and extraversion. The subjective experience of an impulse involves also an increasing sense of arousal or tension before committing/engaging in the act and an experience of pleasure, gratification or release of tension at the time of committing the act. Aggressive behaviour is a conduct that inflicts harm upon oneself or others. Behaviour can be impulsive without being aggressive (for example, a person who engages in pathological gambling is behaving in an impulsive manner, but not in an aggressive manner). Likewise, aggressive behaviour can lack impulsivity (as in a premeditated murder). Impulsive disorders cause large costs to society, and are associated with substantial morbidity, mortality, social/family/job dysfunction, accidents, suicide, violence, aggression, criminality, and excessive utilization of health-care, government and financial resources (Hollander *et al.*, in press).

Largely on the basis of the varying theoretical and clinical approaches of the myriad scientific and professional disciplines studying impulsivity and aggressive behaviours in animal models or in humans, impulsivity and aggressiveness are conceptualized and diagnosed in an unusually broad and disparate fashion. Just as anxiety and depression may be conceptualized either as symptoms or as specific disorders, impulsivity may be distinguished as a symptom or as a distinct disorder.

In psychiatric classification, impulsivity is a core symptom of a broad spectrum of disorders, including the impulse-control disorders (impulse-control disorders not elsewhere classified, comprising pathological gambling [PG], intermittent explosive disorder, pyromania, kleptomania, and trichotillomania, and impulse-control disorder not otherwise specified [NOS]), the impulsive-aggressive personality disorders (borderline, antisocial), the neurological disorders that can be associated with disinhibition of behaviour (such as epilepsy), and substance abuse (Hollander and Rosen, 2000). Of interest, addictive behaviour could also be described as conduct resulting from failure to inhibit impulses that urge and seek tension relief or pleasure. Furthermore, other psychiatric conditions also contribute to the expression of impulsivity, notably attention deficit/hyperactivity disorder (ADHD), mania, and eating disorders (Hollander and Rosen, 2000). The impulse-control disorders may belong to a family of compulsive-impulsive spectrum disorders lying at opposite ends of the dimension of risk avoidance, with impulsive disorders driven by pleasure or arousal, and compulsive disorders driven by reduction of anxiety (Hollander, 1998). Both impulsive and compulsive disorders involve a failure to resist a drive to act in a way that is potentially self-damaging, escalation of anxiety before engaging in the act, and relief of anxiety following the act. In fact, one of the few differences between the two types of disorders is that most compulsive behaviour disorders are perceived by the patient as ego-dystonic, whereas impulsive behaviours are usually viewed as ego-syntonic, at least in the impulsive setting. Rather than being the dimensional opposite of obsessive-compulsive disorders, impulse-control disorders may represent a different phenomenological manifestation of a group of disorders sharing the feature of decreased ability to inhibit motor responses to affective states.

We are at a very early stage in our understanding of the neurobiology of impulsivity and aggression (Kavoussi *et al.*, 1997). Thus, it is noteworthy that meanings and definitions of impulsivity and aggressiveness differ greatly in psychiatry and in neurobiology. Moreover, no simple extrapolation of animal subtypes to humans is possible, mainly because of the influence of complex cultural variables on behaviour. On the whole, research into the subtypes of human impulsivity has been rather limited. Much of this has been conducted in children. Clinical observation, experimental paradigms in the laboratory, and cluster/factor analytical statistics have all been used in an attempt to subdivide impulsivity and aggression. A consistent dichotomy can be identified for aggression between an impulsive-reactive-hostile-affective subtype and a controlled-proactive-instrumental-predatory subtype. Although good internal consistency and partial descriptive validity have been shown, these constructs still need full external validation, especially regarding their predictive power for comorbidity, treatment response, and long-term prognosis (Vitiello and Stoff, 1997).

In attempting to find parallels between aggression in humans and aggression in animals, Gregg and Siegel (2001) observed that whereas both humans and animals exhibit aggressive behaviour, animals do not engage in the premeditated aggressive acts that humans often display. Vitiello *et al.*, (1990), in distinguishing between affective aggression and predatory aggression, emphasize that the first is impulsive, occurring as a result of autonomic arousal, while the second is cold-blooded and premeditated, and is not a result of arousal. Due to the presence of the frontal cortex, humans have the capacity to engage in both affective and predatory aggression, while animals engage only in affective aggression, which is aggression related to survival and increased arousal. Impulsivity has been classified in to three different types: motor impulsivity, impulsivity without programming, and attentive impulsivity; the last may be represented by an exaggerated alert reaction (Barratt and Stanford, 1995).

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NEUROLOGICAL STRUCTURES INVOLVED IN IMPULSIVITY

The neurobiological basis of impulsivity is not fully understood. Frontal lobe abnormalities are associated with an inability to delay or inhibit acting on impulse, and an inability to calculate the odds of negative risk or outcome. Aspects of impulsivity are core symptoms of a number of frontal lobe syndromes, and frontal lobe hypofunction has been observed in impulsive individuals. Studies in subjects with borderline personality disorder have confirmed a fundamental, biologically based, affective hyperresponsiveness in borderline personality disorder (BPD), whereas autonomic underarousal may seriously interfere with a flexible adaptation to environmental stimuli (Herpertz *et al.*, 1999). Damasio (1996) hypothesized that 'somatic states', or emotional changes that occur in response to a stimulus, influence the cognitive process that occurs when an individual decides how to respond to the stimulus. Damasio found that patients with ventromedial frontal damage do not respond to emotionally charged stimuli, thus indicating that the frontal cortex is involved in the decision.

Bechara *et al.* (2000b), investigating gambling, have suggested that gamblers with ventromedial prefrontal cortex lesions are insensitive to future consequences, positive or negative, and are primarily guided by immediate prospects. This 'myopia for the future' in ventromedial prefrontal cortex-lesioned patients persists in the face of severe adverse consequences, as in risking future punishment or declining future reward.

Patients with lesions of the ventromedial prefrontal cortex display decision-making impairment similar to that observed in abusers of cocaine, opiates, and alcohol (Rogers *et al.*, 1999). They also evidence a disregard for or insensitivity to future consequences that is related to, but not entirely explained by, the construct of impulsivity (Bechara *et al.*, 2000a). Metabolic abnormalities have been observed in the orbital frontal, adjacent ventral medial, and cingulate cortex of impulsive-aggressive individuals (Siever *et al.*, 1999). Previous research has implicated the anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC) in impulsivity and in processes regulating choice between alternative reinforcers, and their abnormal function has been observed in some individuals with ADHD (Ernst *et al.*, 1998). Decreased regional cerebral blood flow to the ACC and frontal cortex has been observed in pathological gambling (Goyer *et al.*, 1999). Lesions of the amygdala reveal a complex modulation of aggressive and impulsive behaviours.

Recent lesion studies in animals and humans have begun to elucidate the neurocircuitry of impulsivity. Cardinal *et al.* (2001) reported induction of persistent impulsive choice in rats following lesions of the nucleus accumbens core (AcbC), a key brain region of reward and reinforcement. These lesioned rats consistently chose small or poor rewards that were immediately available in preference to larger delayed rewards. The group also exhibited locomotor hyperactivity, another sign of the hyperactive-impulsive subtype of ADHD, and so this may represent an animal model of the disorder. In contrast to earlier studies, lesions of two of the AcbC's cortical afferents, the ACC and the mPFC, did not induce impulsive choice, suggesting that ventromedial or orbitofrontal afferents may play a role. This finding complements recent work in humans with bilateral ventromedial prefrontal cortical lesions who, in a gambling test, opt for choices that yield high immediate gains in spite of high future losses (Bechara *et al.*, 2000b). Lesions in the amygdala have also been associated with impaired decision-making (Bechara *et al.*, 1999). Thus, the orbitofrontal, nucleus accumbens and amygdala regions appear to play an important role in mediating at least one aspect of impulsivity. Lesion studies to improve the localization of the neurocircuitry of specific impulsive behaviours may be helpful, and Cardinal *et al.*'s tentative localization of the neuroanatomical basis for delayed reinforcement may eventually lead to new diagnostic imaging or therapeutic procedures. Pharmacological manipulation of neurotransmitter peptide systems specifically targeted at the AcbC and/or relevant afferent pathways may moderate impulsive behaviours more specifically than do current therapies. However, while previous lesion and imaging studies suggest a role for the ACC and mPFC in impulsivity (Ernst *et al.*, 1998; Goyer *et al.*, 1999), Cardinal *et al.*'s study suggests that neither the ACC nor the mPFC contribute to the AcbC's ability to promote the choice of delayed reinforcers. Therefore, the obvious next step is to elucidate the afferent paths by which information concerning the value of delayed reinforcers is supplied to the AcbC. It has been suggested that the basolateral amygdala and/or the orbitofrontal cortex may play such a role. Orbital lesions of the prefrontal cortex have elsewhere been associated with increases in reflexive emotional responses to environmental stimuli (Luria, 1980).

NEUROLOGICAL STRUCTURES INVOLVED IN AGGRESSION

There are many literature data linking specific brain structures to aggressive behaviour in mammals and non-human primates (Hess, 1957). It is commonly observed that patients with neurological lesions may present with symptoms of aggression (Weiger and Bear, 1988). Several investigators hypothesize that, for a subgroup of chronically aggressive persons, the root of the aggressive behaviour is brain damage. Lewis *et al.* (1982) reported that every death-row inmate studied by her team had a history of head injury, often inflicted by abusive parents. Her study concluded that death-row inmates constitute an especially neuropsychiatrically impaired prison population. Although the connection between physical abuse, head injury, and aggression is uncertain, many studies do show an association between physical abuse and later aggressive behaviour. Clinical reports of aggressive patients with specific neurological lesions may help delineate the structures that mediate these symptoms. In patients presenting with aggressive symptoms, researchers have demonstrated neurological 'soft signs', a marker of subtle neurological dysfunction (Shaffer *et al.*, 1985).

It has been shown that antisocial personality disorder patients that engage in aggressive behaviour without exhibiting autonomic arousal have a reduction in prefrontal grey matter and less autonomic activity than healthy controls, psychiatric controls, and patients with substance dependence when presented with a social stressor (Raine *et al.*, 2000). Larson and Summers have recently observed that social stress from aggressive interaction is expressed differently in specific brain regions of dominant and subordinate male lizards (Anolis carolinensis). Prior to aggressive behaviour, the outcome is predictable via the celerity of postorbital colouration: dominant males exhibit more rapid eyespot darkening. Serotoninergic activation is manifested rapidly (1 h) in the hippocampus, nucleus accumbens and brainstem of subordinate males, and is expressed more rapidly in dominant males. Amygdalar serotoninergic activation responds rapidly (1 h) in dominant males, but is expressed slowly (1 week) in chronically subordinate males (Larson and Summers, 2001); these data seem to suggest that aggressive behaviour may play a role in ranking the dominance status.

We should also consider that impulsive and aggressive behaviour corresponds to a wide range of adaptative patterns, such as the self-mutilation of trapped wolves or the cannibalism of the praying mantis, and the aggressive component of reproductive behaviour in certain mammals.

Hypothalamus

The hypothalamus monitors internal status and regulates neuroendocrine responses via sympathetic arousal. It is involved in the

regulation of the sleep-wake cycle, appetite, body temperature, and sexual activity. In association with the pituitary, it is the major regulator of the autonomic nervous system. The mesolimbic dopamine (DA) pathway and the ascending serotonergic, noradrenergic, and cholinergic pathways from the brainstem have terminations in the hypothalamus.

The hypothalamus plays a major role in the expression of aggression in animals (Eichelman, 1971; Wasman and Flynn, 1962). Stimulation of the anterior hypothalamus causes predatory attacks in cats, whereas activation of the dorsomedial aspect produces aggression in which the animal ignores the presence of a rat and attacks the experimenter. Destruction of aggression-inhibitory areas, such as the ventromedial nucleus of the hypothalamus, produces permanently aggressive cats and rats (Bard, 1928; Reeves and Plum, 1969). Following cortical ablation, stimulation of the posterior lateral hypothalamus of the cat elicits sham-rage, a posture of preparation for attack. Stimulation of the posterior lateral portion of the hypothalamus shortens the latency of the attack, whereas stimulation of the medial ventral area prolongs it (Eichelman, 1971; Wasman and Flynn, 1962). Hamsters tested for offensive aggression after microinjections of arginine vasopressin (AVP) directly within the anterior hypothalamus in combination with a 5-HT_{1B} agonist have increased aggression, while those injected with AVP and a 5-hydroxytryptamine $(HT)_{1A}$ agonist have a dose-dependent inhibition of AVP-affiliated offensive aggression (Ferris *et al.*, 1999). In humans, structural lesions of the hypothalamus may be associated with unplanned and undirected aggressive behaviours that often appear to be unprovoked but may be in response to physical discomfort (Reeves and Plum, 1969; Killeffer and Stern, 1970; Haugh and Markesbery, 1983; Ovsiew and Yudofsky, 1983).

Recently, Gregg and Siegel (2001), using techniques of electrical brain stimulation, anatomical-immunohistochemical techniques and behavioural pharmacology, have investigated the neural systems and circuits underlying aggressive behaviour in the cat. The authors demonstrate that the medial hypothalamus and midbrain periaqueductal grey (PAG) are the most important structures mediating defensive rage behaviour, and that the fornical lateral hypothalamus clearly mediates predatory attack behaviour. The hippocampus, amygdala, bed nucleus of the stria terminalis, septal area, cingulate gyrus, and prefrontal cortex project to these structures directly or indirectly, and thus can modulate the intensity of attack and rage. Evidence suggests that several neurotransmitters facilitate defensive rage within the PAG and medial hypothalamus, including glutamate, substance P (SP), and cholecystokinin, and that opioid peptides suppress it; these effects usually depend on the subtype of receptor that is activated. A key recent discovery was a GABAergic projection that may underlie the often-observed reciprocally inhibitory relationship between these two forms of aggression. Recently, SP has come under scrutiny as a possible key neurotransmitter involved in defensive rage, and the mechanism by which it plays a role in aggression and rage is still under investigation (Gregg and Siegel, 2001). The possible hypothalamic role of SP in the intraspecific aggressive behaviour of isolated male mice was previously investigated by Bigi *et al.*, who evaluated the effects of a single intravenous administration of this neuropeptide on isolation-induced aggressive behaviour: SP treatment (0.25, 1.0, or $2.5\,\text{mg}\,\text{kg}^{-1}$ dose injected 15 min before testing) induced a decrease in offensive scores, a longer latency to the first attack episode and enhanced defensive displays. In no case did SP treatment affect locomotor activity levels or freezing behaviour (Bigi *et al.*, 1993).

The possible role of the hypothalamus in aggressiveness is also confirmed by Kim *et al.*, who examined the effects of intracerebroventricular injection of pertussis toxin, a specific inhibitor of G(i)/G(o) proteins, on plasma corticosterone levels, aggressiveness, and hypothalamic and hippocampal monoamines and their metabolite levels in mice. Plasma corticosterone levels were markedly increased after injection of pertussis toxin, which induced a progressive increase in aggressiveness, that is, a decrease in attack latency and an increase in number of attacks, on days 1 and 6 after injection. Brain monoamines and their metabolite levels changed on days 1 and 6 after toxin injection: in the hypothalamus, the levels of dopamine and 3,4-dyhydroxyphenylacetic acid were increased, the norepinephrine level was decreased, and the 5-hydroxyindole acetic acid (5-HIAA) level was markedly increased, with no changes in 5-HT level, whereas, in the hippocampus, the 5-HT level was significantly decreased, with no changes in 5-HIAA and catecholamines (Kim *et al.*, 2000).

According to Kim *et al.*, Van Goozen *et al.* have underlined the possible relationship between adrenal androgens and aggressive behaviour occurring in children with oppositional defiant disorder (ODD), showing that children with ODD had higher dehydroepiandrosterone sulphate (DHEAS) levels than either psychiatric control or normal control groups, and hypothesizing that the mechanism should be a shift in balance of ACTH-beta-endorphin functioning in the hypothalamic-pituitary-adrenal axis due to early stress or genetic factors (Van Goozen *et al.*, 2000).

Finally, it has been documented that maternal and mating-induced aggression is associated with elevated citrulline immunoreactivity in the hypothalamic paraventricular nucleus (PVN) in prairie voles. In the monogamous prairie vole, *Microtus ochrogaster*, the males are parental and exhibit a dramatic increase in aggression, termed mating-induced aggression, in association with reproduction. In mice, the gas, nitric oxide (NO), inhibits the males' aggression, but may have an excitatory role in the production of maternal aggression. Gammie and Nelson have combined aggressive behavioural testing of female and male prairie voles with immunohistochemistry for citrulline, a marker of NO synthesis, to examine NO synthesis indirectly during maternal and mating-induced aggression. A significant increase in the number of citrulline-positive cells was identified in the PVN of the hypothalamus in aggressive lactating females compared with unstimulated lactating females. A significant increase in the number of citrulline-positive cells was also observed in the PVN of aggressive mated males compared with nonaggressive unmated males and unstimulated mated males. In other regions of the brain, no changes in the number of citrulline-positive cells were observed. These data suggest that NO is released specifically in the PVN during both maternal and mating-induced aggression in prairie voles (Gammie and Nelson, 2000). Moreover, the intraperitoneal injections of the neuronal NO synthase (nNOS) inhibitor, 3-bromo-7-nitroindazole, significantly impaired the expression of maternal aggression in terms of the average time in aggressive encounters, the average number of attacks and the average latency to the attack first. These data suggest that the central release of NO may play an important role in the production of maternal aggression in prairie voles (Gammie *et al.*, 2000).

Amygdala

The amygdala consists of an anatomically defined region located within the temporal lobe of the brain in mammals. Along with other limbic system structures, the amygdala responds to changing environmental conditions by inducing emotive behaviours that are linked to past experiences. The amygdala is an important component of the limbic system and, as such, is considered a pivotal region for mediating the perception and the expression of fear and anxiety. The limbic system encompasses the amygdala and the temporal cortex. The amygdala activates and/or suppresses the hypothalamus and modulates input from the neocortex. It also has efferents to the extrapyramidal system. The amygdala may have a role in associating sensory experience with (hypothalamically directed) affects and behaviours, including anger (Bear, 1991). In

a study using positron-emission tomography, the amygdala was shown to be more activated during the processing of visually presented linguistic threats than during the processing of neutral words (Isenberg *et al.*, 1999).

Bilateral lesions of the amygdala tame a variety of hostile and vicious animals (Kluver and Bucy, 1939), whereas irritative lesions or electrical stimulation can lead to rage outbursts. Removal of the amygdala from monkeys results in decreased aggression (Downer, 1961). However, amygdalectomy in submissive monkeys may result in increased aggression (Dicks *et al.*, 1969). Aggressive behaviour following stimulation of the amygdala in cats varies accordingly to their pre-existing temperament (Adamac 1990). These findings suggest that the amygdala may not simply function to increase regulatory affects and behaviours, but that it may mediate and balance their control. Moreover, it seems that there is an association between intermale social aggression and cellular density within the central amygdaloid nucleus in rats with lithium/pilocarpine-induced seizures, suggesting that seizure-induced damage within proximal amygdaloid nuclei disinhibits the central nucleus and encourages aggression (Desjardin and Persinger, 1995).

Recently, it has been shown that the monoaminergic activities of limbic regions are elevated during aggression in the lizard *Anolis carolinensis* (Korzan *et al.*, 2000). In monkeys, bilateral temporal lobectomy leads to hyperorality, hypersexuality, absence of fear response, increased touching, and visual agnosia (Kluver-Bucy syndrome). Bilateral temporal lobe damage in humans leads to similar symptoms, including hypersexuality and visual and auditory agnosia. In addition, humans exhibit placidity, apathy, bulimia, and aphasia (Terzian and Ore, 1955; Marlowe *et al.*, 1975; Isern, 1987). This syndrome appears to be a disconnection between sensory information about the environment and the regulation of affects and behaviours (e.g., aggression, sex, food) that usually help the person or animal negotiate that environment.

It has been known since the classical work of Kluver and Bucy (1939) that amygdaloid lesions markedly reduce emotional responsiveness. More localized amygdaloid lesion studies indicate that a specific part of the amygdala, the central nucleus, is important for mediation of autonomic changes associated with stress. Bilateral ablation of the central amygdaloid nucleus attenuates learned heart-rate responses in the rabbit (Kapp *et al.*, 1985). Cryogenic treatment of the central amygdaloid nucleus blocked learned blood pressure and respiratory response in the cat (Zhang *et al.*, 1986). In rats, lesions of the central amygdala impede the increases in heart rate and blood pressure that occur to a tone associated with shock (Iwata *et al.*, 1986). The increases in heart rate and blood pressure to the shock alone were unaffected by amygdaloid lesions. Destruction of the central amygdala in rats also reduced stimulus-induced exaggerated increases in cardiovascular responses (Folkow *et al.*, 1982).

Seizure studies of the limbic area in humans may explain the possible neuroanatomical underpinnings of aggression. Whereas bilateral temporal lobe damage in humans may lead to Kluver-Bucy syndrome, with a decrease in regulatory affects and behaviours, disorders of temporal lobe excitation may result in increased affect and aggression (Serafetinides, 1965; Nachson, 1988). Associations between aggression and temporal lobe epilepsy has been reported: 30% of 286 patients with intermittent violent outbursts had temporal lobe epilepsy (TLE) (Elliot, 1992); 18 of 97 incarcerated delinquent boys with a history of violence presented psychomotor epilepsy (Lewis *et al.*, 1982). TLE patients may demonstrate hyperemotionality and increased aggression. Interictal aggression is much more common than ictal or postictal aggression in TLE. Interictal aggression is often characterized by intense affect in response to environmental stimuli, whereas ictal and postictal aggression is spontaneous and unfocused. In humans, reports of surgical intervention for the relief of mental or structural brain disease or epilepsy have shown that both the amygdala and other temporal lobe and limbic system structures contribute to modulation of aggression. Two patients who underwent bilateral amygdalotomy for intractable aggression showed a reduction in autonomic arousal in response to stressful stimuli and a decrease in aggressive outbursts (Lee and Coccaro, 2001). Limbic system tumours, infections, and blood vessel abnormalities have also been associated with violence and impulsivity. Although it is clear that various limbic system structures have an inhibitory or excitatory effect on aggression, the precise mechanism of the aggression pathway is still far from established.

It has been hypothesized that the amygdala probably does not function in normal homeostatic functions, as it is not active during sleep or anaesthetic states. In addition, the amygdala is not necessary for mediation of cardiovascular response to physical stressors. Rather, the amygdala probably functions to alter autonomic activity during responses to threatening or anxiety-provoking stimuli. Finally, a high density of benzodiazepine receptors has been localized within the amygdala, suggesting that it is an important site for anxiolytic drug actions (Niehoff and Kuhar, 1983). The benzodiazepine receptors are localized within the basolateral amygdaloid nucleus, an important intra-amygdaloid input to the central amygdaloid nucleus.

Prefrontal Cortex

The prefrontal cortex modulates limbic and hypothalamic activity, and is associated with the social and judgement aspects of aggression. The frontal cortex coordinates the timing of social cues, often before the expression of associated emotions. Lesions in this area give rise after minimal provocation to disinhibited anger characterized by an individual's showing little regard for the consequences of affect and behaviour. Weiger and Bear (1988) hypothesize that, whereas TLE patients may express deep remorse over an aggressive act, patients with prefrontal lesions often indicate indifference. Patients with violent behaviour have been found to have a high frequency of frontal lobe lesions (Kandel and Freed, 1989; Lishman, 1968). In a study of Vietnam veterans with a history of penetrating head injuries, patients with ventromedial lesions had higher verbal aggression scores than controls and those with lesions in other brain areas (Grafman *et al.*, 1996). Frontal lesions may result in the sudden discharge of limbic- and/or amygdala-generated affects no longer modulated, processed, or inhibited by the frontal lobe. Patients consequently respond with rage or aggression upon feelings that would have ordinarily been modulated by the individual. Prefrontal damage may cause aggression by a secondary process involving lack of inhibition of the limbic area. Dorsal lesions of the prefrontal cortex are associated with impairment in long-term planning and increased apathy. Orbital lesions of the prefrontal cortex are associated with increases in reflexive emotional responses to environmental stimuli (Luria, 1980).

Recently, neuroimaging studies suggest a role for the prefrontal cortex, along with other regions of the brain, in the expression of aggression, as shown by Lee and Coccaro (2001). Using positron-emission tomography, studies have documented reduced serotonergic function in specific brain regions in subjects with increased aggression and impulsivity. One imaging study showed that in contrast to controls, patients with borderline personality disorder have diminished response to serotonergic stimulation (*d*,1-fenfluramine) in areas of the prefrontal cortex associated with impulsive behaviour regulation, specifically the medial and orbital regions of the right prefrontal cortex, left middle and superior temporal gyri, left parietal lobe and left caudate body (Soloff *et al.*, 2000). Siever *et al.* (1999) found that impulsive-aggressive patients had significantly blunted metabolic responses in the orbital frontal, adjacent ventral medial and cingulate cortex compared to controls. Finally, impulsive murderers have been shown to have lower

left and right prefrontal functioning and higher right subcortical functioning in comparison to predatory murderers (Raine *et al.*, 1998). Van Erp and Miczek (2000) investigated the dynamic changes in DA and serotonin during aggressive confrontations in the corticolimbic areas of rats: DA and serotonin levels in the prefrontal cortex changed in opposite directions, with a sustained decrease of serotonin to 80% of baseline levels during and after the confrontation and an increase of DA by up to 120% after the confrontation. The temporal pattern of monoamine changes, which followed rather than preceded the confrontation, points to a significant role of accumbal and cortical DA and 5-HT in the consequences as opposed to the triggering of aggressive acts. The increase in accumbal DA in aggressive animals supports the hypothesis that this neural system is linked to the execution of biologically salient and demanding behaviour.

Other areas implicated in impulsivity and aggression include the midline thalamus, lateral preoptic region, mammillary bodies, hippocampus and basal ganglia.

PSYCHOBIOLOGY

Transmitter Systems

Several recent studies in genetics, neuropsychopharmacology and neuroimaging have helped to clarify the biological contributions to impulsivity and aggression in humans and animals. Various neurotransmitter systems modulate impulsive disorders. Evidence of serotonergic dysregulation and a presynaptic deficit of available 5-HT has been observed in humans with a variety of impulsive disorders, as well as animal models of impulsivity and aggression. Knockout mice lacking 5-HT_{1B} receptors display increased impulsive aggression, cocaine self-administration and alcohol consumption (Brunner and Hen, 1997), and polymorphisms of tryptophan hydroxylase (the rate-limiting enzyme for 5-HT synthesis) have been associated with impulsive-aggressive behaviours (New *et al.*, 1998). Specific impulse-control disorders, such as pathological gambling, probably involve abnormalities of DA receptors and reward pathways, as well as noradrenergic and serotonergic dysfunction (Hollander *et al.*, 2000). DA function, particularly within the mesocorticolimbic pathways, is critical in the mediation of reward and reinforcement behaviours (reviewed in Hollander *et al.*, 2000). Association studies of genes related to DA receptors have supported a genetic influence in impulsive behaviours (reviewed in Hollander *et al.*, 2000), and μ-opioid receptors are involved in the regulation of these pathways. Peptides such as vasopressin have also been implicated in aggressive behaviours. A better understanding of the role of neurotransmitters in modulating relevant neurocircuitry (i.e., the ventromedial cortex and nucleus accumbens) is needed to develop specific treatments for pathological impulsivity across various conditions.

Various approaches involving pharmacological manipulation of neurotransmitters have been undertaken in an attempt to ameliorate pathological impulsivity in several psychiatric disorders. Much research to date has focused on modulation of 5-HT transmission. Selective serotonin reuptake inhibitors (SSRIs) and other enhancers of serotonergic transmission have reduced impulsive behaviours in a wide range of different disorders, including pathological gambling, borderline personality disorder (BPD), sexual addictions and obsessive-compulsive spectrum disorders (Hollander *et al.*, 1998, Hollander and Rosen, 2000). While serotonin dysregulation and hypofunction appear to play a central role in these disorders, it is likely that impulsivity is influenced to differing degrees by the highly interconnected 5-HT, noradrenergic, dopaminergic, opiate and GABAergic systems. Nevertheless, the neuronal mechanisms producing behaviour are complex and irreconcilable with simplistic constructs, and it is impossible to manipulate one neurotransmitter system to the exclusion of surrounding neurons.

Serotonin Function

Animal and human research suggests that the central serotonin system is involved in the inhibition of impulsive behaviour, and reduced levels of cerebrospinal fluid (CSF) 5-HIAA seem to be associated with impulsive aggression.

A decrease in brain 5-HT has been found in the brainstems of muricidal rats (aggressive rats that spontaneously kill mice introduced into their cages) and other animals made aggressive by isolation. The administration of tryptophan, a 5-HT precursor, reduces or abolishes the violence (Depue and Spoont, 1987).

In primate studies, researchers have noted higher blood 5-HT and 5-HIAA CSF concentration in monkeys who tend to be dominant and high-ranking in their colonies (Higley *et al.*, 1992), and lower 5-HIAA CSF concentration as an antecedent to greater alcohol consumption (Higley *et al.*, 1996). Social stress from aggressive interaction is expressed differently in specific brain regions of dominant and subordinate male *A. carolinensis* lizards. Prior to aggressive behaviour, the outcome is predictable via the celerity of postorbital colouration: dominant males exhibit more rapid eyespot darkening. Serotoninergic activation is manifest rapidly (1 h) in the hippocampus, nucleus accumbens and brainstem of subordinate males, and is expressed more rapidly in dominant males. Amygdalar serotoninergic activation responds rapidly (1 h) in dominant males, but is expressed slowly (1 week) in chronically in subordinate males (Larson and Summers, 2001).

Mice deficient in monoamine oxidase A (MAO-A) have increased brain levels of serotonin and norepinephrine, and they show enhanced aggression (Shih *et al.*, 2000). Pruus *et al.* (2000) demonstrated that the 5-HT_{1A} receptors may be involved in the mediation of the apomorphine-induced aggressive behaviour in adult male Wistar rats; nevertheless, the prominent antiaggressive effect of the 5-HT_{1A} receptor agonist buspirone seems to be mediated by some other mechanisms, evidently via the DA D_2 receptors.

Fairbanks *et al.* (2001) provided evidence for serotonergic influences on social impulsivity in vervet monkeys. Svensson *et al.* (2000) have demonstrated that gonadectomy reduced disinhibitory behaviour in 5-HT-depleted rats, and that gamma-amino-butyric acid (A)/benzodiazepine receptor complexes ($GABA_A$/BDZ-RC) may be involved in this effect.

Studies of genetically engineered mice with targeted disruption of the neuronal nitric oxide synthase (nNOS) gene have established the inhibitory role of nitric oxide (NO) in male impulsive-aggressive behaviour. The molecular mechanism accounting for the aggressive behaviour caused by the lack of neuronally derived NO is not known; the excessive aggressiveness and impulsiveness of nNOS knockout mice is caused by selective decrements in serotonin turnover and deficient receptor function in the brain regions regulating emotion, indicating — as already mentioned — an important role for NO in normal brain 5-HT function (Chiavegatto *et al.*, 2001).

For dogs with dominance aggression, the addition of tryptophan to high-protein diets or change to a low-protein diet may reduce aggression; for dogs with territorial aggression, triptophan supplementation of a low-protein diet may be helpful in reducing aggression (De Napoli *et al.*, 2000).

Thus, there is significant evidence for the role of serotonergic (5-HT) dysregulation or dysfunction in impulsive aggression in both animals and humans (Asberg *et al.*, 1976; Brown *et al.*, 1979, 1982; Sabrie, 1986). In an attempt to dissect the contribution of individual 5-HT receptor subtypes to behaviour, Ramboz *et al.* (1996) have generated mutant mice lacking the 5-HT_{1B} receptor. These mice did not exhibit any obvious developmental or behavioural defect; however, the hyperlocomotor effect of the 5-$HT_{1A/1B}$ agonist, RU24969,

was completely absent in mutant mice, indicating that this effect is mediated by 5-HT_{1B} receptors; moreover, when confronted with an intruder, isolated mutant mice attacked the intruder faster and more intensely than wild-type mice, suggesting an involvement of 5-HT_{1B} receptors in the modulation of aggressive behaviour. These data might be related to the fact that a class of 5-HT_1 agonists, termed serenics, have antiaggressive properties, and to the findings that certain impulsive-aggressive behaviours are associated with deficits in central serotonin (Saudou *et al.*, 1994; Ramboz *et al.*, 1996). Furthermore, Brunner and Hen (1997), using a knockout mouse that lacks 5-HT_{1B} receptors, observed that this animal shows more impulsive aggression, acquires cocaine self-administration faster, and drinks more alcohol than the corresponding wild-type control; for Brunner and Hen, these impulsive characteristics are not due to a change in cognitive functions, since in a cognitive task involving a choice between a small immediate reward and a larger, more delayed reward, knockout mice showed intact choice and timing capabilities and good discrimination of reward amounts.

Scearce-Levie *et al.* (1999) have evaluated the behavioural effects of 3,4-methylenedioxymethamphetamine (MDMA), a psychoactive drug of abuse, which in rats stimulates locomotion while decreasing exploratory behaviour, on knockout mice lacking the 5-HT_{1B} receptor: these animals show a reduced locomotor response to MDMA, although delayed locomotor effects of MDMA are present in these animals. These findings indicate that the locomotor effects of MDMA are dependent upon the 5-HT_{1B} receptor, at least in part. In contrast, MDMA eliminates exploratory behaviour in both normal and knockout mice, suggesting that the exploratory suppression induced by MDMA occurs through mechanisms other than activation of the 5-HT_{1B} receptor.

Moreover, according to Ramboz, the 5-HT_{1A} receptors should be involved in the modulation of exploratory and fear-related behaviours, suggesting that reductions in 5-HT_{1A} receptor density due to a genetic defect or environmental stressors might result in heightened anxiety (Ramboz *et al.*, 1998). These results seem to be confirmed by other authors who observed that 5-HT_{1B} knockout mice are more aggressive, more reactive and less anxious than the wild types, whereas the 5-HT_{1A} knockouts are less reactive, more anxious, and possibly less aggressive than the wild types (Zhuang *et al.*, 1999).

Animal studies suggest that 5-HT_1-receptor stimulation results in a decrease in aggressive behaviour, whereas, in humans, aggressive, personality-disordered patients show a blunted prolactin response to the 5-HT_{1A} agonist buspirone. Antagonism of the 5-HT_2 receptors appears to decrease aggression, and this effect may explain the ability of newer antipsychotics agents (which, unlike older antipsychotic medications, block 5-HT_2 receptors) to produce a dramatic reduction in aggression and agitation independent of the effects on psychotic symptoms (Kavoussi *et al.*, 1997).

In humans, Asberg *et al.* (1976) initially noted an inverse relationship between violent/lethal suicidal behaviour and the 5-HT metabolite 5-HIAA CSF concentration in depressed patients. Subsequent studies on populations in eight different countries confirmed that suicidal depressed patients have lower 5-HIAA CSF than nonsuicidal depressed patients. For example, Lidberg *et al.* (2000) found that homicide offenders with a history of suicide attempts had a lower concentration of spinal fluid 5-HIAA than the remaining murderers. This correlation is particularly strong in those with violent suicide attempts. Low concentration of CSF 5-HIAA has also been shown to be related to aggressive behaviour independent of suicidal behaviour in patients with Axis I disorders (Stanley *et al.*, 2000). In addition, Brown *et al.* (1982) demonstrated a decrease in 5-HIAA CSF in patients with personality disorders and found that this decrease correlated with scores on a lifetime aggression scale. Many studies have confirmed an inverse relationship between 5-HIAA CSF level and impulsive and violent behaviours (Bioulac, 1980; Brown *et al.*, 1982; Linnoila *et al.*, 1983; Lidberg *et al.*, 1984). The individual cases and small study populations studied include psychopathic military personnel, arsonists, murderers, violent suicidal patients, and behaviourally disruptive children and adolescents. Linnoila *et al.* (1983) have reported reduced CSF 5-HIAA concentration in both impulsive violent offenders and impulsive arsonists compared with premeditated violent offenders, suggesting that it is specifically nonpremeditated ('impulsive') aggression that correlates with reduced central 5-HT function in these individuals.

Investigators have also correlated abnormal 5-HT platelet studies with impulsivity and aggression (Biegon *et al.*, 1990; Marazziti and Conti, 1991). Decreased numbers of platelet 5-HT transporter sites are found in aggressive conduct-disordered subjects and in 'aggressive' institutionalized psychiatric subjects (Stoff *et al.*, 1991). In addition, an inverse correlation between platelet 5-HT uptake and the Barratt 'impulsivity' score has been reported in aggressive adult males (Brown *et al.*, 1989). In children and adolescents with conduct disorder, there is a negative correlation between platelet imipramine binding and impulsive aggression (Stoff *et al.*, 1987). In individuals with personality disorders, platelet titrated paroxetine binding has been shown to be inversely correlated with the Life History of Aggression total score and aggression score, and with the Buss-Durkee Hostility Inventory Assault score (Coccaro *et al.*, 1996).

Researchers have noted consistently reduced imipramine binding (Brown *et al.*, 1989) and increased platelet 5-HT_2 binding in suicide victims (Biegon *et al.*, 1990). The reduced imipramine binding may reflect decreased 5-HT release. Increased 5-HT_2 binding may reflect the brain's compensatory response to a decrease in functional serotonergic neurons, with consequent upregulation of postsynaptic 5-HT_2 binding sites. Additional findings that suggest the role of 5-HT in impulsivity and aggression include reports of low serum ratios of tryptophan to other neutral amino acids in alcoholics arrested for assaultive behaviour compared with other alcoholics or nonalcoholic control subjects (Lewis, 1991). Type 2 alcoholism is associated with both violent behaviour and serotonergic deficit (LeMarquand *et al.*, 1994, Virkkunen and Linnoila, 1990). Individuals with a family history of alcoholism may be more sensitive to impulsivity in response to low serotonin levels, as tryptophan-depleted individuals with a family history of alcoholism made more errors in a modified Taylor task than did those with no family history of alcoholism (LeMarquand *et al.*, 1999).

Biver has studied gender differences in the living human brain 5-HT_2 receptor, using positron-emission tomography and a selective radiotracer: he found significantly higher 5-HT_2 receptor-binding capacity in men than in women, especially in the frontal and cingulate cortices; this finding suggests that distinct liability for men and women to suffer from some psychiatric disorders responding to serotonergic agents may be related to differences in brain serotonin receptors (Biver *et al.*, 1996).

Other Neurotransmitter Systems

Neurotransmitters other than serotonin probably influence aggressive and impulsive behaviour; for example, GABA, norepinephrine and DA (Oquendo and Mann, 2000). An alpha-amino-3-hydoxy-5-methylisoxadole-4-propionate (AMPA) receptor antagonist, NBQX, was found to increase impulsivity in rats which was restored by injection of a positive allosteric modulator of AMPA receptors, indicating that the AMPA receptor, a type of glutamate receptor, is involved in the regulation of impulsivity (Nakamura *et al.*, 2000). Some studies demonstrate that increasing norepinephrine levels correlate with impulsive aggression, whereas other studies demonstrate an opposite relationship; thus, the role of norepinephrine in impulsive-aggressive behaviour is still unclear (Oquendo and Mann, 2000).

It has been shown that brain GABA levels are involved in intermale aggression in mice, and that mice selected for differences in sensitivity to a benzodiazepine receptor inverse agonist vary in intermale aggression (Guillot *et al.*, 1999). Moreover, the acute $GABA_A$ receptor agonist THIP and the $GABA_B$ receptor agonist baclofen attenuate the apomorphine-induced aggressive behaviour, indicating the involvement of both $GABA_A$ and $GABA_B$ receptor subtypes in the neurobiology of apomorphine-induced aggressiveness, as this phenomenon is evidently subject to the general inhibitory effect of GABAergic neurotransmission (Rudissar *et al.*, 2000). The involvement of GABA as a possible neurotransmitter of aggressive behaviour is indirectly confirmed by the effect of alcohol: in fact, the aggressive behaviour of certain individual animals can be greatly increased when they are under the influence of low doses of alcohol. One of alcohol's neurochemical actions that may be relevant to alcohol-heightened aggression is its positive modulation of the $GABA_A$ receptor complex. Alcohol prolongs 'bursts' of aggressive acts, and displays and disrupts communication between the aggressive animal and the opponent who defends itself, submits or flees. Pharmacological modulation of the $GABA_A$ receptor with benzodiazepines and neuroactive steroids (allopregnanolone) results in dose-dependent biphasic changes in aggressive behaviour that mimic the dose-effect function of alcohol; benzodiazepines potentiate the aggression-heightening effects of alcohol as well as the behaviourally suppressive effects, and antagonists of benzodiazepine receptors prevent the aggression-heightening effects of alcohol (Miczek *et al.*, 1997). Although alcohol induces aggressive behaviour in mice, it has a suppressive effect upon predatory attack behaviour in the cat. Han's studies support the hypothesis that ethanol's suppressive effects in the cat are mediated, at least in part, by $GABA_A$ receptors in the lateral hypothalamus (Han *et al.*, 1997). Moreover, activation of the mesolimbic DA pathway appears to promote drug- and alcohol-seeking behaviour in laboratory animals (Hill *et al.*, 1999).

The possible role of DA in aggressiveness is suggested by the fact that the blockade of the D_1/D_2 DA receptor produces an antiaggressive action commonly associated with an impairment of other motor behaviours, and the D_3 receptor seems to present opposite actions to D_1 and D_2, since the blockade of this receptor produces a stimulation of motor activity, which has been associated with an increase in DA neurotransmission (Rodriguez-Arias *et al.*, 1999).

Several reports suggest that when there is a dysfunction in a person's brain reward cascade — a dysfunction which could be caused by certain genetic variants, especially in the DA system — leading to a hypodopaminergic trait, that person requires a DA fix to feel good. This trait leads to multiple drug-seeking behaviour. This is so because alcohol, cocaine, heroin, marijuana, nicotine and glucose all cause an activation and neuronal release of brain DA, which may satisfy the abnormal cravings. It seems certain that carriers of the DAD_2 receptor A1 allele have compromised D_2 receptors. Therefore, the lack of D_2 receptors causes individuals to have a high risk of multiple addictive, impulsive and compulsive behavioural propensities, such as severe alcoholism; cocaine, heroin, marijuana, and nicotine addiction; glucose bingeing; pathological gambling; sex addiction; ADHD; Tourette's syndrome; autism; chronic violence; conduct disorder; and antisocial behaviour (Blum *et al.*, 2000).

Shih *et al.* (2000) have observed that mice deficient in MAO-A have increased brain levels of serotonin and norepinephrine and show enhanced aggression, and that the administration of Gingko biloba extract to MAO-A knockout mice reduces their aggressive behaviour in resident-intruder confrontations; this effect on aggression is not due to sedation and may be mediated by $5\text{-}HT_{2A}$ receptors. All these contradictory results suggest the complexity of neuronal mechanisms in producing behaviour. One point to consider is that MAO is relatively nonspecific, as it is involved in the breakdown of a number of monoamines, and the activity of MAO in platelets may not reflect MAO activity in the central nervous system.

The observation that naloxone and naltrexone ameliorate self-injurious behaviour (SIB) has suggested that impairment of opioid secretion may be involved in the physiopathology of this aggressive behaviour (Richardson and Zaleski, 1983). In patients with SIB, Met-enkephalin plasma levels are increased and return to normal after improvement of the disease. Beta-endorphin plasma levels, however, are lower than normal in children with SIB and higher than normal in adults (Sandman *et al.*, 1987). There have been studies of the functional effect of enkephalins isolated from the avian brain on preoptic and hypothalamic neurons in male Japanese quails. Behavioural changes have been observed following injection of naloxone, a non-selective opioid receptor antagonist, and D-Ala2-Met5-enkephalinamide (DALA), a selective delta opioid receptor agonist, into the preopti and anterior hypothalamic regions. Naloxone treatment showed a significant increase in the frequency of several aggressive actions and the effect was dose dependent, whereas DALA treatment significantly decreased the frequency of aggressive actions in a dose-dependent manner (Kotegawa *et al.*, 1997).

Finally, it has been reported that the μ-opioid receptor upregulation in limbic areas is consistent with increased emotional and aggressive behaviours observed in enkephalin knockout mice (Brady *et al.*, 1999). Taken together, all these data seem to suggest that the opioid system may play a role in the neurobiology of aggressiveness.

NEUROENDOCRINOLOGY

Endocrine Studies

Animal studies show that the testosterone levels of male rhesus monkeys correlate positively with behavioural dominance and aggression. If a single male monkey is placed with other aggressive males, he becomes submissive and shows a decrease in plasma testosterone, revealing that endogenous hormone production can be affected by behavioural variables. King's recent data seem to support the hypothesis that early androgen treatment may support the neurobiology of animals with genetic predisposition to hyperactivity, impulsivity and inattention in a manner consistent with the enhanced expression of ADHD-like behaviours (King *et al.*, 2000).

The connection between the endocrine system and aggression and impulsivity is not clear. Some researchers have hypothesized that androgens may play a role in aggression. They suggest that the androgen insensitivity syndrome and the androgenital syndrome are examples of androgen excesses and deficiencies associated with aggressive and inhibited behaviour, respectively. In one study, plasma testosterone levels were elevated in juvenile prisoners who had committed violent crimes. CSF free testosterone has been shown to be correlated with overall aggressiveness, but not with measures of impulsivity (Higley *et al.*, 1996). Oestrogens and antioandrogens have been used to reduce aggressiveness effectively in some violent sex offenders, although these cases clearly need to be better studied. Low salivary cortisol levels have been associated with persistence and early onset of aggression in school-aged boys, suggesting that low hypothalamic-pituitary-adrenal axis activity correlates with aggressive activity (McBurnett *et al.*, 2000).

Van Goozen has recently examined the relationship between adrenal androgens and aggression in children with oppositional and antisocial behaviour, and compared their levels with those of psychiatric and normal controls. Children with oppositional defiant disorder (ODD) had higher dehydroepiandrosterone sulphate (DHEAS) levels than either psychiatric control or normal

control groups; DHEAS levels between the last two groups did not differ; in Van Goozen *et al.*'s opinion, it is possible to classify children as having either ODD or ADHD on the basis of their DHEAS levels, whereas this was not the case on the basis of the Child Behaviour Checklist (CBCL) data. These data indicate that adrenal androgen functioning is specifically elevated in children with ODD, and it is speculated that the mechanism could be a shift in balance of ACTH-beta-endorphin functioning in the hypothalamic-pituitary-adrenal axis due to early stress or genetic factors (Van Goozen *et al.*, 2000).

A positive relationship has been reported in boys between testosterone blood level and acting/receiving aggression in 'social interactions' (serious aggression), but not in playing contests (playful aggression). This indicates that radioimmunoassay testosterone samples in saliva can be a useful biological marker for the risk of serious aggression in preschool boys, reflecting that various levels of sociability are linked to different behavioural patterns (Sanchez-Martin *et al.*, 2000).

Moreover, it has been noted that the ability of estradiol to facilitate transcription from six genes whose products are important for lordosis behaviour in female mice (a typical mating behaviour) proved that hormones can turn on genes in specific neurons at specific times, with sensible behavioural consequences. The use of a gene knockout for oestrogen receptor alpha (ERalpha) revealed that homozygous mutant females simply would not do lordosis and instead were extremely aggressive, thus identifying a specific gene as essential for a mammalian social behaviour. In dramatic contrast, (ERbeta) knockout female mice can exhibit normal lordosis behaviour (Pfaff *et al.*, 2000).

A randomized, double-blinded, placebo-controlled, crossover clinical trial has been used to determine the role of sex steroids on the development of aggressive behaviours in 35 boys and 14 girls. Depot testosterone (to boys) or conjugated oestrogens (to girls) was administered in 3-month blocks, alternating with placebo at three dose levels approximating to early, middle and late pubertal amounts. The Olweus Multifaceted Aggression Inventory was administered after each placebo and treatment period to ascertain the effect of steroids on self-reported aggressive behaviours. The data demonstrated significant hormone effects on physical aggressive behaviours and aggressive impulses, but not on verbal aggressive behaviours nor on aggressive inhibitions in both boys and girls (Finkelstein *et al.*, 1997).

Finally, the impairment of the purinergic system, characterized by reduced adenosinergic activity, has been implicated in the neurobiology of aggressive behaviour. Since there are no direct adenosine agonists available for human use, inhibition of purine degradation by allopurinol was conceived as a possible strategy: Lara *et al.* (2000) reports two cases of adults with refractory aggressive behaviour due to a neurological condition with dramatic response to therapy with allopurinol, 300 mg per day p.o., suggesting the involvement of the purinergic system in the neurobiology of aggression.

Pharmacological Challenge Studies

Animal models have been used to define more clearly the role of specific 5-HT receptors in impulsivity and aggression. To define the contribution of 5-HT receptor subtypes to behaviour, mutant mice lacking the 5-HT_{1B} receptor were generated by homologous recombination. As reported above, these mice did not exhibit any obvious developmental or cognitive defects. They were, however, noted to be extremely aggressive — they attacked intruders faster and more intensely than did wild-type mice (Hen, 1994) — to have increased impulsive aggression, to acquire cocaine self-administration faster, and to exhibit increased alcohol consumption (Brunner and Hen, 1997), suggesting a role for the 5-HT_{1B} receptors in modulating aggressive, impulsive and addictive behaviour (Hen, 1994).

Another research approach involves the use of challenge agents, such as m-chlorophenylpiperazine (m-CPP), that simulate or block serotonin receptors. M-CPP, a non-selective serotonin (5-HT) receptor agonist, has a complex effect on the brain with potent binding to the 5-HT_{2C} receptors and weaker affinity for the 5-HT_{1A} receptors (Kahn and Wetzler, 1991). Neuroendocrine changes following m-CPP stimulation have been represented by increased prolactin, adrenocorticotrophic hormone (ACTH), and corticosterone/cortisol responses in rodents, monkeys and humans (Fuller, 1981; Aloi *et al.*, 1984; Yatham and Steiner, 1993; Meltzer and Maes, 1995). Postsynaptic 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors are thought to mediate these effects (Meltzer and Maes, 1995).

Studies have found that aggressive antisocial subjects had significantly lower excretions of baseline urinary free cortisol and lower CSF ACTH concentration than controls. These findings suggest an inverse relationship between the activity of the hypothalamic-pituitary-adrenal (HPA) axis at baseline and aggressive behaviour (Virkkunen, 1985; Virkkunen *et al.*, 1994). Our group (De Caria *et al.*, submitted) studied 5-HT metabolism alteration, examining behavioural ('high') and neuroendocrine (prolactin and cortisol) responses to single dose ($0.5\,mg\,kg^{-1}$) oral m-CPP and placebo in pathological gamblers and matched controls, and the relationship with clinical severity. Pathological gamblers had significantly increased prolactin response compared to controls at 180 and 210 min post m-CPP. Greater gambling severity correlated with increased neuroendocrine responsiveness to m-CCP, suggesting greater 5-HT dysregulation. Pathological gamblers had significantly increased 'high' response to m-CPP compared to placebo. M-CPP challenge, via the HPA axis, provided a dynamic index of central serotonergic function. An enhanced response to direct postsynaptic serotonergic receptor stimulation is consistent with hypersensitive postsynaptic serotonergic function in male pathological gamblers compared to healthy controls. This would be consistent with a net deficiency of presynaptic 5-HT availability at baseline, and a compensatory increase in postsynaptic 5-HT receptor sensitivity.

Similar results were reported in trichotillomanic (Stein *et al.*, 1995a), alcoholic (Benkelfat *et al.*, 1991), and impulsive and antisocial patients (Moss *et al.*, 1990; Maes *et al.*, 2001). These results are also consistent with reported increased prolactin response and a prolonged peak following m-CPP administration in paedophilics, compared to placebo, relative to healthy controls (Maes *et al.*, 2001).

Pathological gamblers reported that the 'high' that they experienced in response to m-CPP was similar to the 'high' they experience while gambling. An increased 'high' response in other impulsive disorders, such as borderline personality disorder (Hollander *et al.*, 1994) and trichotillomania (Stein *et al.*, 1995a), following m-CPP stimulation has been found. These findings suggest that m-CPP elicits an increased euphoric effect in patients with various types of impulsive behaviours/disorders relative to healthy controls and placebo. In disorders of substance abuse, male alcoholics, some of whom had comorbid antisocial personality disorder (Benklefat *et al.*, 1991), cocaine addicts (Buydens-Branchey *et al.*, 1993), and alcoholics (Krystal *et al.*, 1994) also experienced increased 'high' feelings in response to m-CPP stimulation.

Hollander *et al.* (1992) observed that also a subgroup of patients with obsessive-compulsive disorder (OCD) experienced exacerbation of obsessive symptoms following m-CPP challenge studies. M-CPP has affinity for the 5-HT_{1A}, 5-HT_{2C}, and 5-HT_{1D} receptor subtypes. Patients who underwent challenge studies with MK212, a 5-HT agonist with affinity for the 5-HT_{1A} and 5-HT_{2C} receptor subtypes, but not the 5-HT_{1D} subtype, did not manifest exacerbation of obsessions and compulsions. Because there are behavioural changes in a subgroup of OCD patients following m-CPP, but not following MK212, and because the activity of these two agonists differ with regard to only one receptor subtype, the 5-HT_{1D} receptor, it

has been suggested that this receptor may modulate obsessions, of which sexual and aggressive symptoms may be prominent.

Dynamic changes of DA and serotonin have also been assessed: rats were implanted with a microdialysis probes aimed at the nucleus accumbens or medial prefrontal cortex, and, as already mentioned, DA and serotonin levels in the prefrontal cortex changed in opposite directions. Serotonin decreased to 80% from baseline levels during and after the confrontation, whereas DA increased by up to 120% after the confrontation. The temporal pattern of monoamine changes points to a significant role of accumbal and cortical DA and 5-HT in the consequences, as opposed to the triggering, of aggressive acts. The increase in accumbal DA in aggressive animals supports the hypothesis that this neural system is linked to the execution of biologically salient and demanding behaviour (Van Erp and Miczek, 2000).

NEUROGENETICS

Genetic studies in humans and animals have not yet supported a definitive association among impulsivity, aggression, and reduced 5-HT activity. The Maudsley rat study, however, was an example of genetic breeding for aggressive behaviour. Two groups of rats were bred. The first group (MNR) included rats that had low measures of impulsivity and high measures of inhibition. The second group (MR) had the opposite features. The MR strains bred from the second group were significantly more impulsive and demonstrated increased aggressive behaviour compared with the MNR rats (Eichelman, 1971). Neurochemically, the MNR strain showed lower limbic brain 5-HT levels than the MR strain (Sudack and Maas, 1964).

At the synaptic level, reuptake of serotonin is accomplished by a plasma membrane carrier called the serotonin transporter (5-HTT). The gene for 5-HTT has been mapped to chromosome 17 (Collier *et al.*, 1996). Preliminary evidence for a genetic disturbance in serotonergic function that might predispose individuals to impulsive-aggressive behaviour includes a study of the gene for the rate-limiting enzyme for serotonin synthesis, tryptophan hydroxylase (TPH). The gene for TPH has been mapped to the short arm of chromosome 11 and is one of the major candidate genes for psychiatric and behavioural disorders. Part of the gene for TPH has been discovered to exist as two alleles: U or L, with certain genotypes (UL and LL) being associated with impulsive-aggressive behaviour and suicidal behaviour, and with low levels of CSF 5-HIAA in violent offenders (Nielsen *et al.*, 1994). Persons having the TPH U allele scored significantly higher on measures of aggression than individuals homozygous for the L allele, and peak prolactin response was attenuated among male subjects, but not female subjects, having any U allele relative to LL homozygotes (Manuck *et al.*, 1999). In another study, the TPH genotype was found to be associated with impulsive-aggressive behaviours in male, but not female, patients who had personality disorders (New *et al.*, 1998). There have been reported polymorphisms in the regulatory region of the serotonin transporter, in intron 7 of the TPH gene, and in the MAO-A gene, associated with mood and anxiety disorders, impulsivity and aggression (New *et al.*, 1997; 1998). Nolan *et al.* (2000) examined these polymorphisms in schizophrenic and schizoaffective patients, suggesting the association between the TPH L allele and impulsive aggression in males with personality disorder. Turecki *et al.* (2001), instead, investigated the correlation between TPH and suicidal behaviour: haplotype analysis revealed that one haplotype (−6526G −5806T 218C) was significantly more frequent among suicide subjects than in normal controls, and this haplotype was particularly more frequent among subjects who committed suicide by violent methods. Further studies are needed to clarify the role of TPH alleles in aggression and the differences between the sexes.

Genetic studies involving the 5-HT_{1B} receptor gene in human subjects have been equivocal. In one study, a polymorphism of the 5-HT_{1B} receptor gene was linked to aggressive and impulsive behaviour in alcoholics (Lappalainen *et al.*, 1998). However, Huang *et al.* (1999) found no relationship between suicide, alcoholism or pathological aggression with 5-HT_{1B} receptor-binding indices or a genotype using two common polymorphisms. In summary, serotonin synthesis and regulation are at least partially regulated by genetic controls that probably contribute to an individual's propensity for impulsive and aggressive behaviours.

Homozygosity of a low-enzyme-activity variant of the catechol-*O*-methyltransferase (COMT) gene has been reported to be associated with aggressive behaviour in a group of schizophrenic patients. A similar tendency has been observed by Kotler *et al.* (1999) in a group of 30 schizophrenic subjects who were confined to a maximum-security psychiatric facility for homicide; significant excess (46.7% versus 21.0%) homozygosity of the low activity COMT Met/Met genotype was observed in 30, mostly male (28 of 30), homicidal schizophrenic patients compared with 415 control subjects, and no difference in COMT genotype has been found between nonviolent schizophrenic patients and control subjects. Moreover, the COMT genotype has been determined in 62 impulsive violent recidivist offenders with early-onset (type 2) alcoholism, 123 late-onset nonviolent alcoholics (type 1) and 267 race- and gender-matched controls. The allele and genotype frequencies of these groups were compared with each other and also with previously published data from 3130 Finnish blood donors. The type 2 alcoholics did not differ from either the blood donors or the controls. The low activity (L) allele frequency was higher among type 1 alcoholics than type 2. The odds ratio for type 1 alcoholism as compared with type 2 alcoholism for those subjects with the LL genotype versus the HH was 3.0 (95% confidence interval 1.1–1.84). These results suggest that the COMT genotype has no major role in the development of early-onset alcoholism with severe antisocial behaviour (Hallikainen *et al.*, 2000).

Currently, there are no controlled family history studies of individuals with impulse-control disorders (i.e., intermittent explosive disorder, kleptomania, pyromania, pathological gambling, and trichotillomania). There are studies supporting associations between major mood disorder and alcohol and substance abuse in first-degree relatives of individuals with kleptomania and in first-degree relatives of pathological gamblers (Linden *et al.*, 1986; Ramirez *et al.*, 1983; Saiz *et al.*, 1992).

The relationship between mood swing and impulse control represents a field of great relevance to the understanding and therapy of impulse-control disorders. Other findings include associations between anxiety disorders in the families of individuals with kleptomania and violent behaviour and ADHD in the families of individuals with intermittent explosive disorder (McElroy *et al.*, 1991).

Research involving monozygotic twins reveals a hereditary aspect of aggressive behaviour, with concordance rates for monozygotic twins greater than those for dizygotic twins. Twin studies suggest that antisocial behaviour in adult life is related more to genetic factors than to environmental factors (Cadoret *et al.*, 1995). Twin studies have established that there are substantial genetic influences on alcoholism in both men and women, and it seems that the heritability of alcoholism is substance-specific; the relationship between this and behaviours predisposing to alcoholism, including impulsivity, is still challenging. Refinement of clinical phenotypes and use of intermediate phenotypes will improve the changes of gene identification (Enoch and Goldman, 2001).

Chromosomal studies have looked at the influence of chromosomal abnormalities in aggression, particularly the XYY syndrome (Bioulac *et al.*, 1980). However, the link between XYY and violence has not been confirmed. Inborn metabolic disorders that affect the nervous system can be associated with aggressive personalities. These disorders, which diffusely affect the central nervous system

and are inherited, include phenylketonuria, Lesch-Nyhan syndrome, Prader-Willi syndrome, Vogt syndrome (a neuronal storage disorder), and Sanfilippo's syndrome (increased mucopolysaccharide storage).

THERAPEUTIC INTERVENTIONS

Impulsivity and aggression are behavioural characteristics that encompass a broad range of clinical problems. Studies on impulsivity and aggression have focused on a heterogeneous group of disorders with varied responses to pharmacotherapeutic interventions. In this chapter, we do not focus on the treatment of patients with epilepsy and patients with drug-induced aggression. These areas are reviewed elsewhere.

Controlled studies suggest that a number of medications may be useful in the treatment of impulsivity and aggression. Given the evidence for decreased 5-HT function in impulsive and aggressive behaviours, many, but not all, of these medications involve direct 5-HT mechanisms.

SSRIs have been shown to reduce impulsive, aggressive behaviours in various psychiatric disorders. For example, fluvoxamine resulted in improvement in gambling severity in patients with pathological gambling compared to placebo in one double-blind study (Hollander *et al.*, 2000b). However, in some disorders characterized by impulsivity, SSRIs have a quick onset, but these effects maybe transient, and some patients may require augmentation with compounds such as lithium, buspirone, and anticonvulsants (Hollander and Wong, 1995). Preliminary data from an open-label study conducted by our group reported that nefazodone (100–500 mg per day) is also an effective treatment in a sample of 12 pathological gamblers.

BPD is a common clinical problem in which researchers have used pharmacological interventions to target the characteristic symptoms of impulsivity, aggression, lability, and hostility. Fluoxetine is the best-studied SSRI for the treatment of impulsivity and aggression. A number of open trials of fluoxetine in BPD suggest its efficacy in the treatment of impulsivity and aggression in BPD. Markowitz (1990) reported that BPD patients showed significant decreases in self-injurious behaviour after treatment with fluoxetine 80 mg per day for 12 weeks. Three subsequent double-blind, placebo-controlled trials of fluoxetine confirmed the findings of the open trials (Markowitz, 1992). Overall, controlled studies of fluoxetine, sertraline, and fluvoxamine suggest that these medications are of benefit to patients with impulsivity and aggression in the context of BPD. More studies are needed to assess further which behaviours are associated with responsivity to an SSRI, and to determine the appropriate dosage, and longitudinal efficacy of those agents. The tricyclic antidepressants (TCAs) have been extensively studied in BPD for their effects on depression in BPD patients. Although clearly effective for depressive symptoms, TCAs have not been shown to be particularly helpful in decreasing aggression and impulsivity in BPD (Soloff *et al.*, 1986). Some BPD patients actually experienced increased anger, hostility, and aggression while taking imipramine (Klein, 1968) and amitriptyline (Soloff *et al.*, 1986). There are case reports of using desipramine and clomipramine effectively to treat violent outbursts in some patients, and of using amitriptyline, trazodone, and fluoxetine for aggression associated with brain injury and anoxic encephalopathy. The potential for worsening impulsive, aggressive symptoms and the danger of overdose in patients who have impaired self-control may limit the use of TCAs. The same limits apply to MAOIs, which also have not been shown to decrease the behavioural dyscontrol or impulsivity seen in BPD.

Medications that are not serotonergically mediated, such as carbamazepine and other anticonvulsant mood stabilizers, have also proven to be useful (Haller *et al.*, 1994). Although evidence suggests that impulsivity and aggression are serotonergically mediated, a 5-HT hypothesis of impulsivity is not a definitive model. The complete role of 5-HT activity and its complex interactions with other neurotransmitters and receptors in impulsivity and aggression have not yet been fully delineated. The antagonism of 5-HT2 receptors appears to decrease aggression and may explain the efficacy of newer antipsychotic agents in the reduction of aggression and agitation independently of effects on psychotic symptoms.

The neurotransmitter effects of lithium are complex and include an effect on second-messenger systems related to the 5-HT system. A recent single-blind, randomized study (Pallanti *et al.*, in press) suggests the efficacy of both lithium carbonate and valproate in the treatment of PG. Further studies on psychopharmacological treatments of pathological gambling should be performed, as preliminary studies involving medication in the treatment of this disorder have shown promising results. Researchers and clinicians have used lithium, carbamazepine, valproic acid, and, more recently, gabapentin, lamotrigine, and topiramate to treat the impulsivity, aggression, and mood instability seen in bipolar patients, and they subsequently reasoned that it might stabilize these same symptoms in BPD. In a double-blind placebo-controlled trial (Cowdry and Gardner, 1988), carbamazepine decreased impulsivity in a group of BPD patients.

Our group (Stein *et al.*, 1995b) found that valproate led to significant overall improvement in 50% of a small sample of BPD patients who completed an 8-week, open-label trial, and that valproate may be more effective than placebo was shown in a 10-week, double-blind study (Hollander *et al.*, 2001). The medication was helpful for impulsivity, anger, and irritability, as well as for mood instability and anxiety. The potential efficacy of valproate in the treatment of BPD raises the question of the neurobiological underpinnings of the core features of BPD, namely, impulsivity and aggression. A number of points are relevant. First, a link between impulsive aggression and limbic abnormality has long been postulated. Although only a small percentage of BPD patients have seizure activity, more subtle neuropsychiatric abnormalities have been found in this population, including increased neurological soft signs. The hypothesis that valproate alters limbic dysfunction by interrupting neuronal kindling is therefore of interest. Second, there is increasing evidence that 5-HT hypofunction may play a role in the mediation of BPD symptoms. Although valproate has multiple effects on neurotransmission, it is notable that it increases 5-HIAA levels. Further studies and larger sample sizes for the use of valproate in the treatment of BPD are warranted.

Neuroleptics are among the most studied medications for the treatment of BPD, and they have been effective in treating violence associated with psychosis. Although they are the most commonly used medications for violence and aggression related to psychosis, neuroleptics are often misused as chronic sedatives. In BPD patients, moreover, neuroleptics were not well tolerated and were statistically no better than placebo in the reduction of hostility, anger, and aggression (Goldberg *et al.*, 1986; Soloff *et al.*, 1986). In one 8-week, open-label, pilot study, BPD patients treated with olanzapine had lower Barratt Impulsivity Scale and Buss-Durkee Hostility Inventory scores then those treated with placebo (Schulz *et al.*, 1999). Neuroleptics may result in a number of adverse side effects. They may cause tolerance to sedation and lead to increased doses and thereby increased side effects such as akathisia, extrapyramidal side effects, and anticholinergic toxicity. These specific side effects can worsen aggression in predisposed patients, particularly those with organic brain injury.

Kim has employed the opioid antagonists in the treatment of impulse-control disorders, prescribing naltrexone for up to 9 months to 15 patients who had impulse-control disorder: naltrexone was generally well tolerated, and there were no hepatic side effects. Naltrexone appears to reduce urge-related symptoms and decrease

the problematic behaviours such as PG; the effects appear to be sustained. In general, 50 mg per day of naltrexone was not effective, and most patients required higher doses. These data suggest that naltrexone may be of use in selected impulse-control disorder patients (Kim, 1998).

Moreover, it seems that the disinhibitory behaviour of 5-HT-lesioned rats can be reversed by the commonly used opiate receptor-antagonist naloxone at doses that do not significantly affect behaviour in sham-lesioned controls. Moreover, this effect of naloxone is reversed by a low inert dose of amobarbital. Thus, naloxone may represent a new pharmacological principle for the treatment of impulse-control disorders (Soderpalm and Svensson, 1999).

SUMMARY

Impulsivity is a core symptom of a broad spectrum of psychiatric disorders including impulse-control disorders (pathological gambling, intermittent explosive disorder, pyromania, kleptomania, trichotillomania, and impulse-control disorder NOS), impulsive aggressive personality disorders (borderline, antisocial), neurological disorders that can be associated with disinhibition of behaviour (such as epilepsy), and substance abuse. Moreover, impulsive disorders entail large costs to society, and are associated with substantial morbidity, mortality, social/family/job dysfunction, accidents, suicide, violence, aggression, criminality, and excessive utilization of health-care, government and financial resources.

While the concept of impulsivity has been widely studied and reviewed in clinical psychiatry, our understanding of the neurobiology of impulsivity is still at a very early stage. No simple extrapolation of animal subtypes to humans is possible, mainly because of the influence of complex cultural variables on behaviour. On the whole, research into subtypes of human impulsivity has been rather limited. Much of this has been conducted in children, by clinical observation, experimental paradigms in the laboratory, and cluster/factor analytical statistics.

Animal models have recently helped to clarify both the neuroanatomical and biological basis of specific impulse behaviours. Brain regions such as the nucleus accumbens core, the basolateral amygdala, the hypothalamus, and the prefontal cortex may play an important role in impulsivity, as well as genetic and environmental factors. Serotonin dysregulation and hypofunction seem also to play a central role in impulse-control disorders, with the influence of the highly interconnected noradrenergic, dopaminergic, opioid, and GABAergic systems. Pharmacological therapeutic approaches have focused for this reason on serotonin transmission (serotonin reuptake inhibitors), but various classes of medication acting on different neurotransmitter systems, mood stabilizers, and opioid antagonists have also had encouraging results.

Further and more sophisticated research is needed to elucidate the localization of impulsive choice in animal and human models, and to understand this core behavioural symptom domain that cuts across various disorders and plays an important role not only in clinical psychiatry but also in everyday life.

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XXVI

Personality Disorders

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XXVI-1

Animal Models of Personality

A.R. Cools and B.A. Ellenbroek

PERSONALITY AND ITS DISORDERS

In practice, we need to be able to assess personality in order to predict how patients will respond to illness and to treatment. We also need to be able to manage people with personalities that cause serious problems to themselves or other people. As elaborated in the next paragraph on 'basic dimensions of personality', the term 'personality' refers to the balance between various traits. If one or more dominant traits are strong enough to cause difficulties to the person or to other people, the person is said to have a personality disorder. However, the borderline between normal personality and personality disorders is hard to define. In fact, there is no reliable and valid measure that allows a cut-off for distinguishing between normality and disorder. In clinical practice, therefore, a personality is said to be disordered when it causes suffering to the person or to other people. It is evident that such a criterion is impossible to model in animals. Indeed, animal models of personality disorders are not available, unless one adheres to the traditional classification system for personality disorders: in that case, we are dealing with animal models for anxiety disorders, mood disorders, etc., as discussed in several other chapters of this textbook.

Recently, however, Cloninger *et al.* (1994) have introduced a new classification system that allows differential diagnosis of personality disorders with the help of temperament and character descriptors. As outlined below, it is possible to model a number of these temperament descriptors in animals. In this chapter, we will focus on these and related studies.

BASIC DIMENSIONS OF PERSONALITY

In the first place we should clarify what we want to model. Thus, we should delineate the basic units of personality. As elaborated by Zuckerman (1991), the basic unit in personality psychology is the trait, defined as a group of related habits that, in turn, are defined as consistent behaviours in specific situations. Using this definition, Zuckerman suggests that the term 'basic dimensions of personality' should refer to

> supertraits that have internal reliability and can be identified in terms of their constituent traits across methods, genders, and cultures; these supertraits should be identifiable in childhood but not necessarily in infancy; the basic personality traits should show consistency over time (but because the phenotype and environment interact throughout life, one would not expect perfect or even near perfect consistency over long periods of time); they should be identifiable in species other than the human on the assumption that they have been shaped by natural selection; they should show at least moderate heritability and be related to significant biological markers; and, eventually, their biological basis in the structure and physiology of the nervous system should be identifiable, though classification is a necessary first step in biological bases of traits (pp. 40–41).

Unfortunately, there is no general agreement about the kind of classification system to be used for classifying fundamental traits. In psychiatry, there are at least two types of classification systems: the traditional system that distinguishes 5–7 dimensions resulting from factor analyses of the phenotypic structure of personality, and a more recently introduced functional classification system that is based on largely hypothetical characteristics of central nervous system functioning. The traditional classification system has resulted, among others, in the three-factor model of Eysenck (1947), encompassing the factors introversion-extraversion (E), neuroticism (N) (or emotional instability), and psychoticism (P); the five-factor model of Fiske (1949) and, later, Norman (1963), encompassing the factors extraversion, agreeableness, consciousness, emotional stability, and culture; and the seven personality dimensions of Zuckerman, which are activity, sociability, impulsivity, socialization, sensation seeking, emotionality (subdivided into general, anxiety, and hostility), and social desirability (Zuckerman *et al.*, 1988). As elaborated elsewhere, this traditional classification system suffers from several shortcomings (Cloninger *et al.*, 1994; van Praag, 1986; Tuinier and Verhoeven, 1995), the most important one being the fact that the entities classified are still complex behaviours that are not yet sufficiently broken down into specific and elementary items that are mutually exclusive and, in addition, theoretically tractable in the brain. Anyhow, animal models for some of these dimensions, especially anxiety in particular and emotionality in general, are discussed elsewhere in this book.

More recently, Cloninger has developed a neurobiologically based model to guide the rational development of descriptors for temperament and character (Cloninger, 1994). Temperament refers to those components of personality that are heritable, developmentally stable, emotion-based, or uninfluenced by sociocultural learning (Goldsmith *et al.*, 1987), whereas character refers to those components of personality that are weakly heritable, and are moderately influenced by sociocultural learning (Loehlin, 1982). His model is based on the synthesis of information from twin and family studies, studies on longitudinal development, and neuropharmacological and neurobehavioural studies of learning in human and other animals, as well as psychometric studies of personality in individuals and twin pairs. Though the neurobiological processes are not yet identified, he proposes four temperament dimensions on the basis of individual differences in associative learning that are postulated to be genetically homogeneous and independent of one another: 'novelty seeking', which is associated with the behavioural activation system; 'harm avoidance', which is associated with the behavioural inhibition system; 'reward dependence', which is associated with the behavioural dependence system; and 'persistence', which is associated with the behavioural persistence system. The dimension 'novelty seeking' nicely corresponds with Zuckerman's dimension 'sensation seeking' (McCourt *et al.*, 1983). Cloninger complements his model of personality with three character dimensions, self-directed behaviour, cooperativeness, and self-transcendence.

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Because this definition of character refers to self-concepts and individual differences in goals and values, which influence voluntary choices, intentions, and the meaning of what is experienced in life, it is evident that these character dimensions are difficult to model in animals. For that reason, they are not considered in this chapter.

The authors of this chapter regard Cloninger's classification system as a very powerful model that provides four temperament dimensions that fulfil all criteria summarized by Zuckerman (1991): among others, they are moderately heritable, stable from childhood through adulthood, and structurally consistent in different cultures and ethnic groups (Cloninger *et al.*, 1994). Furthermore, it is interesting to note that Cloninger's four temperaments to some extent correspond to the four basic emotions of anger (*novelty seeking*), fear (*harm avoidance*), love (*reward dependence*), and tenacity (*persistence*), as well as with a modern interpretation of the ancient temperaments the choleric (*novelty seeking*), the melancholic (*harm avoidance*), the sanguine (*reward dependence*), and the phlegmatic (*persistence*). The model of Cloninger *et al.* (1994) is especially powerful, because it has given rise to the development of a validated guide that allows a clear-cut definition of personality disorders with the help of the so-called 'temperament and character inventory' (TCI) scales. In other words, each individual personality disorder is defined as a configural function of all seven temperament and character dimensions. The correlations between TCI scales and various symptoms for individual personality disorders according to DSM-IV classification are illustrated in Table XXVI-1.1 (Svrakic *et al.*, 1993). This table also shows that individuals can fall into disorders in multiple clusters because the temperament dimensions are independent, not mutually exclusive. To what extent Cloninger's dimensions of temperament are identifiable in species other than the human is discussed below. We will also discuss whether the biological basis of these dimensions can be identified in the structure and physiology of the nervous system.

CRITERIA FOR ANIMAL MODELS

Modelling aspects of human personality in animals should result in animal models that meet criteria that allow validation of the models. Though such generally accepted criteria for animal models of normal human behaviour are missing, such criteria are formulated for animal models of abnormal human behaviour. In the latter field, it is quite common to subdivide animal models by specifying the presumed validity of the models. This has led to three different sets of models, which are hierarchically related (Willner, 1984):

1. animal models with predictive validity of the lowest class
2. animal models with face validity of the second class
3. animal models with construct validity of the highest class.

By modifying the set of criteria for each of these models, we can try to formulate criteria for animal models of normal human behaviour. By analogy with the predictive validity of the animal models for human disorders (that is, predicated on the prediction they make with respect to pharmacotherapy), the predictive validity of animal models for normal human behaviour should be based on the prediction they make with respect to treatments and/or environmental conditions that are known to alter specifically the behaviour in question. Though early environmental factors, brain injury, delay in brain development, childhood experience, and the use of alcohol or drugs are assumed to contribute to the occurrence of abnormal behaviour in people with personality disorders, there is no convincing evidence that these factors alter a particular behaviour in a specific and selective manner. Indeed, animal models with predictive validity for normal human behaviour are not yet available. By analogy with the face validity of animal models for human disorders (which are predicated on symptom similarity), the face validity of normal human behaviour should be based on convincing similarity of the behavioural phenomenon between the model and the target behaviour. As mentioned below, there are animal models that fit into this category. Finally, by analogy with the construct validity of animal models for human disorders, the construct validity of animal models for normal human behaviour should be based on convincing similarity of causes and (neuro)biology of the behavioural phenomenon between the model and the target behaviour. In our opinion, the animal models of *novelty seeking* that are discussed below, appear to fit into this category.

Table XXVI-1.1 Correlations between Cloninger's Temperament and Character Inventory (TCI) Scales and number of symptoms for individual personality disorders (Modified from Svrakic *et al.*, 1993)

TCI scale scores Personality disorder symptoms	NS	HA	RD	P	SD	C	ST
Antisocial (adult)	H	—	L	—	L	L	—
Histrionic	H	—	—	—	L	L	—
Borderline	H	H	—	—	L	L	—
Passive-aggressive	H	—	—	L	L	L	—
Avoidant	L	H	—	—	L	—	—
Obsessive-compulsive	—	H	—	—	L	L	—
Dependent	—	H	H	—	L	—	—
Schizoid	—	—	L	—	L	L	L
Schizotypal	—	H	L	—	L	L	—
Paranoid	H	—	L	—	L	L	—

H = high scores.
L = low scores.
P = persistence.
NS = novelty seeking.
SD = self-directedness.
HA = harm avoidance.
C = cooperativeness.
RD = reward dependence.
ST = self-transcendence.

INDIRECT APPROACHES IN ANIMAL STUDIES

In the traditional biological approach to personality, the independent variables are behavioural and the dependent variables biological. Because there are also other approaches in this field that can shed light on selected aspects of personality, it is worthwhile to consider these approaches as well.

Gene-Oriented Approach

Following human studies on the association of various gene alleles with a particular trait or temperament dimension, animal models are developed by assessing behavioural or molecular genetic strategies to trace the genes and their involvement in the trait or dimension under study. The following can illustrate this approach. Studies on the association of dopamine-receptor genes and human behaviour including personality traits have provided evidence that the *Taq* I A D2 dopamine-receptor (DRD2) minor (A1) allele and the 7-repeat (7R) allele of the D4 dopamine-receptor (DRD4) gene D2 polymorphisms individually associate with *novelty-seeking* behaviour (Benjamin *et al.*, 1996; Noble *et al.*, 1998; Noble, 2000; cf. Herbst *et al.*, 2000). Predicated on these findings, two types of animal models are of interest. By the quantitative trait loci (QLT) technique in recombinant inbred mouse strains, it has become possible to show that the QTLs for alcohol preference drinking are localized in the region of the DRD2 (Tarantino *et al.*, 1998). The next question, of course, is whether or not this gene is also associated with the animal counterpart of human novelty

seeking. Another animal model in this respect involves mice that lack functional dopamine D2 receptors: such mice are marked by a severe reduction of alcohol- and opiate-related behaviours (Maldonado *et al.*, 1997). In this case, it is also important to know whether these mice are marked by a reduced amount of the animal counterpart of human novelty seeking. These models can be very helpful in laying the foundation for new hypotheses about the involvement of particular genes in personality dimensions, but at the same time they are less suitable for elucidating the genetic complexity of personality dimensions, because the independent variables are biological rather than behavioural.

Substrate-Oriented Approach

Following human studies in which biological correlates and/or substrates of a particular behaviour, trait, or temperament dimension have been mapped, animal models are developed by manipulating the biological correlate or substrate in question, in the hope that the resulting models can help to elucidate the biological mechanisms of the phenomenon under study, and even to discover new treatments for abnormal behaviours that are inherent in a disorder of that trait or temperament dimension. For example, human studies have shown that there is relationship between sensation seeking (*novelty seeking*), violent suicide, homicide, arson, psychopathology or substance abuse with decreased cerebrospinal fluid levels of the main serotonin metabolite 5-hydroxyindoleacetic acid (Lewis, 1991; Virkkunen *et al.*, 1994). Given these findings, together with the notion that serotonin (5-HT) deficiency may be associated with impulsive, disinhibited, and aggressive behaviours (cf. Soubrie, 1986), two kinds of animal models have been developed. One model involves extensive depletion of brain 5-HT by means of 5-HT synthesis inhibition or selective neurotoxic lesioning of 5-HT neurons (Söderpalm and Svensson, 1999). Given the therapeutic efficacy of the opiate receptor antagonist naloxone in this model, it is suggested that this drug may represent a new pharmacological principle for the treatment of impulse-control disorders (Söderpalm and Svensson, 1999). Another animal model involves the selective breeding of rat lines that show a differential physiological response to stimulation of the 5-HT1A receptors, namely, the high and low sensitivity responding lines (HDL and LDS, respectively), since these receptors have been implicated in a variety of disorders, including anxiety and alcoholism. This approach has generated data suggesting that the HDL line is a useful model for a type of high trait anxiety linked to susceptibility to depression (Knapp *et al.*, 1998). Though such models can certainly provide new insights into the relevance of certain biological mechanisms involved in the traits or temperament dimensions under discussion, they are primarily of heuristic value because the independent variables are biological, but not behavioural. Nevertheless, these models can be very helpful in laying the foundation for new hypotheses about the biological mechanisms that underlie the particular personality dimension under discussion.

Physiology-Oriented Approach

Following the finding in human studies (Zuckerman *et al.*, 1974) that high sensation seekers (*high novelty seekers*) show increasing amplitudes of visual evoked potentials (VEP) as a function of flash intensity (VEP augmenters), in contrast to low sensation seekers (*low novelty seekers*), who show decreasing VEP amplitudes as a function of flash intensity (VEP reducers), Siegel and co-workers have looked at animals with a bimodal distribution of certain behavioural features. Initially, VEP responses were compared in cats that vary in terms of their behavioural response to novel and threatening stimuli: active and explorative cats showing approach behaviour even to threatening stimuli turned out to be VEP augmenters, whereas less active cats showing withdrawal responses turned out to be VEP reducers (Saxton *et al.*, 1987a). An additional interesting finding was that VEP augmenter cats learn a novel and simple bar press task much faster than VEP reducer cats (Saxton *et al.*, 1987b). In this context, it is interesting to note that high responders to novelty, considered as the rodent counterpart of novelty seekers (see below), also learn a simple radial-maze task much faster than low responders to novelty (Cools *et al.*, 1993c). Siegel later reported that the Swiss line of the Roman high avoidance (RHA) rats are VEP augmenters, in contrast to the Swiss line of the Roman low avoidance (RLA) rats, who are VEP reducers (Siegel *et al.*, 1993). Again, these animal models are primarily of heuristic value, because in these models the independent variables are biological rather than behavioural. Still, these models can help us to formulate new hypotheses about the biological mechanisms that underlie the particular personality dimension under discussion.

Behaviour-Oriented Approach

Given the enormous individual variation in human behaviour, traits, or temperaments, animal research has focused attention on individual differences in behaviour in its broadest sense. In practice, three research strategies are assessed:

1. comparing features of individuals belonging to different inbred strains of animals, usually rodents (e.g., RHA vs. RLA rats [Driscoll *et al.*, 1998], Lewis vs. Fischer rats [Sternberg *et al.*, 1989] or C57BL/6 vs. DBA/2 mice [Cabib and Puglisi-Allegra, 1988]);
2. comparing features of intrastrain individuals that are selected on the basis of a particular behaviour or trait (e.g., winner vs. loser rats [Masur *et al.*, 1995]);
3. comparing features of intrastrain individuals that differ in terms of behaviour because of known individual-specific features of the physiological state of the brain (Cools *et al.*, 1990).

The first approach has the disadvantage that one is dealing with different inbred strains, each of them marked by a genotypic uniformity that is not only greater than it is in the initial outbred population, but also accompanied by a loss of the originally present variation that is necessary for survival in changing environments. This does not match the human situation. The second approach is far better in this respect, but has the disadvantage that it remains unknown whether the variable used for the selection is a primary feature or just an intervening variable. The final approach lacks these shortcomings, since in this strategy the behavioural variable that is used for the selection is not an intervening variable but, instead, a basic feature, the consequence of a well-delineated and known physiological state of the brain. As discussed in the next paragraph, the latter approach has resulted in an animal that seems to be a highly interesting animal counterpart of the human novelty seeker. In general, the behaviour-oriented approach has provided a large amount of data that are primarily descriptive and correlative by nature. In principle, individual animals, related lines, or related strains are selected on the basis of a particular behavioural variant. The extent to which the behavioural variant is associated with other behavioural features that are considered to be features of a particular trait or temperament dimension is then investigated. Apart from this, one commonly investigates to what extent the behavioural variant is associated with physiological, neurochemical, and neuroendocrine and pharmacological variables. Using this approach, Gentsch *et al.* (1988) have shown that three distinct kind of pairings of rats resulted in a remarkably similar dichotomy across these pairings: RHA, spontaneously hypertensive (SHR) rats and individually housed Wistar rats are marked by lower emotionality (shown in factors such as decreased defecation and corticosterone secretion) than their respective counterparts, RLA,

Wistar-Kyoto rats and group-housed rats. Of course, the question arises why rats selected for extremes in the shuttle box performance (RHA/RLA), blood pressure (SHR/Wistar Kyoto rat), or variation in housing conditions show similar intrapair differences. Though these findings may lead to the hypothesis that these rats are all characterized by a similar idiosyncrasy in relation to emotionality, the similarities in the differences between the various pairings have to be considered as merely coincidences if there is no evidence that the distinct variables used for the selection are part of a cross-situational consistency in brain and behavioural physiology. Thus, the animal models that belong to this category can provide very useful information, although these models should not be considered as animal models for aspects of personality.

Construct-Oriented Approach

Though this approach does not deal with animal models as such, it assesses animal studies to provide insight into neurobiological processes that underlie various aspects of personality. In general, basic constructs about the functioning of the brain and body that are based on theoretical and empirical knowledge are used to relate temperament dimensions to processes and circuitries in the body and brain. Once such a relationship is hypothesized, animal experiments can be performed to (in)validate such hypotheses. An example that clearly illustrates this approach is provided by studies recently reviewed by Davidson (1999). In short, on the basis of a large number of human studies, Davidson developed a model that features individual differences in prefrontal asymmetry as a reflection of a diathesis that modulates reactivity to emotionally significant events. It was then hypothesized that if individual differences in prefrontal asymmetry are associated with a dispositional affective type, such differences should be related to cortisol levels, since individual differences in baseline cortisol levels have been related to various aspects of trait-related stressful behaviour (Davidson, 1999). They indeed found that individual differences in cortisol levels in nonhuman primates predict biological measures that are related to affective style (Kalin *et al.*, 1998). Though our present-day knowledge about the functioning of the brain is limited and, consequently, the available hypotheses in this respect are by definition speculative, this approach is very promising, because it helps us to develop new constructs about the functional anatomy of fundamental dimensions of personality.

DIRECT APPROACH IN ANIMAL STUDIES

As already mentioned above, in the traditional approach to personality the independent variables are behavioural and the dependent variables biological: we label this strategy the 'trait-oriented approach'. A description of the behavioural features of a particular trait or temperament is used to model these features in animals. The demonstration of a convincing similarity in the behavioural phenomenon between the model and the target behaviour provides an animal model with face validity and perhaps even with construct validity.

As clarified above, the authors of this chapter regard the classification system of Cloninger as the most powerful system for two reasons. First, the resulting four temperaments meet all criteria that are set for basic dimensions of personality. Second, the classification system provides the basis for differential diagnosis of personality disorders. Below we consider animal models for the four temperament dimensions delineated by Cloninger (1994).

Persistence

This dimension is hypothesized to be a heritable tendency to maintain behaviour in response to a) signals of anticipated reward, b) intermittent punishment, and c) intermittent frustrative non-reward. Today, animal models of *persistence* are not yet available. According to Cloninger, this dimension is mediated and modulated by the behavioural persistence system that encompasses the hippocampal subiculum, the anterior cingulate cortex and the orbitomedial cortex. Assessment of the above-mentioned construct-approach might help us to develop new constructs about the functional anatomy of this fundamental dimension of personality.

Reward Dependence

This dimension is hypothesized to be a heritable tendency to respond intensely to signals of reward and to maintain or resist extinction of behaviour that has previously been related to rewards or relief from punishment. It can be suggested that individuals of the monogamous vole species serve as animal model, because they show a greater social affiliation with their partner, a larger distress in response to isolation, and a larger sensitivity to social cues than individuals of the polygamous vole species (cf. Insel *et al.*, 1993). According to Cloninger (1994), this dimension is mediated and modulated by the behavioural dependence system that encompasses the lateral amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens, the midline thalamus, and the prelimbic cortex in which oxytocin plays a key role. Thus, a theoretical and empirical analysis of the function of these structures might help us to develop new constructs about the functional anatomy of this fundamental dimension of personality.

Harm Avoidance

This dimension is hypothesized to be a heritable tendency to respond intensely to signals of aversive stimuli, thereby learning to inhibit behaviour in order to avoid punishment, novelty, and frustrated non-reward. If one regards high frequency of defecation under bright lights in a novel environment and/or immobility with bradycardia in response to foot shock as indicators of *harm avoidance* in animals, one can suggest that individuals of the Maudsley reactive rat line (MR) (cf. Broadhurst, 1975) serve as animal models for a high degree of harm avoidance, because they are higher defecators in a novel environments (MR) than their counterparts, individuals of the Maudsley nonreactive rat line (MNR). In this context, it is interesting to note that the MR share this feature with individuals of the Syracuse low avoidance rat line (SLA) (cf. Brush *et al.*, 1988), the Roman low avoidance rat line (RLA) (cf. Driscoll and Bättig, 1982), the Tsukuba high emotional rat line (THE) (Fujita and Nakamura, 1976), and the Flinders sensitive rat line (FLS) (Russell *et al.*, 1982), although the latter rat lines were selected for a different biological variable, namely, performance in a two-way active avoidance task (SLA and RLA), entering a bright runway (THE) and sensitivity to the anticholinesterase dipropyl fluorophosphates (FLS). Although there is hardly any information about the neurochemical, neuroendocrine, physiological, behavioural, and pharmacological features of the MR, Driscoll and his colleagues have invested an enormous amount of research in analysing these features in the RLA (Driscoll *et al.*, 1998). Ongoing research with the RLA and their counterparts, the RHA, investigates to what extent the biological features in question should be conceptualized as substrates of the various traits of these animals rather than just biological correlates. The finding that neonatal handling produces changes in certain behavioural and endocrine responses of the RLA that are line-specific (Escorihuela *et al.*, 1995) strongly suggests that at least these responses reflect basic aspects of the underlying psychobiology of the feature in question. Still, it is important to note that none of these rat lines are selected for *harm avoidance*: they are selected for a feature of which breeding has resulted in a correlated difference in behaviour that is related to *harm avoidance*. Thus, it remains to be proven

that the feature used for the selective breeding is consistently and causally coupled to *harm avoidance* or to related aspects of this dimension. If so, the available knowledge about the neurochemical, neuroendocrine, physiological, behavioural, and pharmacological differences between the RLA and their counterparts, the RHA, provide unique information for constructing a functional anatomy of the temperament dimension, *harm avoidance*.

Novelty Seeking

This dimension is hypothesized to be a heritable tendency to intense excitement in response to novel stimuli or cues for potential reward, and it is quite likely to correspond with Zuckerman's *sensation seeking* (McCourt *et al.*, 1983). At least two important animal models are available: a) mice that are selectively bred on the basis of their behavioural response to novelty (Gerschenfeld and Paul, 1998; Hall, 1938; Simmel and Bagwell, 1983; Walsh and Cummins, 1976), and b) Wistar rats that are selected on the basis of their behavioural response to novelty (Cools *et al.*, 1990; Dellu *et al.*, 1996; Piazza *et al.*, 1990). Whereas the mouse model can help us to trace the contribution of the heritable component, the rat model can help us to study the biological processes that underlie this trait. Because the latter model is a very basic one that has provided a wealth of biological data, it will be discussed below under a separate heading. Here we discuss the mouse model, a relatively simple behavioural assay, in which the mouse is placed in a brightly lit, novel environment, and its behaviour serves as indicator. The mouse model is generally seen as an animal model for temperament or emotionality. Zuckerman has rejected the possibility that the mouse behaviour actually models *sensation seeking*, because of the negative correlation usually found between activity and defecation at both the phenotypic and genotypic levels. The reader is referred to Zuckerman for a detailed discussion about the pros and cons of open-field activity in mice as a model for *sensation seeking* (Zuckerman, 1984; cf. Gershenfeld and Paul, 1998). Irrespective of the precise nature of the temperament dimension that is modelled by open-field activity in mice, there is little doubt that it does reflect a particular aspect of personality. Although the interpretation of mouse behaviour under these conditions is difficult and ambiguous, it has been found to represent a well-defined, innate behavioural response, and to have broad generalization across species from chickens to infants. In addition, it has been found to differ between individuals and strains, due to heritable components. With this mouse model, it has become possible to partition the behavioural variance into genetic, environmental, and interaction components, and the heritable component has been further demonstrated by selectively breeding mice that differ in their open-field behaviour. By the above-mentioned QTL technique, it has even become possible to map the QTLs for initial ambulation in the open field: these are localized on chromosomes 1 and 15 (Flint *et al.*, 1995; Gershenfeld and Paul, 1998). This approach should help us to narrow down the human chromosomal regions in order to define human candidate genes and to define polymorphisms.

ANIMAL MODEL OF NOVELTY SEEKING

The best animal model for the temperament dimension *novelty seeking* is the so-called high responder to novelty (HR) (Cools, 2000; Dellu *et al.*, 1996), namely, rats that are selected for their intense behavioural response to novelty in an open-field test. According to Cloninger, the following traditional personality dimensions are marked, among others, by a high degree of novelty seeking: antisocial, histrionic, passive-aggressive, and explosive (Cloninger *et al.*, 1994). When in childhood such a high degree of *novelty seeking* is associated with low *reward dependence*, low *harm avoidance* and high *persistence*, these persons are likely to display antisocial behaviour, to become addicted to alcohol and other drugs of abuse, and to develop criminal behaviour as adults (Cloninger *et al.*, 1994). Because the HR are also marked by low *reward dependence*, low *harm avoidance*, and high *persistence*, we regard these animals as a very important source of information for understanding the psychobiology of humans with a predisposition to the latter behaviours. For that reason, particular attention is paid to this animal model.

The animal studies in question are performed with the HR and their counterparts, the low responders to novelty (LR). These two types of rat occur in any population of Wistar rats, and are characterized by individual-specific differences in the structure, function, and reactivity of the limbic-mesolimbic-striatal axis and the hypothalamic-pituitary-adrenal (HPA) axis. These differences underlie the fully distinct behavioural, physiological, and endocrine responses to stress shown by the HR and the LR, respectively. Genetic factors direct the way to cope with stressors during the early postnatal and, probably, prenatal period: the nature and intensity of the stressor to which the individual is exposed, during the perinatal period, appears to direct the structure and function of the brain and the body of these individuals. The available animal studies reveal that three factors direct the behaviour of this personality variant: a) genetic predisposition, b) the early postnatal and, probably, prenatal factors that direct the phenotypic expression of the genotype in question, and c) the degree of stress to which the individual is exposed immediately before, and during the onset of, the performance of the behaviour (Cools and Gingras, 1998). Knowledge about the structure, function, and reactivity of the brain and the body of the HR and the LR might help us to understand which factors can underlie the behavioural lapses that occur in certain individuals under particular conditions. Below we will provide a short summary of the outcome of animal studies on the HR and the LR.

Selection Procedure

The Nijmegen outbred population of Wistar rats has been found to contain at least two distinct types of individual, each of them marked by their own structure, function, and reactivity of the brain and body. There are three validated methods to select these types of rat.

First, we have the assessment of the open-field test (Cools *et al.*, 1990; Cools *et al.*, 1993c; Cools and Gingras, 1998; cf. Piazza *et al.*, 1989), which allows the separation of the HR and the LR. Both the dimensions of our open field — 160 × 160 cm — and the absence of external cues are important features of this open-field test. The HR and the LR behave differently in this novel environment: the HR are bound to the only available external stimulus, the edge of the open field, and they continue their exploratory behaviour for a very long period of time (>840 s). As in the intruder test (see below), the HR interrupt their ongoing behaviour only when a change in their environment occurs: this is considered to reflect a high degree of context-dependency. In contrast, the LR start to explore their novel environment and, after about 480 s, stop their exploratory activity in an otherwise undisturbed environment. Thus, as in the intruder test, the LR can interrupt their ongoing behaviour by themselves: this is considered to reflect a high degree of self-control.

Second, we have the assessment of the so-called intruder test in which 'freezing', defined as 'sitting motionless for over 45 s' and 'fleeing', defined as 'number of fleeing spells seen during the whole observation period of 6 min', serve as dependent variables (Cools, 1988; Cools *et al.*, 1990; Schuurman, 1981). This test allows the separation of rats that primarily flee (FLEE rats) and rats that primarily freeze (NON-FLEE rats). Since FLEE rats — being

the intruders — primarily flee during the direct confrontation with the resident (coping situation), but freeze as long as the intruders can only see and smell the resident without being able to attack him (non-coping situation), and because NON-FLEE rats freeze during the coping situation, but actively explore during the non-coping situation, the nature of the selected coping strategy (active or passive) varies according to the context and therefore is not a trait of the individual.

Third, we have the apomorphine test (Cools *et al.*, 1990), which allows the separation of apomorphine-susceptible rats (APO-SUS) and apomorphine-unsusceptible rats (APO-UNSUS): APO-SUS display more than 500 gnawing spells/45 min following an injection of 1.5 mg kg^{-1} apomorphine (s.c.), and APO-UNSUS display less than 10 gnawing spells/45 min following such an injection. Since 1985, the latter rats have also been bred, using a particular breeding schedule to prevent inbreeding and to maintain the original genotypic heterogeneity, apart from the alleles at the loci (or locus) involved in the determination of the chosen traits. Nevertheless, inbreeding, which reduces the genotypic heterogeneity and, ultimately, creates new substrains and strains, cannot be prevented in the long run; given our interest in the individual variability that occurs within a single strain in order to maintain a situation that approaches the human situation as closely as possible, it became necessary to restart the breeding of these lines, once every 5–8 years.

We have been able to show that the bimodal variation in apomorphine-susceptibility, the original selection criterion for the breeding, is consistently coupled to a bimodal variation in various neuroanatomical, neurochemical, endocrinological, immunological, and behavioural features. Evidence has been provided that rats marked by a high apomorphine-susceptibility (APO-SUS) are high responders to novelty in terms of both their behavioural response and their endocrinological responses, and that rats marked by a low apomorphine-susceptibility (APO-UNSUS) are low responders to novelty in terms of their behavioural and endocrinological responses (Cools *et al.*, 1990, 1993a–c; Rots, 1995; Rots *et al.*, 1996a–c). This individual consistency in behaviour and physiology has revealed that any of the biological or behavioural variables known to differ between these rats can be used as a criterion for selecting these two types of rat. For example, APO-SUS and APO-UNSUS rats can be selected from outbred strains of Wistar rats by establishing their response to novelty and selecting the HR and LR rats, respectively (Cools *et al.*, 1990; 1993). In sum, the male HR, FLEE rats and APO-SUS are marked by idiosyncratic features of one and the same type of individual; the same holds true for male LR, NON-FLEE rats and APO-UNSUS. In other words, there are two distinct types of individuals whose genetic make-up is reflected in the above three responses (Cools *et al.*, 1990).

The HR and LR are not tails of the population, but each group (HR and LR) represents a major part (40–45%) of our outbred strain of Nijmegen Wistar rats; the remaining 10–20% of rats form a heterogeneous group of rats, showing a mixture of HR and LR features, of which no details about the behavioural, neurochemical, and endocrinological features are known. As mentioned below, the ultimate neurochemical and behavioural phenotype of these two types of animal is determined, among others, by early postnatal factors. Owing to this factor and owing to distinct selection procedures, one has to be aware of the fact that the HR and LR that are studied in different research centres are not necessarily fully identical. Nevertheless, it is important to note that the HR/LR studied in other laboratories (Exner and Clark, 1993; Piazza *et al.*, 1989, 1991; Rouge-Pont *et al.*, 1993; Hooks *et al.*, 1991, 1991) share the following features with the Nijmegen HR/LR (Cools *et al.*, 1997; Saigusa *et al.*, 1999; Rots, 1995; Ranaldi *et al.*, 2001):

1. There is a positive correlation between the locomotor response to novelty and the acute behavioural response to dexamphetamine.
2. Environmental or pharmacological stressors produce a greater increase in the extracellular concentration of accumbal dopamine in the HR than in the LR.
3. The HR show a greater release of corticosteroids in response to stressors than the LR.
4. The HR acquire self-administration of psychostimulants such as cocaine and dexamphetamine much faster than LR.

In this chapter, we use the labels HR and LR for the Nijmegen HR (APO-SUS) and the Nijmegen LR (APO-UNSUS), respectively, whereas we refer to 'high responders to novelty' and 'low responders to novelty', respectively, when we refer to the studies of other groups.

CHARACTERISTIC FEATURES OF THE ANIMAL COUNTERPART OF THE *NOVELTY-SEEKING* PERSONALITY DIMENSION: GLOBAL SURVEY

Adult HR and LR animals are male rats that normally occur in every outbred strain of Wistar rats: these rats are neither mutants nor members of different substrains or strains. As mentioned above, these rats are marked by the genotypic heterogeneity that is originally present in every outbred strain of Wistar rats. However, characteristic features differ remarkably between these rats. The most characteristic ones are mentioned below.

1. *Novelty seeking*. The HR explore unfamiliar novel objects and novel environments: they enter the open arms of an elevated plus-maze to an extent that is only displayed by the LR when treated with benzodiazepines (Cools *et al.*, unpublished data). They readily self-administer cocaine (Ranaldi *et al.*, 2001). In sum, there are many features that are characteristic of the high novelty seekers.
2. *Reward dependence*. In studies on the intake of a variety of rewarding solutions, we found that HR consume far less sucrose than LR, possibly indicating that HR respond far less intensely to signals of reward than LR do. In other words, HR may have a low degree of *reward dependence* (Gingras, 1997).
3. *Persistence*. If one regards the repetition of aimless behaviour in response to novelty or psychostimulants as an indicator of *persistence* in animals, it is evident that HR have a high degree of *persistence*, because they show a far more stereotyped response to novelty and dexamphetamine than LR do (Gingras and Cools, 1997a).
4. *Harm avoidance*. If one regards a high frequency of defecation under bright lights in a novel environment and/or immobility with bradycardia in response to foot shock as indicators of *harm avoidance* in animals, HR have a relatively small degree of harm avoidance, because HR produce far fewer droppings in a novel open field than LR (Sluyter *et al.*, unpublished data). In addition, HR show also far less immobility in this open field than LR do (Cools *et al.*, 1990).
5. *Stress-sensitivity*. HR are more stress-sensitive in terms of behavioural (locomotor activity) and endocrine (release of ACTH and corticosteroids) responses than LR. In this context, stress refers to novelty-induced stress as well as to stress measured in the so-called conditioned emotional test (Rots, 1995; Rots *et al.*, 1995, 1996): the locomotor response to novelty, as well as the release of plasma release of ACTH and corticosteroids in response to stress, is far greater and longer-lasting in HR than LR.
6. *Rate of acquiring a new task*. HR that are not habituated to a four-arm radial-maze start to learn a simple cued four-arm radial-maze task during the three initial test days, whereas LR only later start to acquire that task: the overall rate of acquisition, however, does not differ between HR and LR (Cools *et al.*, 1993c, 1994). In fact, it seems that HR need stress

in order to acquire a new task, in contrast to LR, which need to be habituated to stress before learning. This holds especially for tasks known to involve the basolateral part of the amygdala (Tuinstra, 2000).

7. *Retrieval of recently stored information*. Once HR have learned to find a platform that is invisible and submersed in a so-called Morris water maze, with the help of cues around the swimming pool, they have severe difficulty in retrieving the stored information when returning to the pool 24 h after the last trial on the first test day; however, after 2 min, they perform very well. In contrast, LR have no problem in retrieving the stored information under these conditions: in fact, their performance is even better than that shown on the first day. In sum, it seems that stress inhibits HR in retrieving recently stored information, in contrast to LR, in which stress facilitates the retrieval of recently stored information. This holds especially for tasks known to involve the ventral subiculum of the hippocampus (Tuinstra *et al.*, 2000c).
8. *Context-dependency*. HR are very dependent on spatial and contextual stimuli, whereas LR are relatively independent of these stimuli (see sections on Selection procedure and on Early life events).
9. *Self-control*. HR have less self-control than LR do (see sections on Selection procedure and on Early life events).
10. *Predisposition to mental illness*. HR, but not LR, show patterns of behaviour in animal models with construct validity for certain cognitive deficits of schizophrenic patients (Ellenbroek *et al.*, 1995). HR show a reduced latent inhibition as well as a reduced pre-pulse inhibition, whereas LR show normal latent inhibition and pre-pulse inhibition. However, HR display less parkinsonian symptoms in animal models of Parkinson's disease than LR (van Oosten and Cools, unpublished data).
11. *Predisposition to somatoform diseases*. HR respond negatively in animal models for autoimmune diseases such as experimental allergic encephalomyelitis (EAE model), whereas LR respond positively in such models (Cools *et al.*, 1993b; Kavelaars *et al.*, 1997). However, HR show a vigorous, Th2-dependent IgE response after infection with the nematode *Trichinella spiralis*, whereas LR do not (Kavelaars *et al.*, 1997). In other words, HR are relatively insensitive to autoimmune diseases, but very sensitive to infections, whereas LR reveal the opposite pattern.
12. *Predisposition to 'therapeutic' and unwanted effects of drugs*. HR are more sensitive to anti-parkinsonian agents than LR, whereas LR are more sensitive to antipsychotics than HR (Cools *et al.*, 1990; 1993c): systemic administration of dopaminergic agonists such as apomorphine produce a greater and longer-lasting behavioural response in HR than in LR, whereas intra-accumbens administration of neuroleptics, such as sulpiride, produces a behavioural response in HR that is smaller than that seen in LR.
13. *Sensitivity to psychostimulant and/or reinforcing effects of orally or otherwise administered agents*. During absence of stress, HR are far less sensitive to psychostimulant and/or reinforcing effects of ethanol, dexamphetamine, sucrose, and quinine than LR (Cools and Gingras, 1998; Ellenbroek and Cools, 1993; Gingras and Cools, 1995, 1997). Under stress, however, the situation is fully reversed: HR are behaviourally far more sensitive to ethanol, dexamphetamine, and cocaine, and under stress, HR self-administer far more cocaine than LR (cf. Cools and Gingras, 1998).
14. *Structure of the brain*. There are numerous differences in number of receptors (e.g., mineralocorticoid and dopamine receptors: [Rots, 1995; Rots *et al.*, 1995, 1996a–c; Sutanto *et al.*, 1989]), concentrations of neurotransmitters (e.g., noradrenaline and dynorphin: [Cools *et al.*, 1993c; Rots, 1995]), number of synapses (nucleus paraventricularis hypothalami: [Mulders *et al.*, 1995a–b]), etc.
15. *Reactivity of the brain*. The reactivity of both the limbic-mesolimbic-striatal axis and the HPA axis is far greater in HR than in LR. These differences underlie, among others, the fact that during stress HR have a relatively active basolateral part of the amygdala and a relatively inactive ventral subiculum of the hippocampus, with the consequence that HR can easily acquire new information, but have difficulty with retrieving recently stored information. In contrast, LR have a relatively inactive basolateral part of the amygdala and a relatively active subiculum of the hippocampus, with the result that LR show the behavioural mirror image under these conditions (Roozendaal and Cools, 1994; Tuinstra, 2001; Tuinstra *et al.*, 2000b).
16. *Early postnatal development*. A rat of the LR genotype that is deprived of its mother for 24 h on the ninth postnatal day develops into an adult animal with characteristic features of the HR, whereas a rat of the HR genotype raised with an LR foster mother develops into an adult animal with characteristic features of the LR phenotype (Ellenbroek and Cools, 1996). These findings show that the biological variables in question have to be considered as biological substrates rather than biological correlates. These findings also indicate that each distinct genotype is sensitive to a type-specific set of stressors: what is fully harmless for one type appears to have large consequences for the other type. In sum, the nature of the stressors that are present during the early postnatal period have a far-reaching influence upon the phenotypic expression of the genotype.

CHARACTERISTIC FEATURES OF THE ANIMAL COUNTERPART OF THE *NOVELTY-SEEKING* PERSONALITY DIMENSION: BRAIN, BODY, AND BEHAVIOUR

Given the role of telencephalic dopamine and that of corticosteroids in the neurochemical and behavioural responses to novelty (stress) and drugs of abuse (Cools, 1988; Di Chiara and Imperato, 1988; Fahlke *et al.*, 1994, 1995; Goeders and Guerin, 1996; Marinelli *et al.*, 1994; Roberts and Koob, 1982; Shoaib *et al.*, 1995; Wise and Rompré, 1989; Yoshimoto *et al.*, 1991), the most salient type-specific differences in this respect are summarized.

As mentioned, HR are far more sensitive to the dopaminergic agonist apomorphine than LR (Cools *et al.*, 1990). Furthermore, unchallenged HR are more sensitive to noradrenergic agonists. In fact, unchallenged HR behave as if they were sensitized by dexamphetamine; Piazza *et al.* (1990) have also found that their 'high responders to novelty' show this phenomenon. Surprisingly, HR are less sensitive to dopaminergic antagonists such as sulpiride than LR (Cools *et al.*, 1993c). In fact, there are neurochemical features that can explain these differences in susceptibility to aminergic agents (Cools *et al.*, 1990, 1993a; Rots, 1995; Rots *et al.*, 1995, 1996a and c); for example, unchallenged HR have a smaller amount of mesolimbic noradrenaline in the nucleus accumbens than unchallenged LR (Cools *et al.*, 1990; Rots *et al.*, 1996c). As discussed elsewhere in detail (Cools *et al.*, 1987), this explains why unchallenged HR are more sensitive to the accumbal administration of the alpha-adrenergic agonist phenylephrine (Ellenbroek and Cools, 1993). Furthermore, unchallenged HR have more striatal dopamine D1 receptor mRNA and more tyrosine hydroxylase mRNA in the A9 (substantia nigra, pars compacta) and A12 (nucleus arcuatus) cell groups than unchallenged LR, implying that the capacity to enhance the formation of dopamine in response to stress is greater in HR than in LR. Indeed, when the rats are challenged by novelty or tested in the conditioned emotional response test, behavioural and physiological responses mediated by

dopamine are greater in HR than LR (Cools *et al.*, 1990; Rots, 1995; Rots *et al.*, 1995, 1996a). The following example nicely illustrates this dopamine hyperreactivity in HR: the dopaminergic, tuberoinfundibular system that arises in A12 normally inhibits prolactin, and this response is far more strongly inhibited in HR than in LR, when the rats are exposed to stress (Rots, 1995; Rots *et al.*, 1996a).

Given the difference in the behavioural response to stress, it could be predicted that this difference extends to the HPA axis. This is indeed the case. First, the amount of corticotrophin-releasing hormone (CRH) mRNA in the nucleus paraventricularis hypothalami (PVN) of HR is greater than that in the PVN of LR (Rots *et al.*, 1995), implying that these cells in HR have also a greater capacity to generate CRH. Because CRH, which is under the stimulatory control of dopamine (Plotsky *et al.*, 1989), stimulates the release of plasma ACTH, especially during exposure to stress, it is logical to expect a HR-LR difference in the ACTH plasma release in response to stressors such as novelty. This is indeed the case: the stress-induced release of ACTH as well as that of plasma corticosteroids, of which the release is stimulated by ACTH, are greater and longer-lasting in HR than in LR (Rots *et al.*, 1995), a finding that fits in with those reported by Piazza *et al.* (1991). Apart from these data, we found that plasma levels of ACTH in HR are greater than those in LR under baseline conditions, but that the plasma release of free corticosteroids in HR is lower than those in LR under these conditions (Rots *et al.*, 1995; 1996a).

NOVELTY AND THE NEUROCHEMICAL STATE OF THE NUCLEUS ACCUMBENS IN THE ANIMAL COUNTERPART OF THE *NOVELTY-SEEKING* PERSONALITY DIMENSION

As elaborated elsewhere in detail (Cools *et al.*, 1991, 1993a, 1994; Cools and Gingras, 1998; Roozendaal and Cools, 1994), there is anatomical, electrophysiological, pharmacobehavioural, and neurochemical evidence that the neurochemical state of the nucleus accumbens of an unchallenged HR is marked by the following features when compared with LR:

1. The functional activity at the level of beta-adrenergic receptors that can stimulate the release of dopamine at the level of dopamine D2 receptors is relatively low.
2. The functional activity at the level of these dopamine D2 receptors, presynaptically localized on glutamatergic hippocampus-accumbens neurons, is relatively low.
3. The functional activity at the level of alpha-adrenergic receptors that can inhibit the release of dopamine at the level of so-called inhibitory dopamine receptors (DAi) — a subtype of dopamine receptors not yet linked to the two more recently discovered families of dopamine D1 and D2 receptors (Cools and van Rossum, 1980) — is relatively low.
4. The functional activity at the level of these DAi receptors — localized on glutamatergic amygdala-accumbens neurons — is relatively high.
5. The neurochemical state of the nucleus accumbens of an unchallenged HR greatly differs from that of an unchallenged LR: for all neurotransmitters, functional activity that is relatively low in HR is relatively high in LR, and vice versa.

Very recently biochemical evidence gained by the microdialysis technique has shown that the noradrenaline-dopamine interaction in the nucleus accumbens differs completely between HR and LR (Saigusa *et al.*, 1999; Tuinstra and Cools, 2000a and b). Finally, under challenge by a mild physiological, pharmacological, or environmental stressor, the neurochemical state of the nucleus accumbens and, probably, of other parts of the brain and body as well, is temporarily reversed (Roozendaal and Cools, 1994; Tuinstra and Cools, 2000a and b). Thus, the state of a challenged HR goes in the direction of that of an unchallenged LR, whereas that of a challenged LR goes in the direction of that of an unchallenged HR.

EARLY LIFE EVENTS AND THE DEVELOPMENT OF THE ADULT PHENOTYPE OF THE ANIMAL COUNTERPART OF THE *NOVELTY-SEEKING* PERSONALITY DIMENSION

Before discussing the consequences of early postnatal life events, it is important to consider characteristic changes that occur during the normal development of HR and LR (Rots *et al.*, 1996b). In 10-day-old rats, type-specific differences in dopaminergic variables (e.g., D1 receptor mRNA and TH mRNA) and in variables of the HPA axis (e.g., ACTH and corticosteroids) are not yet present. However, in 18-day-old rats, variables of the HPA axis already show some type-specific differences known to occur in 60-day-old, adult rats. For instance, the ACTH plasma level under baseline conditions is greater in HR than in LR, and a trend towards lower free corticosterone plasma levels is present in HR; in contrast, there are still no type-specific differences in the dopaminergic variables in 18-day-old rats (Rots, 1995; Rots *et al.*, 1996b). Thus, the divergence in the dopamine phenotype of HR and LR develops subsequent to distinct differences in the HPA axis.

To investigate to what extent early experiences direct the development of the adult phenotype, two paradigms were used (Ellenbroek *et al.*, 2000): cross-fostering on day 1 and maternal deprivation on day 9. These manipulations are chosen because they are known to function as mild stressors at that time. Remarkably, cross-fostering influences only the adult phenotype of HR, and not of LR (Ellenbroek and Cools, 1996), whereas maternal deprivation influences only the adult phenotype of LR (Ellenbroek *et al.*, 2000). In fact, cross-fostering reverses HR into LR, whereas maternal deprivation reverses LR into HR, as far as their apomorphine-susceptibility is concerned. In addition, we found that maternal deprivation affects various characteristics of the biochemical phenotype: adult rats that were deprived show a higher ACTH plasma level under baseline conditions and a greater amount of TH mRNA in A9 cells than their controls (Rots, 1995; Rots *et al.*, 1996b). Very recently, we found that this reversal also holds for the individual-specific sensitivity to diseases such as periodontitis (Sluyter *et al.*, unpublished). These data together suggest that this early life experience reverses the biochemical, immunological, and behavioural phenotype of LR into that of HR.

EPILOGUE

This review reveals that there are many animal studies that deal with animal analogues of various aspects of personality dimensions of the recently introduced functional classification system of personality. However, most of the approaches are indirect in the sense that the independent variables are biological, but not behavioural. Nevertheless, these animal models can be very helpful in laying foundations for new hypotheses about the biological mechanisms and substrates that underlie particular personality dimensions. However, there is only one mouse model and one rat model that use behavioural measures as independent variables, and biological ones as dependent variables. These two models deal with *novelty seeking*. The available mouse model has made it possible to partition the behavioural variance into genetic, environmental, and interaction components. However, the available rat

model has made it possible to provide basic insights into various biological processes and substrates that underlie the personality dimension in question. As summarized below, the rat model also helps us to understand to what extent genetic background, perinatal conditions, and the currently present environment determine the individual-specific set of personality dimensions of an individual.

The rat studies on *novelty seeking* show that the animal counterpart of a high novelty seeker, namely, the high responder to novelty (HR), and the animal counterpart of a low novelty seeker, namely, the low responder to novelty (LR), are characterized by individual-specific differences in structure, function, and reactivity of both the limbic-mesolimbic-striatal axis and the HPA axis. These differences underlie the individual-specific manner of coping with stress. The features of these individuals at adult age are determined by a) genetic predisposition, b) early postnatal and, probably, prenatal factors that direct the phenotypic expression of the genotype, and c) the degree of stress to which the individual is exposed immediately before, and during the onset of, the performance of the behaviour.

Available data show that the structure, function, and reactivity of the brain, the endocrine system, and the immune system differ completely between both types of individual. Analysis of behaviour as in the open-field test, the intruder test, the swimming task, the radial-maze task, etc., has clearly revealed that these two types of individuals organize their ongoing behaviour in a fully distinct manner: HR predominantly display extrinsically directed behaviour: they have a very well-developed capacity to use external cues for organizing their behaviour and are, in practice, dependent on these cues. In contrast, LR predominantly display intrinsically organized behaviour: they have a very well-developed capacity to control their own behaviour and are relatively independent of external cues. It is due to these features that HR perform optimally in tasks in which contextual stimuli have to be used for solving problems, but have severe problems with tasks in which internal cues have to be used for solving the problem. This also explains why LR display the mirror behaviour in these tasks. In other words, each of these types has its own optimal niche in which it can flourish, but also its own niche in which it will perish. This explains why this disruptive selection, in which more than just one phenotypic expression of a particular trait has surplus value, is present in such a stable balance within non-selected strains of Wistar rats. This makes it possible that dimorphisms, such as HR and LR, in rat populations and polymorphisms in humans exist.

One of the most remarkable findings is that both these types of individuals deteriorate as soon as they end up in each other's niche. This illustrates that certain stressors are harmful for one type, but not for the other. Nevertheless, one should not forget that a challenged HR behaves as an unchallenged LR, and vice versa. In short, the statement 'once a HR, always a HR' is incorrect. The key message of these findings is that genetic predisposition and early life events are decisive for the psychobiology of the personality dimensions, but that it is ultimately the current situation that gives rise to behavioural lapses.

Finally, HR are marked not only by a high degree of *novelty seeking*, but also by low degree of *harm avoidance*, a low degree of *reward dependence*, and a high degree of *persistence*. The coexistence of this particular configuration of scores on these dimensions is rather unexpected, because the human counterparts of these dimensions are genetically independent. To what extent these findings imply that one is dealing with a species-specific difference in this respect remains to be investigated. Anyhow, persons with a high degree of novelty seeking are marked, among others, by traditional personality dimensions such as the antisocial, histrionic, passive-aggressive, and explosive. When in childhood such a high degree of novelty seeking is associated with low *reward dependence*, low *harm avoidance*, and high *persistence*, these persons are likely to display antisocial behaviour, to become addicted to alcohol and other drugs of abuse, and to develop criminal behaviour at adult age. In view of these considerations, we believe that HR might help us to understand the biological processes and substrates that underlie the behaviour of persons marked by this particular (dys)balance of personality dimensions.

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XXVI-2

Neurotransmitter Systems in the Personality Disorders

Matt Eks, Harold W. Koenigsberg and Larry J. Siever

INTRODUCTION

According to DSM-IV (American Psychiatric Association, 1994), a personality disorder is 'an enduring pattern of inner experience and behaviour that deviates markedly from the expectations of the individual's culture'. This enduring pattern is 'inflexible and pervasive over a broad range of personal and social situations'. It 'leads to clinically significant distress or impairment in important areas of functioning'. The pattern is 'stable', 'of long duration' and 'its onset can be traced back to adolescence or early adulthood'.

Personality disorders represent a relatively new field of research within psychiatry, particularly with a neurobiological approach. People intuitively react and adapt differently to their surroundings. Hippocrates explained these differences in terms of the 'four humors', different combinations and balances of which led to different personality traits. Bumps on the head, shape of the skull, hair colour, and body stature are among other traits that have been studied in the search for biological correlates of personality. Biological factors, such as the ones mentioned above, and environmental factors, such as family environment, social class, or early life experiences, have been considered to be factors which contribute to 'create' our personalities. Psychoanalysts point to 'drives' and defence mechanisms to explain individual differences. Psychologists have defined psychometric dimensional factors in normal personality and proposed that their measures correlate with biological differences. Investigators are now focusing on finding these biological correlates in relation to different temperamental and character traits. This does not necessary imply that personality disorders are explainable by biology alone. A more likely perspective is that we inherit basic traits or endophenotypes that predispose to psychiatric disorders in interaction with the environment.

Clearly defined diagnostic criteria are an important prerequisite for studying the role of biology and environment in the personality disorders. To clearly demarcate them from the more symptom-oriented psychiatric disorders, personality disorders were placed on Axis II in the DSM axial system. Personality disorders are categorized into three clusters: A, B, and C. Cluster A represents a group of personality disorders that are marked by isolation and eccentricity. Disorders in Cluster B are characterized by behaviours of acting out in a dramatic, emotional, and erratic way. Diagnoses in Cluster C include individuals who are anxious and fearful. It is important to emphasize that the DSM criteria are clinically derived schema for categorizing personality disorders. While we seek external validators for the diagnostic system, our knowledge of the biological correlates, pathophysiology, genetics, or aetiological factors has not yet progressed to the point that we can obtain biological validation of the personality disorder diagnoses. The DSM has, however, made it possible to diagnose personality disorders in a more uniform way and in that way identify more homogeneous patient populations for research studies. The advances in neuroscience and its adaptation to study the pathophysiology of the Axis I disorders also have benefited these research efforts. With the help of neuroimaging and neuroendocrine measurements, the mapping of the brain and the activity of its neurotransmitters is possible.

What makes this area interesting and difficult is the fact that what we are trying to study is the essence of what makes all of us who we are. What are the underlying reasons for the extreme problems some people have in relation to themselves and/or their surroundings? These are fundamental questions regarding personality and particularly personality disorders. As our knowledge about the central nervous system is increasing rapidly, evidence of correlations between personality traits and brain function is also increasing. We can see resemblances between Axis I disorders and personality disorders on Axis II, both in personality traits and in brain function (or dysfunction). We can see the therapeutic effects of medication on dysfunctional personality traits, but we still do not have a comprehensive knowledge of the exact mechanisms underlying personality and personality disorders.

DIMENSIONAL APPROACHES TO PERSONALITY

Personality dysfunction may be conceptualized from either a categorical or a dimensional perspective. The categorical model defines a set of specific personality disorder diagnostic categories to which an individual belongs or does not belong. DSM-IV uses this approach with a polythetic system in which individuals may fit into a diagnostic category if they possess a subset of criteria characteristics from a larger set. Thus, individuals with differing clusters of symptoms may share the same diagnosis. In contrast to a categorical system, a dimensional approach identifies a set of features which vary in magnitude among individuals. Individual personality disorders are characterized by high levels of one or more of these dimensions. The temperamental traits that are critical in the different personality disorders show strong evidence of heritability (Torgersen, 1994). Patients rarely represent 'clear-cut' cases; they often demonstrate a mix of traits that do not fit under one single diagnosis. This makes it more fruitful to evaluate personality traits rather then specific disorders to identify such associations. However, the nature of the key traits or dimensions in personality and personality disorders and the specific neurotransmitters and brain systems that can be associated with these key dimensions have not been definitively established. While researchers do not fully agree on what dimensions are critical to the study of personality, there is a fair correspondence between different dimensional systems. Our knowledge of abnormalities in neurotransmitter function in psychiatric disorders such as depression, bipolar disorder, and anxiety has suggested avenues of approach in understanding the biology of the personality disorders (Ressler and Nemeroff, 2000).

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There are a number of dimensional approaches to personality disorder. Some of these are derived from studies of normal personality such as the systems of Eysenck (Eysenck, 1991) or the five factor model of personality (Costa and Widiger, 1994). Other approaches have identified key dimensions from analyses of personality disorders. Finally, other schema, such as the temperament and character (TCI) model of Cloninger (Cloninger *et al.*, 1993), are based on a theoretical construct of the psychobiology of personality disorder as well as a framework for describing the relationship of character to temperament. For the purposes of this review, we employ a broad, face-valid model of key domains of personality disorders that may be dimensional and that cut across the boundaries of specific disorders. For example, cognitive disorganization is the hallmark of Cluster A, the schizophrenia spectrum personality disorders, including schizotypal, schizoid, and paranoid personality disorder, and is manifest in traits of suspiciousness, social isolation, and paranoid thinking, and, in some cases, psychotic-like phenomena. More extreme forms of cognitive disorganization are found in schizophrenia, where reality testing is severely distorted. Cluster B is characterized by impulsivity, defined as a relative disinhibition of action-oriented, particularly aggressive responses to the environment, and affective instability, defined as intense affective shifts in response to environmental precipitants. Anxiety, defined as apprehension and anticipation of negative consequences of behaviour often associated with autonomic arousal, is most characteristic of the anxious cluster, Cluster C. These dimensions have been related to specific neurotransmitters in brain systems on the basis of research of Axis I disorders (Siever and Davis, 1991; Kirrane and Siever, 2000). These clinical domains are consistent with those derived from factor models of personality disorders. They may also be related to other models of personality disorders, such as the temperament and character model of Cloninger, in that low harm avoidance could be considered to be coincident with impulsive aggression, while high novelty seeking may map into impulsivity and high reward dependence into affective instability (see Chapter XXVI-6).

THE ECCENTRIC CLUSTER

The eccentric cluster (Cluster A) consists of paranoid, schizoid, and schizotypal personality disorders. Patients that are diagnosed with these disorders share odd and eccentric behaviour as well as unusual thinking. The temperament and character model characterizes this group as having a temperament of low reward dependence (Cloninger *et al.*, 1993; Svrakic *et al.*, 1993). Cognitive disorganization in these patients is a central problem because it interferes with effective interaction with others in social and occupational arenas. Research suggests that the dopaminergic system, an important neurotransmitter system, is involved in cognition. Cognitive function in schizotypal personality disorder has been studied because these patients have a well-demonstrated association with schizophrenia, in both their cognitive symptoms (Siever and Davis, 1991) and their genetic basis (Kendler *et al.*, 1981), yet they are free from the potential confounding effects of chronic neuroleptic treatment and downward social drift. The major metabolite of dopamine, homovanillic acid (HVA), measured in plasma and cerebrospinal fluid (CSF), has been shown to correlate with psychotic-like symptoms in both schizotypal and non-schizotypal personality disorder subjects (Siever *et al.*, 1991a; 1993). Studies using SPECT imaging of IBZM displacement by amphetamine-stimulated dopamine release and studies of plasma HVA responses to 2-deoxyglucose (2-DG) indicate that subcortical dopamine activity in schizotypal patients is comparable to or lower than that seen in normal subjects, and is markedly lower than that seen in schizophrenic patients (Siever *et al.*, 2002). Thus, schizotypal personality disorder patients may be spared from psychosis because of reduced subcortical dopaminergic activity. Among schizotypal patients, those with higher dopaminergic activity tend to have more psychotic-like symptoms.

THE DRAMATIC CLUSTER

The dramatic cluster (Cluster B) consists of antisocial, borderline, histrionic, and narcissistic personality disorders. Common to these disorders are dramatic, emotional, and erratic behaviours. Individuals with these disorders are impulsive and aggressive, as well as affectively unstable. The TCI describes their temperament as one of high novelty seeking and low harm avoidance (Cloninger *et al.*, 1993; Svrakic *et al.*, 1993). Associations have been found between the traits of impulsivity, aggression, and affective liability and the monoaminergic systems, but other systems, such as the cholinergic system, also seem to play a role (Gurvits *et al.*, 2000).

IMPULSIVITY AND AGGRESSION

Aggressive behaviour is common in the personality disorders (Berman *et al.*, 1998), in part because of the interaction between irritability and the interpersonal environment. Borderline personality disorder (BPD) is characterized by prominent impulsive/aggression.

Early evidence suggested that borderline personality disorder (BPD) runs in families (Loranger *et al.*, 1982). Later it was shown that it is not BPD, but the personality trait of impulsive aggression, common in BPD, that is heritable (Torgersen, 1994). Impulsive aggression has been shown to be heritable in the general population through identical twin studies (Coccaro *et al.*, 1993; 1994), adoptive studies of criminal offenders and antisocial personality-disordered subjects (Cloninger *et al.*, 1975; Crowe, 1974), and family studies. The risk of having affective and impulsive personality disorder traits is greater in relatives of BPD than in relatives of people with other personality disorders (Silverman *et al.*, 1991). In order to understand the underlying biology of impulsivity and aggression, a number of neurotransmitter systems have been studied.

Serotonin

Serotonin is involved in self-directed, restitutive behaviours, such as grooming, feeding, and sleeping. Animal studies show that these behaviours are associated with increases in the firing of serotonergic neurons, neurons that normally fire at a consistent, continuous rate (Jacobs and Fornal, 1995). Low levels of the serotonin metabolite 5-HIAA in the CSF have been associated with impulsive, violent crimes and suicide attempts, examples of externally and internally directed aggressive behaviour.

Violent offenders who committed impulsive crimes had a lower concentration of 5-HIAA in the CSF than violent offenders who committed premeditated crimes (Linnoila *et al.*, 1983). It is interesting that the lowest levels were found in the subjects who had attempted suicide. Later several other studies confirmed the hypothesis that a low level of serotonergic activity correlates with an increased risk of suicide or parasuicide. Asberg *et al.* (1987) showed that low serotonin metabolite levels increased the risk of later suicide attempts. This association was confirmed in a short-term prospective study (Nordstrom *et al.*, 1994) and appears to occur regardless of psychiatric diagnosis (Siever *et al.*, 1991b). Reduced 5-HT levels were also found in parasuicidal patients (Coccaro and Astill, 1990). A meta-analyses of 27 research reports (Lester, 1995) and later a review of 20 studies (Asberg, 1997) concluded that low levels of 5-HIAA in the CSF are strongly correlated to suicidal behaviour. Mann *et al.* (1996d) found that low 5-HIAA correlated

with a history of planned suicide and with suicide attempts that resulted in greater medical damage, suggesting a more violent method.

Externally directed aggression also correlates to reduced serotonergic metabolites, although not as consistently. Subjects with antisocial personality disorder responded with more aggression when using alcohol than subjects without antisocial personality disorder (Virkkunen and Linnoila, 1993; Moeller *et al.*, 1998b). Finnish alcoholic, impulsive, habitually violent offenders have been found to have low brain serotonin concentrations (Virkkunen *et al.*, 1996). Decreased serotonin metabolism correlates with externally directed aggressive behaviour in criminal offenders and in clinical and normal populations (Coccaro *et al.*, 1990b). Lower concentrations of 5-HIAA in the CSF are found in people with a history of problematic aggressive behaviour (Brown *et al.*, 1979; 1982). Other studies have, however, shown the opposite, that is, that aggression scores correlate with high levels of 5-HIAA in the CSF (Moller *et al.*, 1996). The same study found that low levels of 5-HIAA in the CSF correlate with internally directed aggression scores (Moller *et al.*, 1996). Another study found that homicide offenders did not differ from control subjects in the concentrations of 5-HIAA in the CSF (Lidberg, 2000). However, the group of homicide offenders that had made one or more suicide attempts had a significantly lower concentration of 5-HIAA in the CSF than the rest of the group, with the exception of subjects with impulse-control disorder, suggesting that serotonin regulates suicidal tendencies and impulse control. Thus, the most consistent finding is that serotonergic activity has a strong inverse relationship with internally directed aggression.

It is relatively difficult to obtain CSF samples in order to study serotonergic activity, and these measures reflect only the presynaptic component of serotonergic activity. Since prolactin secretion is regulated by serotonergic neurotransmission (Coccaro *et al.*, 1998a), measurement of prolactin in response to a serotonergic agent could provide an indirect measure of serotonergic activity. It has been suggested that this method might be a more sensitive measurement of serotonergic activity than the measurement of 5-HIAA levels in the CSF in that it also reflects the postsynaptic responsiveness of the serotonergic system (Coccaro *et al.*, 1997a). Studies using the neuroendocrine paradigm report a consistent correlation between blunted prolactin response to serotonergic agents and impulsivity or aggression in the personality disorders.

A blunted prolactin response to *d*-fenfluramine, a serotonin releaser and reuptake inhibitor, was found in personality disorder subjects compared to controls and was associated with a history of suicide attempts and impulsive aggression in the personality disorder subjects (Coccaro *et al.*, 1989; Coccaro, 1989; Siever and Trestman, 1993). A blunted response to fenfluramine was also found in subjects with antisocial personality disorder convicted of murder (O'Keane *et al.*, 1992), and it correlated to both self-reported and behavioural measures of aggression (Coccaro *et al.*, 1996a). In a study employing m-CPP (*m*-chlorophenylpiperazine), a 5-HT_{1a} and 5-HT_{2c} agonist, the prolactin response varied inversely with assaultiveness (Coccaro *et al.*, 1997c) and was blunted in antisocial personality disorder (Moss *et al.*, 1990). Buspirone and ipsapirone, two 5-HT_{1a} agonists, also produced blunted prolactin responses that varied inversely with aggressiveness (Coccaro *et al.*, 1990a; Moeller *et al.*, 1998a). Thus, a blunted prolactin response to a serotonergic challenge seems to be a consistent correlate of impulsive aggression in personality disorder patients.

The 5-HT_{2a} and -$_{2c}$ receptors appear to mediate the prolactin response to *d*-fenfluramine (Coccaro *et al.*, 1996c), while 5-HT_3 receptors seem to play little if any role in the prolactin response (Coccaro *et al.*, 1996b; Mann *et al.*, 1996a). An increase of 5-HT_2-receptor density has been reported in depressed patients and in suicidal patients (Pandey *et al.*, 1990). The increase was localized to the prefrontal cortex in a group of suicide victims (Arango *et al.*, 1990). This could reflect a compensatory increase due to reduced 5-HT release (Mann *et al.*, 1990). The density of 5-HT postsynaptic receptors correlates with the tendency to assaultive behaviour in non-criminally aggressive, personality-disordered subjects (Coccaro *et al.*, 1997c). The density of 5-HT_{2a} receptors positively correlates to assaultiveness in personality-disordered subjects. However, the affinity seems to be decreased (Coccaro *et al.*, 1997b). The number of 5-HT transporter (SERT) sites has been shown to vary inversely with self-mutilation in patients with personality disorder (Simeon *et al.*, 1992). The SERT sites in the brain are coded for by the same single-copy gene as the platelet 5-HT uptake site (Lesch *et al.*, 1993), a fact which makes it possible to study SERT in psychiatric disorders by studying platelets. Coccaro and colleagues have found a correlation between life history of aggressive behaviour and a reduced number of platelet 5-HT transporter sites in personality disorder patients (Coccaro *et al.*, 1996d).

These studies of serotonergic activity pointed the way to candidate gene studies of serotonin-related genes. One candidate gene (Burmeister, 1999) codes for the rate-limiting enzyme, TPH, in the synthesis of serotonin. A particular polymorphism of the TPH gene has been found to be associated with suicide, but not with CSF 5-HIAA concentration, in criminal offenders (Nielsen *et al.*, 1994; 1998). However, this association with suicide was not duplicated in a more general suicidal population (Abbar *et al.*, 1995). There are two alleles of the TPH gene, the 'L' and 'U' alleles. One study has shown that the 'L' allele of the TPH gene appears to be associated with impulsive aggression (New *et al.*, 1998). However, another study contradicted this by showing an association between the presence of at least one 'U' allele of the TPH gene and overt aggression in men (Manuck *et al.*, 1999). Thus, it remains unclear to what extent the TPH gene is involved in aggression. Other studies have shown association between the 'G' allele of the 5-HT_{1b} receptor gene and a history of suicide attempts in Caucasian patients with personality disorders (New *et al.*, 2001), and an association between alleles of the 5-HT_{2a} receptor gene and self-directed aggression (New *et al.*, 1999).

Positron emission tomography (PET) studies can demonstrate activity in regions of interest in the brain through measuring glucose metabolism. Patients with impulsive-aggressive behaviours show decreased activity in orbital frontal cortex at baseline and after fenfluramine compared to controls (Goyer *et al.*, 1994; Siever *et al.*, 1999). By giving subjects fenfluramine, a serotonin releaser and reuptake inhibitor, and measuring the metabolic activity of regions of interest with a PET scan, one can obtain an index of that region's serotonergic responsiveness. Fenfluramine caused an increase in metabolic activity in an area centred on the anterior cingulate and an area in the lateral prefrontal cortex involving the inferior, middle, and superior frontal gyri in the left prefrontal cortex and in the left temporoparietal cortex in normal subjects (Mann *et al.*, 1996c), but no increase in activity was found in depressed inpatients by the same method (Mann *et al.*, 1996b). Raine *et al.* (1994) showed that seriously violent offenders pleading not guilty by reason of insanity or incompetent to stand trial had a reduced metabolic activity in both the lateral and medial prefrontal cortex. Patients with impulsive-aggressive personality disorders have reduced metabolic activity compared to healthy volunteers in the orbital frontal, ventral medial frontal, and cingulate cortex after administration of *d*,l-fenfluramine (Siever *et al.*, 1999).

Pharmacotherapeutic studies have shown that fluoxetine hydrochloride, a selective serotonin-uptake inhibitor, reduced the aggressiveness in impulsive-aggressive personality disorder subjects (Coccaro and Kavoussi, 1997; Salzman *et al.*, 1995), again supporting a relationship between serotonin and aggression.

Dopamine

Animal studies have suggested a connection between dopaminergic activity and aggression (Coccaro, 1996). Human studies show either

a negative correlation or no correlation between the dopamine metabolite HVA in the CSF and aggression. CSF HVA levels have been observed to be closely linked to 5-HIAA levels in the CSF, which are known to be reduced in impulsive aggression (Coccaro, 1998), and CSF HVA may thus be reduced as well (Linnoila, 1983). A positive correlation between plasma HVA and novelty seeking among subjects with an anxious cluster personality disorder has been found in our laboratory (Siever *et al.*, unpublished data). The dopamine receptor D4DR gene has been shown to relate to novelty seeking in volunteers in some studies (Benjamin *et al.*, 1996; Ebstein *et al.*, 1996), but not in others (Malhotra *et al.*, 1996). In schizotypal and non-schizotypal personality disorder subjects, HVA concentrations in the CSF and plasma correlate with psychotic-like symptoms rather than aggressive behaviours (Siever *et al.*, 1993; Siever *et al.*, 1991a).

Vasopressin

Animal studies report a correlation between CSF vasopressin levels in the central nervous system (CNS) and aggression (Ferris and Delville, 1994). Vasopressin is associated with aggressive behaviour in humans in that a positive correlation has been reported with a life history of aggression even when controlling for the decreased serotonergic activity (Coccaro *et al.*, 1998b).

Other Peptides

While opiate withdrawal often elicits aggressive behaviour, there has been little study of the relationship of opiates to aggression although some inconsistent correlations have been found (Coccaro and Siever, 2002). Testosterone appears to play a facilitative role for aggressive behaviours in non-psychiatric subjects (Archer, 1991; Rubinow and Schmidt, 1996).

Norepinephrine

While lower concentrations of the serotonin metabolite (5-HIAA) have been found in the CSF of people with a history of problematic aggressive behaviour, a higher level of the norepinephrine metabolite [methoxy-4-hydroxy-phenylglycol (MHPG)] has been found in aggressive subjects than controls, and is correlated with a life history of aggression (Brown *et al.*, 1979; 1982). Plasma norepinephrine has also been correlated with self-reported impulsivity in personality disorder patients (Siever and Trestman, 1993), but reductions in CSF MHPG have also been reported in one study (Virkkunen *et al.*, 1987), although this was not replicated in another later study by the same group (Virkkunen *et al.*, 1994). Increased responses to clonidine were reported to be associated with self-reported 'irritability', but not with assaultiveness (Coccaro *et al.*, 1991).

AFFECTIVE INSTABILITY

In bipolar and depressed patients, PET studies have shown decreased activity in the prefrontal cortex, ventral to the genu of the corpus callosum, a finding partly explained by a corresponding reduction in the grey matter, raising the possibility of reduced cortical activity in relationship to affective symptoms (Drevets *et al.*, 1997). Pleasant and unpleasant emotions both increased cerebral blood flow (CBF) in the vicinity of the medial prefrontal cortex (Brodmann's area 9), thalamus, hypothalamus, midbrain, and head of the left caudate nucleus in healthy women (Lane *et al.*, 1997a; 1997b). Unpleasant affects also activated the bilateral occipito-temporal cortex and cerebellum, and left parahippocampal gyrus, hippocampus, and amygdala (Lane *et al.*, 1997a). Thus, intense affects have been associated with limbic and subcortical activity. Both in healthy sadness and in depressive illness, there is a decrease of CBF in the neocortical (right dorsolateral prefrontal, inferior parietal) and the limbic-paralimbic (subgenual cingulate, anterior insula) regions (Mayberg, 1997; 1999). In remission of depression, a decrease of paralimbic blood flow and an increase of neocortical blood flow was observed (Mayberg, 1997; 1999). These considerations suggest that the cortex may play an inhibitory modulatory role in relation to limbic activation associated with intense affects. Thus, affective instability in patients with BPD may reflect increased paralimbic activity, possibly in response to poor modulation by higher cortical structures.

Acetylcholine

Cholinergic neurons are found in limbic structures, paralimbic structures, the cortex, and the nucleus basalis (Ketter *et al.*, 1996). Acetylcholine is ubiquitous in the CNS and is involved in modulating REM sleep and autonomic nervous system activity. Animal studies suggest a role for central nicotinic receptors in depression (Tizabi *et al.*, 1999).

Procaine activates limbic structures, in part, by means of cholinergic activation (Kellner *et al.*, 1987; Kling *et al.*, 1987). When procaine was administered to healthy volunteer subjects, it induced fear in one-third and euphoria in one-third of the subjects. These effects correlated with the degree of activation of the left amygdala in PET studies (Ketter *et al.*, 1996). When physostigmine, a cholinesterase inhibitor, was given to healthy male volunteers, it induced a dysphoric and cardiovascular response that correlated with high emotional lability, high irritability, feelings of stress, and low life contentment (Fritze *et al.*, 1990). Physostigmine also induced a stronger depressive response in BPD patients than in normal controls and non-BPD patients (Steinberg *et al.*, 1997). The depressive symptoms in response to physostigmine correlated to BPD traits related to affective instability (affective instability, identity disturbances, chronic feelings of emptiness and boredom, and turbulent relationships), but they did not correlate to traits related to impulsivity. Exaggerated affective responses to cholinergic agonists have been shown in affective disorder patients compared to normal controls (Janowsky *et al.*, 1994). BPD patients without current depression showed a decrease in REM latency, similar to depressed patients, as compared to non-BPD and non-psychiatric controls (Akiskal *et al.*, 1985; Bell *et al.*, 1983; McNamara *et al.*, 1984). This is consistent with the possibility of altered cholinergic activity in BPD.

Norepinephrine (NE)

Animal models show an increase of noradrenergic activity in the locus coeruleus when the rodent is confronted with threatening stimuli (Levine *et al.*, 1990). Noradrenergic neuronal function is increased in anxiety and fear states in human subjects (Charney *et al.*, 1992). Administration of dextroamphetamine, a norepinephrine and dopamine releaser/reuptake inhibitor, to healthy subjects induces a dysphoric response (Kavoussi and Coccaro, 1993). This response correlated proportionally to measures of affective lability. By using the neuroendocrine measure of growth hormone response to a clonidine challenge, Coccaro *et al.* (1991) showed that central alpha$_2$-adrenergic receptors play a role in irritability, but not in assaultiveness. Gerra *et al.* (1999) found a positive correlation between sensation seeking, and NE and NE-dependent hormones.

GABA (Gamma-Aminobutyric Acid)

GABA-receptors are found in the amygdala and seem to be involved in the intense affects induced by procaine (Ketter *et al.*, 1996). Medications that successfully treat patients with affective instability,

such as lithium, valproate, and carbamazepine, increase GABAminergic neurotransmission (Shatzberg and Nemeroff, 1989), suggesting that GABA plays a role in affective instability.

CLUSTER C

The anxious cluster consists of avoidant, dependent, and obsessive-compulsive personality disorders. Anxious and fearful personality features are common to these disorders. Individuals with these disorders try to avoid situations that can lead to embarrassment, but at the same time they want to be part of their social group and long for close relationships. According to the TCI, they have a temperament of high harm avoidance (Cloninger *et al.*, 1993; Svrakic *et al.*, 1993). Harm avoidance has been hypothesized to be associated with the neurotransmitters serotonin and dopamine.

Serotonin

Blunted prolactin responses to fenfluramine were found in males with compulsive personality disorder compared with males with non-compulsive personality disorders, but these responses correlated with impulsivity in these patients (Stein *et al.*, 1996). A blunted prolactin response to fenfluramine has been shown in obsessive-compulsive disorder (OCD) patients and in patients with major depression compared to normal controls (Lucey *et al.*, 1992). An inverse correlation between harm avoidance and 5-HT_2 receptor sensitivity has also been demonstrated (Pierson *et al.*, 1999). The s/s genotype, but not the s/l or l/l genotypes, of the serotonin transporter is associated with higher scores of neuroticism on the NEO personality inventory, a measurement that includes anxiety (Greenberg *et al.*, 2000; Murakami *et al.*, 1999).

CONCLUSIONS

In general, surprisingly strong correlations have been shown between neurotransmitter activity and the personality disorders, particularly dimensions of personality such as impulsive/aggression, cognitive disorganization, and affective instability. New imaging techniques may permit more specific delineation of the circuits that may be altered in relation to these disturbances in neurotransmitters. As the genome is mapped in more detail, we may have better means of identifying specific candidate genes. Linkage techniques may also help to develop specific identification of altered genes. Not only will such studies inform our clinical interventions with these difficult-to-treat individuals, but they may also help us to understand better the relationship between individual differences in behaviour and personality and neurotransmitter activity.

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XXVI-3

Neuroendocrinology of Personality Disorders

Roger T. Mulder and Peter R. Joyce

INTRODUCTION

Disorders of personality are currently defined as categories of deeply ingrained and enduring behaviour patterns that are persistently maladaptive and encompass multiple domains of behaviour and psychological functioning. Personality types were described in ancient Greece, yet, despite this lengthy history, it may be argued that we are still far from adequate in describing the phenomena that constitute the clinical core of personality disturbance (Tyrer, 1995). Personality pathology has been conceptualized in a number of ways, including categories, dimensions and clusters, but none of the classification systems have been validated from an aetiological or a biological perspective. We begin by discussing ways of classifying personality pathology and measurement of central nervous system monoamines since the patterns of association are critically influenced by these factors.

CLASSIFICATION OF PERSONALITY PATHOLOGY

Personality Categories

The separation of personality disorders (PDs) as a discrete axis of classification by the American Psychiatric Association (APA) in DSM-III (American Psychiatric Association, 1980) has focused attention on this neglected group of disorders and stimulated research in this area. However, the large increase in literature (Gorton and Akhtar, 1990) has not been accompanied by a corresponding increase in systematic models to aid in understanding the underlying behavioural abnormalities. The 10 current DSM-IV categories — paranoid, schizoid, schizotypal, antisocial, borderline, narcissistic, histrionic, avoidant, dependent and obsessive compulsive — are derived from a mixture of theory, opinion and historical precedent. The *10th Revision of The International Classification of Diseases* (World Health Organization [WHO], 1992) has replicated most features of DSM-IV. Diagnoses in both systems involve a list of operational criteria, of which a specific number must be present for an individual to receive a diagnosis of personality disorder.

Although it is implied that the categories group patients into mutually exclusive diagnostic entities, most studies have reported high rates of co-occurrence (e.g., Pfohl *et al.*, 1986; Joffe and Regan, 1988; Mulder *et al.*, 1994), and the measured behaviours appear to be distributed dimensionally with no evidence of the discontinuity a categorical model would imply (Zimmerman and Coryell, 1990). The overlap and clinical heterogeneity which result hamper attempts to link personality disorder diagnoses with specific neurobiological processes.

Personality Dimensions

Unfortunately, the dimensional models have fared little better. Many have emerged from academic psychology, the most important being the work of Eysenck and the five-factor model currently being refined by Costa and McCrae (1992). Eysenck's model initially consisted of two dimensions labelled introversion/extraversion (E) (sociability, stability, activity) and neuroticism (N) (temperamental sensitivity to negative stimuli). His model included a biological explanatory schema based on autonomic nervous system reactivity and ease of conditionability. He suggested that arousal stems from the ascending reticular activating system and is linked with E, while N reflects activation from the limbic system (Eysenck, 1967). Later critics have noted that these two systems of brain circuitry are interconnected and are thus unlikely to explain two supposedly independent dimensions (e.g., Claridge, 1986; Gray, 1982), but it remains an initial attempt to link behavioural dimensions to underlying biological processes. In 1976, Eysenck added a third dimension, psychoticism (P), defined as tender-minded versus tough-minded (high P is similar to psychopathy) (Eysenck *et al.*, 1976).

Costa and McCrae's (1992) five-factor model consists of five dimensions — neuroticism, extraversion (both similar to Eysenck's dimensions), agreeableness (trust, altruism), conscientiousness (self-discipline, competence, order) and openness to experience (aesthetics, fantasy, values). Although this model is being increasingly related to the DSM PD classification (Nestadt *et al.*, 1994; Widiger and Costa, 1994), it has only recently been linked with biological processes, and then mainly genetics.

More recently, a model of temperament with specific theoretical relationships to neurobiology was proposed by Cloninger (1986; 1987). This model began by postulating brain systems based on animal and genetic studies and then worked out their behavioural manifestations (Carey and DiLalla, 1994). There are three temperament dimensions, namely, novelty seeking (NS), harm avoidance (HA) and reward dependence (RD). Initially, NS was hypothesized to reflect heritable differences in the behavioural activation system and to be associated with central nervous system (CNS) dopaminergic activity, HA was said to reflect individual differences in behavioural inhibition and to be associated with serotonergic neural firing, and RD was held to reflect differences in behavioural maintenance and was associated with noradrenergic activity. More recently, Cloninger's theory has been modified, so that HA is related to gamma-aminobutyric acid (GABA), as well as serotonin; RD is related to serotonin (median raphe) as well as noradrenaline; and persistence (a new temperament dimension based on a reward-dependence subscale) is related to serotonin (dorsal raphe). The tridimensional model of

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temperament has been related to DSM PD categories (Cloninger, 1987; Svrakic *et al.*, 1993; Mulder *et al.*, 1999) and has been useful in describing personality pathology (Svrakic *et al.*, 1993; Mulder and Joyce, 1997) and comorbidity (Mulder *et al.*, 1994; Battaglia *et al.*, 1996).

Clusters and Factors

A third approach to classification has been to group PD diagnoses into broad clusters with the aim of decreasing redundancy and creating more valid categories. At one extreme, Rutter (1987) suggested that, since all PDs have a pervasive and persistent abnormality in maintaining social relationships, there should be one overall category defined in terms of relationship abnormalities. More commonly, researchers, using a variety of factor and cluster-analytical methods, have identified three or four major groups with reasonable consistency (Walton and Presly, 1973; Tyrer and Alexander, 1979; Kass *et al.*, 1985; Hyler and Lyons, 1988; Tyrer and Seivewright, 1988; Dowson and Berrios, 1991; Schroeder and Livesley, 1991; Livesley and Jackson, 1992). DSM has also suggested three clusters named A, B and C. In a recent study, we identified four factors we named the 'Four As' — antisocial, asocial, asthenic and anankastic — from an item level factor analysis of DSM-III-R Axis II symptoms (Mulder and Joyce, 1997).

The possible relationships of these four factors to other empirically derived groups and the DSM-IV clusters are shown in Table XXVI-3.1, while the relationship of the 'Four As' to dimensional models of personality is shown in Table XXVI-3.2.

In this chapter, we will present the neuroendocrinology of PDs using the four groups — antisocial, asocial, asthenic and anankastic. This retains the accumulated clinical experience and meaning to clinicians of PD symptoms and signs, as well as a relationship to dimension models. The prototype PDs of the Four As are borderline PD (antisocial), schizoid PD (asocial), avoidant PD (asthenic) and obsessive-compulsive PD (anankastic).

NEUROENDOCRINE MEASUREMENT

Accessing brain function in human subjects presents significant methodological problems. Despite this, a variety of approaches have been used, including measuring monoamines and their metabolites in cerebrospinal fluid (CSF), plasma and urine; neuroendocrine probes of monoamine function; and the use of platelets and other cells as models for CNS monoamine function. Each technique has limitations, and they are all indirect estimates of what it is occurring in the CNS.

Many studies do not consider potentially important interactions between neurotransmitters or physiologically significant relationships with other compounds (Linnoila *et al.*, 1988). For example, CSF 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, and CSF homovanillic acid (HVA), a dopamine metabolite, are usually significantly correlated. This suggests that when studies report serotonergic abnormalities related to PDs, there are probably dopaminergic abnormalities as well.

If monoamine measures are to be used as an index of personality, they should represent stable biological traits. It is not always clear whether such measures reflect temporary reactions to stressful or pleasant events, that is, state rather than trait.

Finally, there is evidence that neuroendocrine measures are influenced by seasonality, clinical variation, diet and weight,

Table XXVI-3.1 Relationship of Four As to DSM clusters and other factor analyses

Four As 1997	DSM-III R PDs (Mulder and Joyce, 1997)	DSM-III R Clusters	Walton and Presly (1973)	Tyrer and Alexander (1979)	Schroeder and Livesley (1991)
Antisocial	Antisocial Borderline Narcissistic Histrionic Paranoid	B Antisocial Borderline Narcissistic Histrionic	Hysterical	Sociopathy	Psychopathic entitlement
Asocial	Schizoid	A Paranoid Schizoid Schizotypal	Social avoidance	Schizoid	Social avoidance
Asthenic	Avoidant, Dependent	C Avoidant Dependent Obsessive-compulsive	Submissiveness	Passive Dependent	Dependent emotionality
Anankastic	Obsessive-compulsive		Obsessional/schizoid	Anankastic	Compulsiveness

Table XXVI-3.2 Relationship of Four As to personality dimensions

Four As	Descriptors	Eysenck (Mulder and Joyce, 1997)	Cloninger (Mulder and Joyce, 1997)	Costa and McCrae, 1992 (theoretical)
Antisocial	Impulsive, unstable, easily bored	↑Psychoticism	↑Novelty seeking	↓Agreeableness ↓Conscientiousness
Asocial	Socially indifferent, lacks empathy	↑Psychoticism	↓Reward dependence	↓Extraversion
Asthenic	Fearful, anxious, insecure	↑Neuroticism ↓Extraversion	↑Harm avoidance	↑Neuroticism ↓Extraversion
Anankastic	Rigid, conscientious, perfectionistic		↑Persistence ↓Reward dependence	↑Conscientiousness

gender, and menstrual cycle, as well as other variables (Mulder, 1992). Given all these factors, results need to be viewed sceptically, and these considerations may, in part, also explain some of the inconsistencies and contradictory results found in this review.

ANTISOCIAL FACTOR

Most neuroendocrine research into PDs is focused on subjects who would fall within the antisocial personality factor. The behaviours studied have been impulsivity, aggression and affective lability. The principal finding, indeed the major finding, in the neuroendocrinology of PDs, is in the relationship of impulsivity and aggression to CNS serotonin. Much of the initial evidence of a link came from animal studies that had repeatedly demonstrated that serotonin-depleted animals are more aggressive, impulsive and disinhibited (e.g., Brody, 1969; Miczek *et al.*, 1975). The first published study in humans was in 1976, when Asberg *et al.* (1976) reported lower levels of CSF 5-HIAA in those who had made violent suicide attempts than in a control group. Since then, there have been a number of studies linking reduced levels of CSF 5-HIAA in personality-disordered men with a history of aggressive behaviours (Brown *et al.*, 1979), violent prison offenders (Linnoila *et al.*, 1983), impulsive arsonists (Virkkunen *et al.*, 1987) and impulsive suicide attempters (Roy, 1996). Pharmacochallenge studies have reported similar results. Coccaro and others reported a blunted prolactin response to fenfluramine in depressed patients with a history of suicide attempts and/or aggression (Coccaro *et al.*, 1989), and in aggressive and personality-disordered men (Coccaro *et al.*, 1996). There have been reports of reduced prolactin response to fenfluramine in patients with PDs (New *et al.*, 1997) and criminal offenders (O'Keane *et al.*, 1992).

There are some conflicting results. Wetzler *et al.* (1991) failed to find a relationship between self-reported aggression and the prolactin response to fenfluramine in depressed and panic disorder subjects, while Fishbein (1989) reported a positive relationship between prolactin response to fenfluramine and aggression in substance abusers. We have recently found no relationship between repeated self-harm, antisocial or borderline PD, and the prolactin response to fenfluramine in depressed subjects (Mulder and Joyce, 2002, in press).

Another neurotransmitter increasingly studied in relation to antisocial and impulsive behaviour is CNS dopamine. Overall, the evidence is weaker, with some studies showing a relationship between CSF HVA and antisocial and recidivist violent offending (Linnoila *et al.*, 1983; Virkkunen *et al.*, 1989), while others have demonstrated no relationship between this measure and aggression (Brown *et al.*, 1979; Virkkunen *et al.*, 1987). As discussed earlier, this evidence is complicated by the fact that CSF 5-HIAA and CSF HVA are usually correlated. There is some debate over which metabolite is more relevant. Agren (1983), for example, has suggested that suicidal behaviours correlated more closely with low CSF HVA than with low CSF 5-HIAA levels. Animal studies clearly suggest a direct relationship between dopamine function and aggression (Coccaro *et al.*, 1996).

Cloninger's dimension of NS, which is correlated with the antisocial factor, should also theoretically be related to dopaminergic activity. One study (Wiesbeck *et al.*, 1995) reported an association between growth hormone response to apomorphine (an indirect measure of CNS dopamine) and NS scores, but a later study by Hansenne and Ansseau (1998) failed to replicate this finding. Patients at risk of Parkinson's disease have low premorbid NS scores (Menza *et al.*, 1995), a finding which can be seen as support for the importance of dopamine in incentive activation of pleasurable activity. Other behaviours associated with the antisocial cluster, such as hyperactivity, sexual hedonism, drinking and smoking, have also been associated with high NS scores (Bardo *et al.*, 1996; Fergusson and Lynskey, 1996; Downey *et al.*, 1996). However, there is very little study of the relationship of these behaviours to indices of dopaminergic functioning. A study in depressed patients found a correlation between CSF dopamine and extraversion (King *et al.*, 1986).

There have also been some reports linking decreased dopamine β-hydroxylase (DBH) (an enzyme that catalyses the conversion of dopamine to noradrenaline in the brain) with unsocialized conduct disorder in boys (Rogeness *et al.*, 1982; Rogeness *et al.*, 1987; Bowden *et al.*, 1988).

Hyperactivity of noradrenergic functioning has been found to correlate with aggressive behaviour in human beings as well. Increased beta-adrenergic receptor binding has been found in the brains of violent suicide victims compared with accident victims (Stanley and Mann, 1983). Brown *et al.* (1979) reported a positive correlation between CSF 3-methyl-4-hydroxyphenolglycol (MHPG) concentrations and a history of aggression in male subjects with PDs. CSF MHPG levels have also been reported to be elevated in violent suicide attempters compared with non-violent suicide attempters (Traskman-Bendz *et al.*, 1992).

Androgens may also play a role in aggressive behaviour. Violent criminals appear to have higher testosterone levels than criminals who commit non-violent crimes, and are also more aggressive in prison settings (Dabbs *et al.*, 1995). Among violent alcohol offenders, those with antisocial personality disorder (ASPD) and increased aggressiveness appear to have higher CSF testosterone concentration (Virkkunen *et al.*, 1987).

In summary, all three major neurotransmitters and male androgens have been implicated in the impulsive and aggressive behaviour found within the antisocial cluster. The most convincing evidence is for serotonin abnormalities. These biological abnormalities may be confined to individuals with extreme and persistent impulsive/aggressive behaviours.

ASOCIAL FACTOR

The most interesting findings related to asocial behaviour come from animal studies, particularly those on prairie voles (Insel, 1997). The neurohypophysial neuropeptides, oxytocin and vasopressin, appear to be important for the formation of long-term social attachments and some aspects of infant attachment behaviour. For example, blocking oxytocin neurotransmission results in a significant inhibition of maternal behaviour, while a vasopressin antagonist administered to male prairie voles blocks the development of particular mate preference and selective aggression. The pathways appear to be species-specific, but these data suggest that the study of these neuropeptides in highly asocial individuals might be rewarding. There are some inconsistent findings that oxytocin levels may be abnormal in subjects with autism (Gilberg and Coleman, 1992).

There are also suggestions that opioid pathways may be involved in social attachment. There is some literature on an opioid model of autism, although therapeutic trials with opiate agonists have been disappointing (Insel *et al.*, 1996).

The asocial factor may be associated with low reward dependence (Mulder and Joyce, 1997). One study has reported that the noradrenaline metabolite MHPG was significantly correlated with TPQ reward-dependence scores (Garvey *et al.*, 1996). Another has related higher reward dependence scores with increased excretion of harman (a by-product of monoamines) in alcoholics (Wodarz *et al.*, 1996). Neither of these studies utilized measures of asocial behaviour.

Overall, despite the evidence that asocial behaviour or social indifference is consistently reported as a stable and persistent human personality trait, there is remarkably little research on its underlying neurobiology.

ASTHENIC FACTOR

The pathophysiology of this cluster of PDs has also seldom been investigated and is poorly understood (Kirrane and Siever, 1998). There are a few studies in social phobia, which, it could be argued, overlaps with and may not be distinct from avoidant PD. Patients with social phobia may have a greater fenfluramine-induced rise in cortisol than controls (Uhde, 1994), suggesting serotonin receptor supersensitivity. The hypothalamic-pituitary-adrenal (HPA) axis appears to be normal (Tancer *et al.*, 1990), but reduced dopamine metabolism has been suggested in one study (Potts and Davidson, 1992). The fact that the monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs) sometimes reduce symptoms of avoidant personality disorder has led some to suggest that the monoamines, particularly serotonin, are implicated, although the evidence beyond this is vague.

The asthenic cluster is consistently associated with higher Eysenck's neuroticism (N) scores and Cloninger's harm-avoidance (HA) scores. Despite the importance of these dimensions, particularly in predicting the severity and course of mood and anxiety disorders, their neuroendocrinological underpinning has barely been researched. Two studies (Nelson *et al.*, 1996; Pfohl *et al.*, 1990) reported no significant correlation between platelet imipramine binding (an indicator of serotonergic function) and TPQ scores. However, in the Nelson *et al.* study, an alternative measure of serotonin functioning, L-lysergic acid diethylamide (^{125}L-LSD), was strongly related to HA scores. In patients with eating disorders, there was no relationship between blood serotonin and HA (Waller *et al.*, 1993).

Studies of the relationship between biochemical measures and N scores are few. Ballenger *et al.* (1983) reported that serum cortisol correlated negatively with N, as did plasma MHPG and CSF calcium. Recently, McCleery and Goodwin (2001) reported that individuals with high N scores had a smaller cortisol response to the combined dexamethasone–corticotrophin-releasing hormone (CRH) test. This suggests that individuals with a high N may have downregulation of the HPA axis.

ANANKASTIC FACTOR

Specific neurobiological research into the anankastic, or obsessive-compulsive, personality is essentially non-existent. There is some research into the obsessive-compulsive spectrum disorders, which include anankastic personality as well as obsessive-compulsive disorder, Tourette's syndrome, and other stereotypic movement disorders. Whether this characterization of such a varied range of psychopathology is valid remains contentious (e.g., Rasmussen, 1994), but it does hint at possible directions for further research into anankastic personality.

The possible importance of the serotonin system is supported by the response of this obsessive-compulsive spectrum, including anankastic personality, to clomipramine and SSRIs. However, the few studies using CSF 5-HIAA measures or pharmacological challenge techniques do not show evidence of a consistent serotonergic abnormality (Stein, 2000). Although dopamine metabolism may play a part in tics and abnormal movements associated with the obsessive-compulsive spectrum, it seems less likely to have a role in anankastic personality (Stein, 2000).

One interesting hypothesis is that, while impulsivity is associated with decreased serotonergic function, compulsiveness is associated with hyperresponsivity of the serotonergic receptors. This contrast may be overly simplistic in that both impulsive and compulsive disorders have been reported to show neuroendocrine blunting on serotonergic challenge and both respond to serotonergic antidepressants (Stein, 1996).

Millon and Davis (1996) have speculated that the neural connections in the limbic system that control fear and anger may contribute to the indecisiveness and excessive conflict that characterizes decision-making in this personality style. This is an interesting but untested speculative hypothesis.

DISCUSSION

We have noted limitations to formal classifications of personality pathology. These limitations have restricted identification and quantification of behaviours that may be associated with neuroendocrinology. PDs, as currently defined, are non-specific diagnostic categories with no theoretical relationship to neuroendocrinology. Trying to find patterns of associations between such categories and specific neurochemical processes is unlikely to be successful.

Grouping PDs into clusters such as the 'Four As', creates more valid categories from a psychometric point of view, but there is limited evidence that these clusters are related to neuroendocrine abnormalities. The monoamine abnormalities reported in violent, impulsive individuals seem to relate to a broad cluster of personality pathology, including antisocial, borderline, narcissistic, histrionic and paranoid symptoms, rather than to individual personality disorder categories, so that the 'antisocial' cluster has superior face validity. However, the lack of biological research into the other personality groups (i.e., asocial, asthenic and anankastic) means that we do not know whether each has a distinct neuroendocrinology, and whether such groups are likely to be helpful for biological research.

Dimensional classifications appear to offer the most promising model for biological processes. However, most current models are derived through the statistical modelling of answers to questionnaires with little thought given to underlying biological principles. The exception is Cloninger's tridimensional model, which was specifically designed to encompass knowledge about the biology of behaviour. However, there is only limited evidence that CNS neuroendocrinology is related systematically to Cloninger's dimensions, as we have already reported. More convincing evidence is in the neuroanatomical and genetic studies related to TPQ measures (Cloninger, 1998).

It may be time to question whether biological research is building on an adequate base. There are increasingly accurate and sophisticated means for measuring brain processes, but what seems to be lacking is an adequate system for personality description. The current DSM categories may be useful for certain tasks, but they reflect simplistic static notions that seem to relate distantly, if at all, to the core processes involved in psychopathology. The self-report questionnaires used in dimensional models are more self-representations than accurate personality descriptions (Kagan, 1994; Hogan, 1996) and lack any behavioural observation.

The move toward objectification in psychiatric diagnosing was a necessary first step but appears to have come to a premature closure (van Praag, 1992). To advance research into the biology of PDs, detailed scrutiny outside our current symptom representation may be necessary. There are some hints of this already. For example, the serotonin abnormalities correlated with impulsive aggression are largely found in individuals whose aggressive behaviour is not premeditated (Linnoila *et al.*, 1983; Virkkunen *et al.*, 1987). This suggests that the key component may be lack of behavioural inhibition rather than aggression or impulsivity.

It may be better to have skilled clinicians closely observing behaviour and assessing detailed personality pathology rather than using structured interviews and questionnaires. Hypotheses derived from clinical models, genetic studies and brain imaging could provide more specific testable models for linking behaviour

and neuroendocrinology. For example, selecting a group of very socially indifferent individuals for study, regardless of their clinical psychopathology (some may have adapted very well to their temperament), might be useful. This could involve using a screening tool such as low RD scores on Cloninger's TPQ questionnaire and then validating these scores by behavioural observation. Animal and genetic studies suggest that neurochemicals such as vasopressin, oxytocin, serotonin and the opioids would be of interest.

The potential significance of finding consistent links between neurochemistry and personality lies in three main areas. First, if maladaptive personality traits can be shown to be shaped by neurotransmitter functions which relate to an individual's ability to regulate impulse, anxiety, mood, sociability and so on, then a sound theoretical basis for pharmacotherapy becomes possible (Soloff, 1990). Rather than targetting PDs, medications could target specific biological trait vulnerabilities and help to modify and regulate the particular behavioural symptoms that make up the psychopathology of PDs.

Second, there is the possibility that much of the relationship between neuroendocrinology and Axis I disorders is mediated by personality traits. There is considerable evidence that certain Axis I disorders are associated with specific personality pathology. For example, individuals with antisocial personality traits are more likely to have alcohol and substance-abuse disorders but less likely to suffer from generalized anxiety disorder, while those with anankastic personality traits are more likely to have obsessive-compulsive disorder, and anxiety and mood disorders but less likely to have alcohol or substance-abuse disorders (Nestadt *et al.*, 1989). These associations may reflect neuroendocrine abnormalities. For example, the serotonergic abnormalities sometimes reported in depressed patients may be largely confined to those with impulsive suicidal behaviours (De Meo *et al.*, 1989). Samples with a high proportion of such individuals report differences in serotonin functioning compared with a control group; those with a low proportion do not. In other words, serotonin disturbances may be non-specific from a functional/categorical perspective but specific from a functional/dimensional perspective, correlating with particular personality traits such as aggression, impulsivity or obsessionality across diagnoses (van Praag, 1986).

Third, linking personality with neuroendocrinology may be a way to integrate biology and psychosocial experience. There is a small but increasing literature reporting associations between negative early life experiences and abnormalities in adult neuroendocrinology. Measuring urinary catecholamine excretion showed that sexually abused girls secreted greater amounts of HVA (De Bellis *et al.*, 1994), and that abused children had higher concentrations of noradrenaline and dopamine than healthy controls (De Bellis *et al.*, 1999). Hospitalized boys who had experienced neglect in the first 3 years of life have been found to have reduced DBH (Galvin *et al.*, 1991), a finding also associated with antisocial cluster behaviour (Galvin *et al.*, 1995; Rogeness *et al.*, 1987), and, in particular, a low valuation of authority and peer-derived rules of conscience (Galvin *et al.*, 1995). Here is a link between an experience (neglect), through a particular behaviour (lack of conscience), to a personality abnormality (conduct disorder).

HPA axis regulation also appears to be related to the quality of early maternal care (Caldji *et al.*, 1998). De Bellis *et al.* (1994) found a reduced adrenocorticotrophic hormone (ACTH) response to CRH stimulation in their sexually abused girls, and Stein *et al.* (1997) reported enhanced dexamethasone suppression in women with a history of childhood sexual abuse. High neuroticism (and its associated reduced cortisol response to dexamethasone-CRH), while partly heritable, may be a measurable vulnerability factor linking such experiences with later post-traumatic stress disorder (PTSD) and depression (McCleery and Goodwin, 2001).

SUMMARY AND CONCLUSION

Over the past 20 years, there has been increasing interest in the neuroendocrinology of PDs. Early results related CSF monoamine metabolites to specific behaviours. There has been a gradual expansion in the types of neuroendocrine measures and behavioural subtypes studied. Despite this, most research has focused on the broad area of antisocial behaviour, particularly impulsivity and lack of inhibition. These behaviours have been linked with abnormalities in all three major CNS biogenic amines and changes in male androgens. Other consistently identified human behavioural traits — asociality or social indifference, asthenic or neurotic behaviours, and anankastic or obsessive-compulsive behaviour — have been much less studied.

Plausible, testable, aetiological models of personality pathology are a necessary step for the science of neuroendocrinology to contribute to an integrated understanding of personality. Attempts to find patterns of association between current DSM PD categories and specific neurochemicals have had limited success. Broader empirical groupings with less overlap such as the 'Four As' may be more useful, but until increased research on the non-antisocial factors is undertaken, it is difficult to be sure. Cloninger's biosocial model is the most sophisticated attempt so far to construct a theoretical, testable model relating biology to behaviour. Results have been somewhat inconsistent, but it offers a base for future research. New developments in measuring the temperament dimensions while incorporating behavioural factors may lead to more valid parameters. Results from animal and imaging studies have already helped refine the initial model of the three monoamines as 'emblematic' neurotransmitters into more complex theoretical relationships between neurochemistry, neuroanatomy and behaviour.

The neuroendocrine abnormalities associated with some adverse early life experiences offer another potentially important model for research. Personality pathology might form the link between such experiences and later psychopathology. Enduring changes in CNS neurochemistry and HPA axis function associated with abuse may be correlated with personality characteristics such as high N or conduct disorder, which in turn make an individual vulnerable to PTSD, depression and ASPD as an adult. Screening a large population for high and low behavioural measures, such as N, NS or RD, and studying these individuals' neurochemistry, early life experiences and current psychopathology might lead to useful results.

The neuroendocrinology of PDs remains an important area of psychiatric research. It could lead to more rational treatment for both Axis I and Axis II disorders, help construct a more valid classification for psychopathology, and provide a link between early life experience and adult development.

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The Psychophysiology of Personality Disorders

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The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994), defines a personality disorder (PD) as 'an enduring pattern of inner experience and behavior that deviates markedly from the expectations of the individual's culture, is pervasive and inflexible, has an onset in adolescence or early adulthood, is stable over time, and leads to distress or impairment (p. 629)'. In general, PDs involve personality traits that have become severe and rigid enough to be dysfunctional or maladaptive, and are typically manifested in at least two of the following areas: cognition, affectivity, interpersonal functioning, or impulse control.

The DSM-IV distinguishes 10 specific PDs that are grouped into three broad clusters, based upon similarities in descriptive features. Cluster A includes the paranoid, schizoid, and schizotypal PDs, which describe individuals who often appear odd, eccentric, or suspicious. Cluster A PDs are also thought to be part of the schizophrenia-spectrum disorders due to their similarity in symptoms with schizophrenia, though milder in form. Cluster B includes the antisocial, borderline, histrionic, and narcissistic PDs, which describe individuals who appear dramatic, emotional, impulsive, or erratic. Finally, Cluster C includes the avoidant, dependent, and obsessive-compulsive PDs, which describe individuals who often appear anxious or fearful. Clusters B and C have been described as reflecting externalizing (i.e., dramatic, reactive, and aggressive) or internalizing (i.e., anxious, avoidant, and withdrawn) dimensions of personality, respectively, based upon their primary symptomatology (Scarpa *et al.*, 1999).

Psychophysiology involves the study of cognitions, emotions, and behaviour as related to physiological principles and events (Cacioppo and Tassinary, 1990; see also Chapter IX in this volume). As such, psychophysiology can provide unique information on the cognitive, affective, interpersonal, and impulsive features of PDs that is relatively objective in nature. This chapter will describe psychophysiological studies of PDs, as grouped by cluster. Although such studies provide rich information on psychological processes related to PDs, relatively few PDs have been examined by psychophysiological techniques. Specifically, the schizotypal and antisocial PDs have been most abundantly studied in relation to psychophysiology; thus, this chapter must necessarily focus on these two. Whenever possible, this information will be supplemented with psychophysiological studies of the other PDs. Findings related to characteristics associated with the Cluster C PDs are also discussed.

CLUSTER A PERSONALITY DISORDERS

As described above, Cluster A includes the paranoid, schizoid, and schizotypal PDs, which are thought to reflect psychosis-proneness or schizophrenia-spectrum disorder. In the DSM-IV, paranoid PD is defined as a pattern of suspiciousness whereby others' motives are interpreted as hostile or malevolent; schizoid PD is defined as a pattern of social detachment or indifference with a restricted range of affect; and schizotypal PD is defined as a pattern of cognitive and perceptual distortions, interpersonal deficits, and odd behaviour (American Psychiatric Association, 1994). Indeed, schizotypal and paranoid PDs are found with greater frequency in the relatives of probands with schizophrenia, although not all studies have been consistent (see Webb and Levinson, 1993 for a review).

Schizotypal PD, in particular, has been a focus of attention due to its symptoms seeming to reflect a milder form of those seen in schizophrenia. These include negative (or deficit) symptoms, such as social withdrawal or blunted affect, and positive (or excess) symptoms, such as perceptual aberrations or magical ideation. Social anhedonia (i.e., social indifference) and magical ideation seem most predictive of later psychosis (Chapman *et al.*, 1995). As such, researchers have focused on comparing schizotypal PD to findings in schizophrenia, with the hope of discovering either a common genetic marker for vulnerability or a factor protective against the progression to full-blown schizophrenia. In this vein, the primary psychophysiological variables that have been studied in schizotypal PD are the skin conductance orienting response (SCOR), smooth pursuit eye movement (SPEM), and event-related potential (ERP), all of which have been found to be deviant in individuals with schizophrenia. Each of these variables and research on their relationship to schizotypal PD will be summarized in turn below.

The Skin Conductance Orienting Response (SCOR) and Schizotypal PD

Changes in the electrical activity of the skin generally occur in response to the presentation of novel stimuli in one's environment. For example, the presentation of a new tone generally causes an orienting response that is accompanied by increased electrical activity in the skin, and thus, a change in skin conductance levels called the SCOR. One useful model for understanding the SCOR has been that of template-matching (Ohman, 1979; 1985). In this model, novel stimuli are stored in short-term memory where they create neural 'templates'. Subsequent stimuli are matched against the template currently stored in short-term memory. If the two stimuli differ, preattentive mechanisms will fail to recognize the newly presented stimulus, thus requiring controlled processing of the new stimulus. However, if the stimuli match, habituation occurs. In addition to cases in which there is a mismatch between the 'stored' stimulus and the novel stimulus, controlled processing also occurs when the novel stimulus is recognized as significant, necessitating further processing. In either case, augmented controlled processing of information would produce a SCOR. Thus, the SCOR is a useful index of how one attends to and processes novel environmental stimuli (Dawson and Nuechterlein, 1984; Dawson *et al.*, 1989).

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A review of previous studies that have assessed the relationship between SCOR and schizotypy reveals diverse and sometimes inconsistent findings (see Raine *et al.*, 1995 and Yaralian and Raine, in press, for reviews). As in findings with schizophrenia (Dawson and Nuechterlein, 1984), significant effects have shown that negative-symptom schizotypy is marked by hyporesponding while positive-symptom schizotypy is marked by hyperresponding. For example, Simons (1981) and Bernstein and Riedel (1987) both found a greater incidence of SC non-responding in a group of individuals characterized by high scores on physical anhedonia. Whereas neither of these studies found associations with positive symptoms, others have indeed found a significant association between hyperresponsive SCOR amplitudes and positive symptoms of schizotypy, such as cognitive distractibility and distortions (Lipp and Vaitl, 1992; Nielsen and Petersen, 1976). However, significant findings also indicated that positive-symptom schizotypy, as reflected by schizophrenism, perceptual aberrations, and cognitive distortions, is also sometimes associated with non-responding or hyporesponding (Lencz *et al.*, 1991; Raine, 1987; Simons *et al.*, 1983). While the above findings clearly indicate some abnormal orienting in individuals with schizotypal features, it must be noted that none of these studies directly assessed for the syndrome or diagnosis of schizotypal PD. This is particularly noteworthy for studies of negative-symptom schizotypy, where the primary index of physical anhedonia is not even listed as a DSM-IV symptom of schizotypal PD. Thus, the finding of most relevance to DSM-IV symptoms of schizotypy is that either hypo- or hyper-SC responding may characterize cognitive and perceptual deficits.

There is also evidence of a link between erratic responding and schizotypy. One example involved an analysis of SCORs over nine orienting trials for normal subjects characterized by high scores on schizophrenism and physical anhedonia and a group of controls (Wilkins, 1988). The results indicated normal habituation over trials 1–3 for the control and schizophrenism groups, but increased response amplitudes from trials 1–2 in the physical anhedonia group. Similarly, the physical anhedonia group showed an increase in amplitudes, rather than normal habituation, from trials 4–5, which comprised a reorienting set of trials. Thus, negative-symptom schizotypal subjects, characterized in this study by physical anhedonia, failed to display the expected SCOR habituation patterns seen in normal controls. As mentioned above, physical anhedonia is not a DSM-IV symptom of schizotypal PD, and thus it was unclear how these results might generalize to the categorical classification of schizotypy.

To address this issue, this study was replicated, using both a sample of 13 DSM-III-R clinically diagnosed schizotypals (i.e., categorical approach) and a sample of 30 subjects who self-reported on a screening scale for schizotypy (i.e., dimensional approach) (Raine *et al.*, 1997a). The results indicated a similar failure to habituate over orienting trials, regardless of whether schizotypy was defined categorically or dimensionally. Cluster analysis of the 13 diagnosed schizotypals revealed two subclusters, the cognitive-perceptual and the interpersonal deficits subclusters, which respectively reflect positive and negative symptoms of schizotypal PD. The failure to habituate was noted only for the cognitive-perceptual subcluster, as reflected by an initial low orienting response followed by increasingly larger responses for both an orienting trial (i.e., over trials 1–3) and a reorienting trial (over trials 4–6). The interpersonal deficits subcluster, however, showed an expected pattern of habituation, with a large initial SCOR followed by diminished responses. This suggests that erratic SCORs may be associated primarily with positive, rather than negative, symptom features of diagnosed schizotypal PD.

Several interpretations of SC orienting abnormalities have been proposed (see Raine *et al.*, 1995 for a review). One interpretation involves deficits in working memory and frontal brain systems, since reduced SC orienting has been shown to be correlated with both of these indices. In addition, template mismatching has been speculated to underlie abnormal orienting. Template mismatching may result from either the degradation of the accurate representation of the initial stimulus, or from difficulty in initially generating the neuronal template. Inhibitory deficits have also been proposed to explain abnormal orienting. It is possible that the underlying deficit lies in the balance between excitatory and inhibitory processes; too much inhibition would account for the high incidence of SC non-responding in schizotypals, while a failure of inhibitory mechanisms would explain erratic orienting and hyperresponding. It is also thought that the balance between these two contrasting processes affects the particular syndrome. Positive syndrome schizotypy may result from disinhibition, while negative syndrome schizotypy may occur because of an excess of inhibition.

Smooth Pursuit Eye Movement (SPEM) and Schizotypal PD

SPEM measures an individual's visual tracking of a smoothly moving target, such as a pendulum. It can be assessed either qualitatively (by visually inspecting the number of saccadic intrusions and visual lags) or quantitatively (by computerized assessments of tracking accuracy, such as the log of the signal-to-noise ratio). SPEM has been thought to reflect cognitive difficulties with nonvoluntary attention and inhibition (Holzman *et al.*, 1976; 1978) and with working memory and prefrontal disorder (Park *et al.*, 1991), all of which are often seen in schizophrenia.

Indeed, impaired SPEM is one of the most consistent findings in schizophrenia, having been observed in 50–85% of patients with chronic schizophrenia compared with 8% of a control population (Holzman *et al.*, 1974; 1984). Impaired SPEM is also greater in relatives of probands with schizophrenia (Holzman *et al.*, 1974) and in patients whose schizophrenia has remitted (Iacono *et al.*, 1981), and is thus speculated to be a genetic marker or diathesis for schizophrenia-spectrum disorders. As such, an interest arose in its relationship to schizotypal PD.

A relationship between SPEM impairment and schizotypal PD first came from studies of individuals selected from the general population on the basis of either poor tracking ability (Siever *et al.*, 1984) or physical/social anhedonia and perceptual aberration scores (Simons and Katkin, 1985). In the former study, volunteers who were selected on the basis of qualitatively poor eye-tracking accuracy had a greater prevalence of diagnosed schizotypal PD than a control group with high eye-tracking accuracy (11% versus 29%). The difference was even more pronounced for individuals whose eye tracking was reassessed quantitatively, with a prevalence rate of 54% with schizotypal PD. No differences were observed between the two groups on other non-schizophrenia psychopathology, including other PDs. This finding suggested that impaired SPEM was specific to the schizophrenia-spectrum disorder of schizotypal PD.

In the study by Simons and Katkin (1985), subjects were selected by dimensional scores on anhedonia and perceptual aberration. Those with high scores on anhedonia demonstrated heightened prevalence of poor SPEM compared to those with low anhedonia, a finding which suggests a relationship of SPEM to negative symptoms of schizotypy. However, impaired eye tracking has also been noted in relationship to positive psychotic symptoms (Siever *et al.*, 1989). Since this study was not conducted on individuals with clinically diagnosed schizotypal PD, it was still unclear how findings would generalize to the classification of the disorder.

To address the issue of eye-tracking abnormalities in clinically identified schizotypal PD, a study was conducted that compared patients who met DSM criteria for the disorder with three other comparison groups — a group with other non-schizophrenia-related PDs, a group with no psychopathology, and a group with schizophrenia (Siever *et al.*, 1990). The results indicated that the

groups with schizotypal PD and schizophrenia displayed greater impairment of SPEM than the other PD group and the normal control group. This finding of poor SPEM in diagnosed schizotypal PD was replicated by Lencz *et al.* (1993), who showed impaired eye tracking in a group of undergraduates who met DSM criteria for the disorder.

In the study by Siever *et al.* (1990), an analysis of specific symptoms showed that impaired SPEM was predicted only by the criterion of social isolation, with low accuracy trackers also reporting reduced desire for social contact. This would seem to support the notion of impaired SPEM in relation to negative-symptom schizotypy. Other studies, however, show a relationship between eye-tracking abnormalities and positive-symptom schizotypy. Raine *et al.* (1995), for example, reported a reanalysis of the data by Lencz *et al.* (1993), finding that those diagnosed with schizotypal PD who displayed cognitive/perceptual symptoms showed significantly poorer eye tracking than those with interpersonal deficit symptoms. These same individuals exhibited a loss of normal SC habituation (described in the above section on SCOR), and Raine *et al.* speculated that this reflected a loss of inhibitory functions related to both saccadic intrusions in eye tracking and SC attentional deficits.

On the basis of these studies, it is still unclear whether impaired SPEM is specific to certain types of symptoms (i.e., negative versus positive) or instead reflects the entire schizotypal syndrome more generally. It is clear, however, that poor eye tracking is consistently found in individuals with clinically diagnosed schizotypal PD. Together with the literature on SPEM in schizophrenia, this finding supports the notion of impaired SPEM as a marker for schizophrenia-spectrum disorders that may reflect a common genetic vulnerability.

Event-Related Potentials and Startle-Blink

Despite the emphasis on SCOR and SPEM presented above, there has more recently been research on event-related potential (ERPs) and eye-blink correlates of Cluster A PDs. Perhaps the best-replicated electrophysiological correlate of schizotypal personality is reduced amplitude of the P300 ERP. At least five studies find evidence of P300 amplitude reduction in DSM diagnosed schizophrenia-spectrum disorders (Salisbury *et al.*, 1996; Trestman *et al.*, 1996; Black *et al.*, 1992) and individuals high on schizotypal personality (Kimble *et al.*, 2000; Klein *et al.*, 1999). However, Blackwood *et al.* (1992) did not find reduced P300 in individuals who had been diagnosed as schizoid in childhood, suggesting that schizoid PD, unlike schizotypal and paranoid PDs, may not be part of the schizophrenia-spectrum of disorders. P300 deficits have been repeatedly found in schizophrenics and relatives of schizophrenics, and reflect a broad attentional deficit.

Three other ERP findings are of note. First, high scorers on the schizotypal personality questionnaire have failed to show the right-sided predominance in the post-imperative negative variation (PINV) observed in normals (Klein *et al.*, 1998). Second, individuals with schizotypal PD show less gating (suppression) of the P50 ERP to the second of a pair of click stimuli than controls (Cadenhead *et al.*, 2000), indicating a failure of sensory gating at a relatively early stage of information processing, and consequently reduced central nervous system (CNS) inhibition. The same deficits have been observed in both schizophrenics and their relatives. Third, there is growing evidence in schizotypal individuals of a deficit in N400, an ERP component that reflects the degree of search for a semantic match to a word. Two recent studies have shown reduced N400 amplitudes in both individuals high on schizotypy and those with schizotypal PD (Kimble *et al.*, 2000; Niznikiewicz *et al.*, 1999). These two studies suggest a language-processing deficit in schizotypals that is similar to that observed in schizophrenics.

Research has also been conducted on startle eye-blink modification (SEM) and psychosis-proneness (Cadenhead *et al.*, 1993; Dawson *et al.*, 1995; Simons and Giardina, 1992). These findings again show some consistency with findings in schizophrenia, whereby SEM is impaired in subjects with schizotypal features. Findings are inconsistent, however, regarding whether the deficits occur in the controlled or automatic attentional components of SEM. Nevertheless, deficits in sensorimotor gating are consistent with the P50 ERP deficit outlined above, indicating a failure to gate stimuli.

Summary

Overall, two primary findings emerge on the relationship between psychophysiological measures and schizotypal PD. First, they are generally consistent with those found in schizophrenia. That is, SCOR hyporesponding and hyperresponding; failure to habituate; and deficits in SPEM, P300, P400, P50, and SEM have paralleled the literature on schizophrenia. These findings imply an underlying psychophysiological commonality between schizophrenia and schizotypal PD, and suggest that the latter does indeed reflect part of the schizophrenia-spectrum of disorders. Second, all of these psychophysiological measures tap some cognitive capacity, primarily related to attentional, inhibitory, and working-memory processes. They converge in suggesting specific deficits in such cognitive processes in individuals with schizotypal PD. The findings are not consistent, however, in regard to whether the impairments are related to positive or negative symptoms, and this issue needs to be further examined. It is possible, for example, that three-factor models of symptomatology in schizophrenia and schizotypal personality may provide clearer findings than the two-factor positive-negative model (Raine *et al.*, 1994). Moreover, the relationship to the other Cluster A PDs is still understudied.

CLUSTER B PERSONALITY DISORDERS

Cluster B includes the antisocial, borderline, histrionic, and narcissistic PDs. Antisocial PD is defined as a pattern of disregarding and violating the rights of others; borderline PD is defined as a pattern of unstable relationships, self-image, affect, and behaviour in the form of impulsivity; histrionic PD is defined as a pattern of excessive emotionality and seeking of attention; and narcissistic PD is defined as a pattern of grandiosity, need for admiration by others, and lack of empathy with others (American Psychiatric Association, 1994). These PDs seem to share symptoms that reflect dramatic, erratic, and highly emotional behaviours, although antisocial PD may also be associated with a lack of emotionality.

Of this cluster, antisocial PD has been most extensively studied in relation to psychophysiology, especially in regard to heart rate (HR), skin conductance (SC), and electroencephalogram (EEG) as a reflection of underarousal (see Chapter 7 in Raine, 1993 for a comprehensive review). As such, this will be the focus of the following review. It should be noted, however, that the majority of these studies do not use DSM criteria for measurement of PD, but instead use measures of psychopathy or repetitive antisocial behaviour. Thus, though obviously related, the exact nature of the findings in regard to clinically diagnosed antisocial PD is blurred. There has also been some limited psychophysiological research on borderline PD and one known psychophysiological study of narcissism, and this information will be included as well.

HR Studies and Antisocial Behaviour

HR reflects both sympathetic and parasympathetic nervous system activity and can be measured both tonically (i.e., beats per

minute at rest) or phasically (i.e., change in response to a stimulus). Accelerations in HR to a stimulus are thought to reflect sensory rejection or 'tuning out' of noxious environmental events, while decelerations are thought to reflect sensory intake or an environmental openness (Lacey and Lacey, 1974). Emotionally, HR has been associated with the experience of anxiety. As such, high tonic HR is thought to reflect fear, while low tonic HR may reflect fearlessness.

Regarding studies on tonic HR, one of the best-replicated findings to date is that of reduced resting HR in antisocial individuals. In his review of this topic, Raine (1993) noted that of 14 relevant studies, there were no failures to replicate the finding of reduced resting HR in the antisocial groups. Low HR is a robust marker independent of cultural context, with the relationship having been established in the UK (e.g., Farrington, 1987), Germany (Schmeck and Poustka, 1993), New Zealand (Moffitt and Caspi, 2001), the USA (e.g., Rogeness *et al.*, 1990), Mauritius (Raine *et al.*, 1997b), and Canada (Mezzacappa *et al.*, 1997). It is also diagnostically specific (i.e., it is not found in other disorders), and multiple confounds have been ruled out (Raine *et al.*, 1997b).

All of these studies used child or adolescent samples, but consistent with this finding, low HR recently has also been associated with self-reported aggression in uninstitutionalized young adults (Scarpa *et al.*, 2000). In addition, 21 adult individuals from the community with a diagnosis of antisocial PD were found to have low HR during a social stressor (Raine *et al.*, 2000). However, this effect seems specific to antisocial behaviour and personality in general rather than psychopathic personality in particular. Reviews by Hare (1970; 1975; 1978) revealed no successes and at least 15 failures to obtain low resting HR in institutionalized, psychopathic criminals.

Low resting HR has recently been found to characterize life-course persistent offenders (Moffitt and Caspi, 2001), as well as those with diagnoses in institutionalized psychopathy, indicating that low HR is related to a pervasive pattern of severe rule violations. It also characterizes milder forms of antisocial and aggressive behaviour in children and adolescents that preface more serious criminal violations later in life. For example, in one of the few prospective studies examining this issue, psychophysiological measures were obtained at age 15 years in an unselected sample of male students, and government records were obtained 9 years later to measure any criminal violations. Only relatively serious offences were registered, ranging from theft to wounding, with the most common offence being burglary. By this criterion, 17 of the 101 subjects were found to have a criminal record. These 17 criminals were found to have significantly lower resting HR than the non-criminal controls, indicating reduced cardiovascular arousal as measured 9 years earlier. Low HR at age 3 years is also predictive of aggressive behaviour at age 11 years in both males and females (Raine *et al.*, 1997b). Reduced HR may reflect autonomic underarousal, as suggested by Raine *et al.* (1990a), or, alternatively, fearlessness in novel situations (Kagan, 1989; Venables, 1987), which theoretically could predispose a child to early social transgressions that cycle into later serious antisocial behaviour.

Regarding studies on phasic HR changes, the literature is less extensive, but suggests that psychopathic criminals exhibit anticipatory HR acceleration prior to an aversive event. In general, the findings of early studies indicated that psychopaths gave significantly larger acceleratory HR responses in anticipation of a signalled aversive stimulus such as a loud noise or electric shock, followed by reduced SC responses to the aversive stimulus itself (Hare, 1982; Hare and Craigen, 1974; Hare *et al.*, 1978). Hare (1978) interpreted these findings to suggest that psychopathic individuals have a very proficient active coping mechanism that allows them to 'tune out' aversive events. Although interesting, there has been very little further research on this phenomenon. Again, the relationship has not been studied specifically in relationship to antisocial PD, but it would be predicted that these individuals may engage in similar sensory rejection of aversive events. In terms of theories of socialization, this pattern may lead to poor conditioning in response to cues of punishment and thus decreased learning of appropriate social behaviour (Eysenck, 1977).

SC Studies and Antisocial Behaviour

Besides measuring SC phasically, as discussed above in the description of the SCOR, SC can also be measured tonically or in a resting state. In this regard, SC is measured in terms of resting level (SCL) or number of nonspecific SC fluctuations (NSF). NSFs are changes in SC that look like orienting responses, but do not occur in response to a known stimulus. Both SCL and NSF are thought to reflect some baseline level of physiological sympathetic arousal, and thus have often been associated with fear emotions in the fight/flight response.

Recent reviews of SC studies (Raine, 1993; Scarpa and Raine, 1997) indicate that

1. There is some evidence for SC underarousal in antisocial individuals, particularly with respect to nonviolent and non-impulsive forms of crime.
2. SCOR deficits seem specific to antisocial individuals with concomitant schizotypal personality features.
3. Prior findings up to 1978 of reduced SC responsivity to aversive stimuli in psychopaths generally have not been observed in more recent studies, either in psychopathic or non-psychopathic antisocial populations.
4. The strongest findings lie with respect to reduced SC classical conditioning in antisocial populations.

These findings are summarized below.

With regard to SC underarousal, both SCL and NSFs have been found to be reduced in antisocial groups in a number of studies, though this has not been entirely consistent. As with HR, the findings are primarily in uninstitutionalized subjects with mild forms of aggressive or other antisocial behaviour. Again, however, in the prospective study by Raine *et al.* (1990a), it was found that a reduced number of NSFs at age 15 years predicted criminal behaviour 9 years later. Raine and colleagues suggested that this pattern, along with low resting HR, reflected autonomic underarousal in criminals-to-be. In an early review of the literature on psychopathy, Hare (1978) concluded that these individuals are characterized by reduced SCL, but not NSFs. Thus, it is possible that reduced SC arousal is also related to antisocial PD in adulthood. This has recently been confirmed in a study showing that individuals with antisocial PD show reduced SC activity during a social stressor (Raine *et al.*, 2000).

Regarding SCORs, Hare (1978) had concluded that the research to that date showed reduced SC responses to aversive, but not neutral tones (i.e., a reduced defensive response, but no difference in orienting response). This pattern of findings, however, has not been consistently replicated in research conducted after that review. Nevertheless, deficits in SCOR have been consistently observed in individuals who display both antisocial behaviour and features of schizotypal PD. For example, antisocial adolescents with schizotypal features had significantly lower SCORs than those with only schizotypal features (Raine and Venables, 1984). In his review, Raine (1993) also noted that, with the exception of one study, reduced frequency of SCORs was found only in studies where antisocial behaviour was combined with schizotypal or schizoid characteristics. Thus, it seems that SCOR deficits may be specific to schizotypal criminals. Since the number of SCOR responses may be related to frontal lobe functioning (Raine *et al.*, 1991), Raine interpreted these results as possibly reflecting frontal dysfunction in this particular subgroup of antisocial individuals. Further support for interpreting reduced SC activity in terms of prefrontal deficits

comes from a recent study showing that individuals with antisocial PD have both reduced prefrontal grey matter and reduced SC activity (Raine *et al.*, 2000). Furthermore, when antisocials were divided into those with and those without reduced prefrontal grey, it was the subgroup with prefrontal grey deficits who showed SC deficits compared to those without prefrontal deficits.

Perhaps the strongest findings for SC activity lie with respect to reduced classical conditioning in antisocial populations. Classical conditioning involves learning that an initially neutral event (i.e., a conditioned stimulus [CS]), when closely followed in time by an aversive event (i.e., an unconditioned stimulus [UCS]), will develop the properties of this UCS. Eysenck (1977) argued that the socialization process and development of a 'conscience' stems from a set of classically conditioned negative emotional responses to situations that have previously led to punishment. In this way, socialized individuals develop a feeling of uneasiness at even contemplating antisocial behaviour, presumably because such thoughts elicit representations of punishment earlier in life. Furthermore, according to Eysenck's theory, arousal levels are related to conditionability, such that low levels of arousal predispose to poor conditionability and high levels to good conditionability.

The central idea in Eysenck's theory is that antisocials are characterized by poor classical conditioning. Classical conditioning has frequently been assessed with SC: a neutral tone (CS) is presented to the subject, followed a few seconds later by either a loud tone or an electric shock (UCS). The key measure is the size of the SCR elicited by the CS after a number of CS-UCS pairings. In a review by Hare (1978), 13 out of 14 studies reported significantly poorer SC conditioning in antisocial populations. In a review of studies since 1978 by Raine (1993), all six studies showed some evidence of significantly poorer SC conditioning in antisocials, including psychopathic gamblers, other psychopaths, conduct-disordered children from high social-class backgrounds, and criminals from good homes. These results are also consistent with findings of reduced SC arousal in antisocial populations, as presented above.

EEG, ERPs, and Antisocial Behaviour

The EEG reflects the electrical activity of the brain recorded from electrodes placed at different locations on the scalp according to the standardized international 10–20 system. The EEG can be broken down into different frequency components, most commonly delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (13–30 Hz). Alpha and beta are also often subdivided into slow and fast components. The EEG can be either clinically scored by observing the chart record to detect excessive theta or slow-wave activity, or subjected to a more quantitative computerized analysis, which more objectively delineates the EEG into different components.

EEG frequency has been aligned with a continuum of consciousness, with delta associated with sleep, theta associated with drowsiness and low levels of alertness, alpha associated with relaxed wakefulness, and beta associated with alertness and vigilance. As such, individuals with a predominance of theta or slow alpha activity would be viewed as having relatively reduced levels of cortical arousal, while those with relatively more fast alpha and beta activity would be viewed as relatively more aroused.

In a review by Mednick *et al.* (1982), the authors concluded that there is a high prevalence (25–50%) of EEG abnormalities in violent criminals, especially recidivistic offenders, compared with the 10–15% normally found in the general population. Volavka (1987) and Milstein (1988) drew similar conclusions for crime in general and violent crime in particular. Reviews of the EEG literature on psychopathy are less consistent. Hare (1970) initially concluded that psychopaths are characterized by excessive slow-wave activity. Later reviews, however, concluded that the evidence of EEG abnormalities in psychopaths is inconsistent (Blackburn, 1983; Syndulko, 1978), with one study even showing the opposite pattern that primary psychopaths were *more* aroused than secondary (schizoid) psychopaths.

The ERP refers to averaged changes of electrical activity of the brain in response to specific stimuli. ERP responses typically follow a sequence of early, middle, and late components which are thought to reflect the psychological processes of environmental filtering, cortical augmenting, and attention, respectively. ERP studies of antisocial behaviour have primarily involved psychopathic populations, and a review of this literature (conceptually broken down into early, middle, and late latency studies) was presented by Raine (1989). The main conclusions of this review are findings of

1. long early latency brainstem averaged evoked responses, reflecting excessive environmental filtering and reduced arousal
2. increased middle latency ERP amplitudes to stimuli of increasing intensity, which has been linked to sensation seeking
3. enhanced late latency ERP P300 amplitudes to stimuli of interest, suggesting enhanced attention to stimulating events.

Raine suggested that these processes may be causally linked, such that individuals with chronically low levels of arousal (possibly caused by excessive filtering of stimuli) would seek out stimulating events (including risky situations) in order to increase their levels of arousal to more optimal levels. This stimulation-seeking may partly account for the enhanced attention shown to events of interest.

While the evidence is unclear in terms of antisocial PD, there is again some reason to believe that impaired EEG and ERPs may reflect an early risk of later criminal behaviour. As reflected in the prospective study by Raine *et al.* (1990a; 1990b), criminals had significantly more slow-frequency EEG theta activity and larger N100 ERPs to target stimuli, as measured nine years earlier, than non-criminals. These findings are consistent with notions of underarousal and enhanced early stimulus attention in criminals and psychopaths.

Psychophysiological Study of Borderline PD

There are only two known studies examining the psychophysiological correlates of borderline PD. In the first study, a group of female patients with borderline PD were compared to female controls with no psychopathology on emotional reactions to pleasant, neutral, and unpleasant slides as measured by self-report, HR, SC, and eye-blink startle response (Herpertz *et al.*, 1999). In the second study, a comparison group of patients with avoidant PD was added to the borderline PD and normal control groups (Herpertz *et al.*, 2000). It is unclear whether the same individuals were used in both studies to form the borderline PD and normal control groups. The eye-blink startle response measures the automatic blink response that occurs to a startling probe, and is typically magnified when viewing unpleasant stimuli, but diminished when viewing pleasant stimuli. On the basis of previous theories of borderline PD, the authors predicted heightened affective responsivity in the subjects with this diagnosis.

Contrary to predictions, the borderline PD and control groups did not differ in startle response or HR responsivity in both studies, and the borderline PD group exhibited significantly reduced SC responses to all three stimulus categories. Moreover, the borderline PD group reported less pleasant affect to pleasant and neutral stimuli and less arousal to neutral stimuli. Self-report ratings on impulsivity, sensation seeking, and aggressiveness also did not account for the findings of SC hyporesponsivity. The authors speculated that this finding of decreased electrodermal responsiveness instead reflected problems in attentional processing.

Psychophysiological Study of Narcissism

There is one known study relating psychophysiological variables to narcissism (Kelsey *et al.*, 2001). As with many of the other studies reviewed here, narcissism was measured by a dimensional scale in this study, and the diagnostic syndrome of narcissistic PD was not assessed. Nonetheless, this study provides an initial peek at the psychophysiology of the major features of this disorder, including egocentricity, grandiosity, arrogance, a need for admiration, and a lack of empathy.

In this study, 40 undergraduate men who scored high or low on the Narcissistic Personality Inventory were compared on measures of pre-ejection period (PEP), frequency of SC responses, and HR reactivity in anticipation of an aversive stimulus. PEP was included as a measure of beta-adrenergic cardiac reactivity, primarily reflecting sympathetic cardiac activity; SC was included as a measure of sympathetic activity; and HR was included as a measure of both sympathetic and parasympathetic influences. Because narcissism has been posited to be an underlying feature of psychopathy, the authors hypothesized reduced sympathetic activity overall and enhanced parasympathetic control of the heart.

The results indicated greater PEP shortening, HR deceleration, and SC response habituation in the high narcissism group in anticipation of the aversive stimulus. Consistent with one hypothesis, the cardiac deceleration was interpreted as reflecting parasympathetic dominance. Contrary to the other hypotheses, however, the PEP and SC results suggested a fractionation of the sympathetic response. Shortened PEP reflects heightened sympathetic reactivity, while reduced SC activity reflects reduced sympathetic activity. The authors interpreted these findings according to a model of arousal proposed by Pribram and McGuinness (1975), suggesting that low SC activity in narcissism reflects physiological underarousal, HR deceleration reflects internally generated attention and activity, and PEP reactivity reflects heightened effort during the processing of aversive information.

Summary

For antisocial, psychopathic personality, the psychophysiological results suggest several conclusions. First, psychophysiological underarousal may reflect an early predisposition to mild antisocial behaviour that cycles into more serious recidivistic crime later in life. Second, as suggested by poor SC classical conditioning and low tonic SC, psychopathic and antisocial personality may be characterized by poor passive avoidance learning, which would interfere with the ability to learn to avoid misbehaviour in response to the anticipation of punishment. Because SC and HR have been associated with anxiety and fear, it is conceivable that these findings, along with underarousal, may also reflect the callousness and unemotionality that is characteristic of the psychopathic personality. Parallel findings of reduced SC responsivity in borderline PD and in narcissism suggest that they instead may relate to behaviour that is characterized by impulsivity and risk-taking (common to both antisocial and borderline PD) or may reflect an attentional 'tuning out' of aversive environmental stimuli in order to manage negative affect or maintain self-esteem (common to both antisocial and narcissistic PD). Third, low resting HR is the best-replicated psychophysiological correlate of antisocial behaviour. Fourth, findings in antisocial personality using ERPs are consistent with the notions of both increased sensation seeking and focus on events of interest, a fact which could explain the increase in impulsivity and risky behaviour seen in this personality style. Lastly, findings suggest that antisocial individuals with schizotypal PD may reflect a special subgroup of criminality characterized by frontal lobe dysfunction which is reflected in SCOR hyporesponsivity. Further study is necessary to see how the above findings generalize to clinically diagnosed antisocial PD, and a clear need is present to study the psychophysiology of the other Cluster B PDs.

CLUSTER C PERSONALITY DISORDERS

Cluster C includes the avoidant, dependent, and obsessive-compulsive PDs. Avoidant PD is defined as a pattern of social inhibition, feelings of inadequacy, and hypersensitivity to negative evaluation; dependent PD is defined as a pattern of submissive and clinging behaviour related to a need be taken care of; and obsessive-compulsive PD is defined as a pattern of preoccupation with orderliness, perfectionism, and control (American Psychiatric Association, 1994). Each of these PDs has some association with underlying feelings of anxiety and internalizing behaviours. To the authors' knowledge, the only Cluster C PD that has been studied psychophysiologically is avoidant PD, which will begin the following section. Inhibited temperament in children, which includes shy and cautious behaviour, along with fear of novel or unfamiliar people and situations, is also discussed as a possible developmental precursor to avoidant PD. No studies are known that directly assess obsessive-compulsive PD in relation to psychophysiological measures, but the DSM-IV notes that many features of this PD overlap with 'type A' personality (e.g., hostility, competitiveness, and time urgency). Type A personality has been studied extensively in relation to cardiovascular disease risk. As such, a brief review of this literature is also provided.

Psychophysiological Studies of Avoidant PD

As described above, avoidant PD involves a pervasive pattern of social inhibition or avoidance of social situations that seems to revolve around feelings of being inadequate and fears of consequent criticism and rejection by others. According to DSM-IV, there appears to be a great deal of overlap between this PD and the Axis I disorder of social phobia, generalized type. As such, several studies have been conducted to assess for differences or similarities between social phobia with and without avoidant PD, and they do indeed suggest a distinction between these two forms of social phobia in terms of severity.

In general, studies which compare these subgroups of social phobia on measures of subjective and behavioural anxiety find greater reports of anxiety and behavioural impairment in those diagnosed with avoidant PD or the generalized subtype of social phobia (Herbert *et al.*, 1992; Holt *et al.*, 1992). If social phobia with avoidant PD indeed reflects a more severe form of social phobia, one would predict greater psychophysiological reactivity to fearful social situations in these individuals. There seems to be a discrepancy, however, between the subjective anxiety reported and the psychophysiological profile exhibited in this population. For example, one study compared groups with discrete social phobias, generalized social phobia with avoidant PD, and generalized social phobia without avoidant PD on HR recorded during an impromptu speech (Turner *et al.*, 1992). The results indicated no HR differences among these three groups. Similarly, two other studies showed no HR differences during a public-speaking task between socially phobic individuals with avoidant PD and normal controls, but those with a specific social phobia did show higher HR with the task (Heimberg *et al.*, 1990; Hofmann *et al.*, 1995). Interestingly, in these studies, the group with avoidant PD reported the greatest subjective anxiety and fear, although their HR responses did not differ from controls and were lower than the group without avoidant PD.

Overall, these findings indicate a discordance between the subjective and HR responses in socially phobic individuals with avoidant PD. Although the reason for this discordance is unknown, Hofmann *et al.* (1995) suggest several possibilities — that social phobias with avoidant PD have a less coherent cognitive fear structure, that other emotions may be blending with fear to attenuate HR responses, or that the tonic presence of fearful cognitions in avoidant PD may inhibit further processing of fearful material

because the feared event provides no new emotional information. A possibility that was not considered is that excessive tonic worry in avoidant PD may inhibit the information processing of feared stimuli because it simply serves as an attentional distraction, thus leading to reduced HR reactivity. Either of the latter two interpretations is supported by a study by Herpertz *et al.* (2000) in which eye-blink startle, HR, and SC responses to emotional stimuli were compared in groups with avoidant PD and borderline PD, or normal controls. Relevant to avoidant PD, the results showed increased startle magnitude relative to the borderline PD and control groups, but only at baseline (i.e., before the emotional stimuli were presented). No group differences were found for SC and HR responses. Since the eye-blink startle response is specifically sensitive to anxious/fearful mood, this finding suggests that individuals with avoidant PD may have a tonically high level of anxiety that interferes with ongoing information processing.

Psychophysiological Studies of Inhibited Temperament in Children

Behavioural inhibition in childhood reflects a temperamental style of avoidance in situations that are novel or unfamiliar. According to DSM-IV, avoidant PD is described as often starting in infancy or childhood with characteristics of shyness, isolation, and fear of strangers and new situations. Thus, inhibited temperament in children may reflect a developmental precursor to avoidant PD, although it is clear that not all children with such shyness will go on to develop the PD.

In a series of studies, Kagan and his colleagues examined children who were classified as either behaviourally inhibited or uninhibited at 21 months of age by their tendency consistently to withdraw or interact respectively across various experimental situations. At 21 months, inhibited relative to uninhibited children were found to have higher and more stable HR while looking at unfamiliar pictures and listening to unfamiliar sounds (Garcia-Coll *et al.*, 1984). These children were again examined at ages 31 months, and 4, 5.5, and 7.5 years. At every age, it was observed that the behavioural differences which originally led to the classification as inhibited or uninhibited at age 21 months were still maintained between the two groups, and the inhibited children showed higher and more stable HR to a series of cognitive procedures than the uninhibited children (Kagan *et al.*, 1984; Kagan *et al.*, 1988; Reznick *et al.*, 1986). The results were replicated on a second cohort of children who were tested at ages 2, 3.5, 5.5, and 7.5 years, suggesting a stability of inhibited/uninhibited temperament over time, with those classified as inhibited having higher HR at each age than those classified as uninhibited (Kagan, 1989; Kagan, *et al.*, 1987). Taken together, these results indicate that high HR is associated with increased behavioural inhibition.

Kagan (1989; 1994) has suggested that this heightened sympathetic reactivity in inhibited children stems from a lower threshold of excitability in limbic sites, particularly the amygdala and hypothalamus. If behavioural inhibition is thus reflected in increased sympathetic activity, other sympathetic measures should show similar group differences in inhibited individuals. Indeed, Kagan (1994) reports findings of increased sympathetic activity reflected in other measures such as salivary cortisol, urinary norepinephrine, and facial skin temperature. Furthermore, in a study of 3-year-old children, those who were inhibited exhibited heightened levels of both resting HR and SC relative to those who were uninhibited (Scarpa *et al.*, 1997), again providing support for the relationship between inhibition and physiological arousal through limbic activation.

If behavioural inhibition does developmentally precede avoidant PD in adulthood, similar sympathetic arousal and reactivity would be expected in adults with avoidant PD. As described in the preceding section, however, there is no evidence for increased sympathetic levels or responsivity in adults with avoidant PD. In fact, of the few studies examining psychophysiology of avoidant PD, all indicated similar levels and reactivity of HR and/or SC compared to normal controls (Herpertz *et al.*, 2000; Hofmann *et al.*, 1995; Turner *et al.*, 1992). It is possible, therefore, that inhibited temperament in children is not a childhood precursor of adulthood avoidant PD. Alternative explanations could also be that the physiological responses of inhibited children who grow up to develop avoidant PD differ from those who do not develop the disorder, or that their physiological responsivity changes as they grow into adulthood.

Psychophysiological Studies of Type A Personality

As originally described by Friedman and Rosenman (1959), the type A behaviour pattern is characterized by excessive competitiveness; impatience; hostility; overcommitment to work; and a loud, rapid, and pressured vocal style. This pattern has many behavioural similarities to the symptoms of obsessive-compulsive PD, suggesting a possible overlap. We are aware of no psychophysiological studies of obsessive-compulsive PD; however, psychophysiological reactivity in relation to type A personality has been extensively studied (see Houston, 1983 and Harbin, 1989 for reviews). Although other measures have been included, blood pressure (BP) and HR have been the foci of these investigations due to the link between type A behaviour and coronary heart disease (e.g., Cooper *et al.*, 1981). A brief summary of this literature follows.

Results from reviews have generally concluded that, relative to type B individuals (i.e., people who are easy-going, patient, and soft-spoken), type A individuals show greater BP and HR responses to stressors (Houston, 1983; Harbin, 1989). In a re-examination of the same data presented by Houston (1983), Holmes (1983) emphasized that the most consistent findings were with respect to systolic BP and that this effect was of small magnitude. That is, 13 out of 20 experiments revealed reliable differences in systolic BP, while only 4 of 18 experiments revealed reliable diastolic BP effects and only 6 of 24 experiments revealed reliable HR effects. Moreover, the median group difference in systolic BP was 6 mmHg, a difference that is not typically considered to be physiologically meaningful.

Despite some inconsistency in findings and the suggestion that this relationship is weak to moderate at best, the quantitative review by Harbin (1989) still showed an overall effect for increased cardiovascular activity in those with the type A behaviour pattern. It appears that more consistent findings are obtained when the individual is faced with a stressor task that is challenging, where there is an intermediate chance of failure, where there is moderate external incentive to do well, or where there is an interpersonal encounter that is annoying or harassing to the subject (Houston, 1992). Moreover, the most reliable BP responses have been found when type A behaviour is measured according to a structured interview that assesses behavioural qualities, rather than by self-report measures. Finally, it may be that not all aspects of type A personality are equally related to physiological reactivity. Hostility, for example, has been identified as the most powerful type A characteristic to predict coronary heart disease (Dembroski and Costa, 1987), and it has been associated with increased cardiovascular reactivity in response to stress (see Smith and Leon [1992] for a summary).

As summarized by Smith and Leon (1992), several psychological explanations have been posited for the increased cardiovascular reactivity found in type A personality. In general, these explanations imply that the stressor tasks activate a relevant type A characteristic that leads these individuals to increase their efforts, and this is associated with increased sympathetic nervous system activity. Such a behavioural response on the part of the person with type A personality may be due to excessively high standards for success,

perceptions of threat of loss of control over the environment or other people, or a high level of self-involvement. It is also possible that these individuals simply create more frequent stressful situations in their lives, the net result of which is more frequent physiological reactivity and consequent increased risk of coronary heart disease.

Summary

None of the Cluster C PDs have been studied extensively enough to form substantive conclusions in relation to psychophysiological variables. Nonetheless, studies on associated features, such as inhibited temperament and the type A behaviour pattern, as well as the few studies of avoidant PD, suggest some directions. Sympathetic reactivity is implicated in both inhibited temperament and type A personality. However, it is more pervasive in relation to behavioural inhibition, and seems to be primarily associated with systolic BP in relation to type A behaviour. Moreover, no studies found HR differences between avoidant PD, in particular, and normal control groups. Thus, it appears that inhibited temperamental style, avoidant PD, and type A personality (which is the closest personality analogue to obsessive-compulsive PD) are distinct constructs with distinct psychophysiological profiles. As such, one suggested direction for further research in this area includes the study of whether behavioural inhibition does indeed serve as a developmental precursor to adult avoidant PD, and, if so, why is there a change in psychophysiological response over time? Another suggested direction is to examine whether the cardiovascular findings in type A personality generalize to obsessive-compulsive PD. Lastly, more work overall needs to be done on the psychophysiology of all of the Cluster C PDs.

CONCLUSIONS

As can be seen from the above review, the psychophysiological findings with respect to PD are quite diverse. While this might be expected given the diverse characteristics of each of the 10 PDs described in the DSM-IV, it is also notable given the high degree of overlap in PD symptomatology and the resulting high PD comorbidity. As such, this psychophysiological research provides some insight into the differences that may underlie the various PDs. Caution, however, is warranted due to the fact that there has been little work on the diagnostic syndromes of *any* of the PDs. That is, what work has been done typically has focused on dimensional characteristics, specific symptoms, or associated features of the disorders rather than the clinical diagnosis as a syndrome. Further research is definitely needed in this area, but despite this major limitation, the following conclusions are suggested.

First, the psychophysiological findings generally indicate cognitive abnormalities in Cluster A disorders, especially schizotypal PD, that mirror findings in schizophrenia and thus suggest a common underlying biological marker. Second, the findings generally indicate arousal and cognitive learning deficits as well as emotional deficits in Cluster B disorders, which particularly reflect impulsive, antisocial behavioural styles. Third, primarily emotional contributions are suggested in relation to Cluster C PDs, particularly to fear in avoidant PD and to hostility/anger in obsessive-compulsive PD. These findings suggest that the behavioural similarities within PD clusters and differences across clusters may indeed be reflected in corresponding psychophysiological profiles.

It is also noted, however, that certain paradigms for measuring psychophysiology do not cut across the study of PDs. SC conditioning, for example, has in the past been extensively studied in relation to antisocial behaviour, but not in the other PDs. Similarly, SPEM has been studied in relation to schizotypal PD, but not specifically in the other PDs. As such, psychophysiology still has much to offer in terms of elucidating the cognitive and emotional psychology of PDs. This review establishes a clear need for further work on the psychophysiology of PDs.

Finally, probably the most striking and consistent finding in the field of the psychophysiology of PDs is the low autonomic arousal of antisocial behaviour and personality. Furthermore, low autonomic arousal is diagnostically specific to antisocial personality within the diagnostic subfield of PDs in that no other PD is characterized by low autonomic arousal. In particular, low resting HR is the best-replicated biological correlate of antisocial behaviour in community samples. A challenge for future research lies in placing this finding, linked to impairments of the prefrontal cortex, within a wider, neurophysiological, neuropsychological, and psychosocial context. A similar challenge exists with respect to the larger literature on the psychophysiology of PDs overall.

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XXVI-5

Neuropsychology of Personality Disorders

Martina M. Voglmaier

INTRODUCTION

Intuitively, one might expect individuals with personality disorders to have neuropsychological deficits. The hypersensitivity of the paranoid and avoidant personalities, the rigidity of the obsessive-compulsive personality, the perceptual distortions of the schizotypal personality, and the limited social and emotional responsiveness of the schizoid personality suggest areas of deficit fascinating to the researcher interested in brain–behaviour relationships. Yet the study of neuropsychological functioning in personality disorders is in its infancy, primarily because of methodological problems associated with studying these disorders. The focus of this chapter is a general review of studies of clinical neuropsychological test performance in DSM-IV personality disorders as well as a description of the methodological problems associated with their study. Information processing, psychophysiological, and structural anatomical studies are dealt with elsewhere in this text.

GENERAL METHODOLOGICAL CONSIDERATIONS

Before reviewing empirical studies of neuropsychological test performance in personality-disordered individuals, a number of general methodological concerns must be addressed. Typically, in such studies, personality disorders are diagnosed by structured clinical interview, and subjects are administered a battery of neuropsychological tests measuring one or more cognitive domains, such as attention, memory, language, visuospatial perception, executive functions, and motor skills. Test performance of a group of individuals with a personality disorder of interest is then compared to that of one or more groups of subjects with either a different personality disorder, a mixed group of 'other' personality disorders, another psychiatric disorder, or no psychiatric disorder.

Research in this area is fraught with methodological difficulties. Samples differing in selection criteria, inpatient status, medications, and gender may result in inconsistent findings. Moreover, diagnosing personality disorders is a decidedly unclear science (Strack and Lorr, 1997). The clinical definition of character traits, severity, and impact on functional ability can be subjective, and whether a personality disorder can be diagnosed by self-report to an examiner in a preset period of time has been questioned (Oldham and Skodol, 1992). The DSM-IV requires a specific number of character traits to meet clinical diagnostic criteria for a personality disorder, and some studies include individuals who have fewer than the cut-off number of traits, such as those defined as having a 'probable' disorder.

Another concern is determining the specificity of any cognitive deficits given the co-occurrence of the 10 personality disorders with each other (Stuart *et al.*, 1998), the co-morbidity of personality disorders with Axis I disorders (Oldham and Skodol, 1992), and the possibility of misdiagnosis of behavioural traits associated with neurological disorders, such as head trauma, substance abuse, and temporolimbic epilepsy (Devinsky and Najjar, 1999). For example, increased rates of depression have been associated with a number of personality disorders (Table XXVI-5.1). Even with detailed historical and diagnostic information, it could be difficult to determine whether cognitive deficits evinced by a group of subjects are related to the personality disorder under examination, to concurrent symptoms of depression, to cognitive vulnerabilities related to the predisposition to depression, or to treatments for depression. A related concern is that individuals with personality disorders may be vulnerable to neuropsychological dysfunction from secondary sources such as precursors or lifestyle behaviours associated with maladaptive personality traits. For example, risk-taking behaviours, substance use, impulsivity, and aggression in antisocial personality disorder (ASPD) may increase the risk of head trauma in this population, and childhood trauma (physical, emotional, or sexual) may predispose an individual to head injury, borderline personality traits, symptoms of post-traumatic stress, and dissociative disorders (Paris, 1997).

The battery of neuropsychological tests employed in research studies is also of importance. Often, only a few tests or cognitive domains are examined, resulting in a limited view of cognitive functioning. For example, if only executive-function tasks are employed, it cannot be determined whether any weaknesses in this domain are due to a generalized deficit associated with having a psychiatric illness, or to a domain-specific deficit associated with the personality disorder being examined. Finally, there is some evidence for sex differences in cognitive functioning in some personality disorders (Voglmaier *et al.*, submitted). Grouping males and females together in such studies may result in inconsistent findings.

DSM-IV CLUSTER A: THE ODD/ECCENTRIC PERSONALITY DISORDERS

General Findings

Schizotypal, paranoid, and schizoid personality disorders make up this cluster of odd and eccentric personalities. By far, the most research on cognitive functioning in this cluster has been on schizotypal personality disorder (SPD). SPD is characterized by oddities in appearance, perception, and behaviour, as well as marked discomfort in close relationships. On the hypothesis that this disorder is biologically related to schizophrenia, studies have focused on cognitive domains known to be impaired in schizophrenic subjects, that is, abstraction, attention, language, and verbal learning and memory (e.g., Gur *et al.*, 1991; Saykin *et al.*, 1991).

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Table XXVI-5.1 Methodological considerations in the study of personality disorders

	Axis I	Axis II	Precursors	Lifestyle
Cluster A				
Schizotypal (SPD)	Depression, dysthymia, social phobia, agoraphobia	Cluster A BPD, OCP	—	—
Schizoid (SZD)	Depression, substance abuse	Cluster A APD	—	—
Paranoid (PPD)	Depression, delusional disorder, agoraphobia, bulimia	Cluster A	—	—
Cluster B				
Borderline (BPD)	Depression, bipolar, PTSD, substance abuse, dissociative disorders	Cluster B SPD, OCP, DPD	Physical/sexual abuse, emotional trauma, head trauma	Reduced impulse control, substance abuse
Antisocial (ASPD)	Substance abuse, depression, anxiety	Cluster B ADHD, OCP	Conduct disorder	Reduced impulse control, increased risk taking, violence, substance abuse
Histrionic (HPD)	Depression	Cluster B	—	—
Narcissistic (NPD)	Depression	Cluster B	—	—
Cluster C				
Avoidant (APD)	Depression, anxiety disorders, social phobia, GAD, panic disorder	Cluster C SZD	—	—
Dependent (DPD)	Depression, anxiety disorders	Cluster C	—	—
Obsessive-compulsive (OCP)	Anxiety disorders, bulimia	Cluster C SPD, BPD, ASPD	—	—

PTSD [post-traumatic stress disorder], GAD [generalized anxiety disorder], and ADHD [attention deficit hyperactivity disorder].

There have been relatively few comprehensive studies of neuropsychological function in individuals who meet full DSM diagnostic criteria for SPD. Generally, the extant studies have shown deficits in sustained attention (Roitman *et al.*, 1997), working memory (Farmer *et al.*, 2000; Roitman *et al.*, 2000), abstract reasoning (Voglmaier *et al.*, 1997), and verbal learning (Voglmaier *et al.*, 1997; Bergman *et al.*, 1998) that are similar to but less severe than those evident in schizophrenic subjects (Table XXVI-5.2). These findings have been hypothesized to reflect involvement of similar frontal, left hemisphere, and temporal-limbic brain areas.

My colleagues and I examined the neuropsychological profile of SPD (Voglmaier *et al.*, 1997). We studied a wide array of cognitive functions in 10 right-handed men with DSM-IIIR-defined SPD and 10 matched non-psychiatric comparison subjects (Voglmaier *et al.*, 1997). All subjects were unmedicated and were carefully screened for Axis I disorders, neurological disorders, and substance abuse. Cognitive domains included abstraction, verbal and spatial intelligence, language, learning, memory, attention, and motor skills. Analyses covaried the effects of general ability (IQ) and symptoms of depression, variables that were found to differ between subject groups. The resultant profile revealed a mild, general decrement in most cognitive domains, as well as more severe dysfunction in verbal learning and abstract reasoning. Specifically, SPD subjects showed significant decrements in performance on the California Verbal Learning Test (CVLT) (Delis *et al.*, 1987), a word-list learning measure which requires semantic organization for efficient performance, and on the Wisconsin Card Sort Test (WCST) (Heaton, 1981), a measure requiring concept formation, abstraction, and mental flexibility. The CVLT involves five presentations of a list of 16 'shopping items', which can be grouped into four semantic clusters (fruits, spices, clothing, and tools) to enhance learning. The SPD subjects learned significantly fewer words over the five trials, and used the clustering strategy less frequently than comparison subjects. There was no evidence of increased rate of forgetting. On the WCST, subjects were asked to sort cards by category (number of items, shape, or colour) and shift the categorical set based on limited feedback from the examiner. SPD subjects formed significantly fewer categories and made more perseverative responses than control subjects. Overall performance by male SPD subjects was similar to but less severe than in schizophrenic subjects. Females with SPD were found to have less severe cognitive deficits than males (Voglmaier *et al.*, submitted).

Questions were raised about the source of the apparent deficit in verbal learning in SPD. Because the CVLT requires intact language, verbal learning, and memory, and semantic organization skills for efficient performance, it was unclear to us whether the SPD group's reduced performance on this task reflected a primary deficit in language processes, learning, or concept formation and organization. For further examination of the verbal learning deficit in SPD, we evaluated selected components of verbal and non-verbal attention; learning and memory, including short-term retention (trigram versus pattern recall); supraspan learning (serial digit learning versus spatial block span); persistence (verbal versus design fluency); learning (CVLT versus Continuous Visual Memory Test [Trahan and Larrabee, 1988]); and memory retention (WMS-R Logical Memory versus Visual Reproductions [Wechsler, 1987]). In addition to a mild generalized deficit, 16 carefully screened and matched male SPD subjects revealed deficits on verbal measures of persistence, short-term retention, and learning (Voglmaier *et al.*, 2000). Non-verbal analogues of these tests, supraspan learning, and long-term retention of newly learned information were relatively preserved. The results suggested that the verbal learning deficit apparent on the CVLT is at least in part the result of a deficit in the early processing (encoding) stages of verbal learning, and not solely due to a primary deficit in organization or conceptualization.

Language functioning in SPD remains unclear. Standard neuropsychological tests of language ability have not revealed specific deficits in this domain, such as naming, repetition, comprehension, reading, and spelling (Voglmaier *et al.*, 1997). Reduced WAIS-R (Wechsler, 1981a) Vocabulary subtest performance was found in our group of 16 male SPD subjects, and we speculated that a primary deficit in verbal learning could account for such performance (Voglmaier *et al.*, 2000). Based on language samples, we have documented an increased amount of thought disorder in male SPD subjects that is qualitatively similar to that of schizophrenics

Table XXVI-5.2 Neuropsychological test performance in personality disorders

	General ability/IQ	Attention/working memory	Learning	Memory	Language	Visuoperception/ construction	Abstraction/executive functions	Sensory and motor skills
CLUSTER A Schizotypal (SPD)	(+)Mild General Decrement, WAIS-R VIQ < PIQ	(+)CPT-IP (+)COWAT (CFL) (+)Trigram Recall (+/−)Visuospatial Working Memory (−)Design Fluency (−)Digit Span (−)Pattern Recall (−)HRB Trail-Making Test	(+)CVLT, total words learned; semantic clusters (−)CVMT (−)Serial Digit Learning (−)Corsi Block Tapping	(−)WMS-Logical Memory, % retained (−)WMS-Visual Reproductions, %retained	(+/−)WAIS-R Vocabulary (−)BNT (−)BDAE-Complex Ideation (−)Sentence Repetition (−)WRAT-R Spelling (−)WRAT-R Reading	(−)WAIS-R Block Design (−)WAIS-R Picture Arrangement (−)Judgment of Line Orientation	(+)WCST, fewer categories (+)WCST, perseverative responses	(−)Finger Tapping Test
CLUSTER B[a]		(+)Serial 7's (−)Digit Span Forward		(+)Delayed Memory for 3 items at 10 min (−)Orientation	(+)Naming Common Objects (−)Word Repetition		(+)Similarity/ Dependence Comparisons (+)Proverb Interpretation	
Borderline (BPD)	(+/−)WAIS-R	(+/−)Stroop Color-Word Test (−)Trail-Making Test, Part B (−)Digit Span (−)Digit Symbol Test		(−)WMS-R Logical Memory (−)WMS-R Figural Memory (−)Story Recall (−)Verbal Recall with Interference	(−)WAIS-R Vocabulary	(+/−)Rey-Osterreith Copy/Recall (+)Matching Familiar Figures Test (−)WAIS-R Block Design (−)Embedded Figures Test	(−)Porteus Mazes	(+)Left side soft signs
Antisocial (ASPD)		(+)Object Alternation (+)Stroop Color Naming (+)Divergent Thinking (+/−)COWAT (+/−)Visual GoNoGo (−)Mental Rotation (−)Trail-Making Test		(+)Sequential Matching Memory	(+)Visual Naming (+)Token Test (−)Aural Comprehension of Words	(+)Necker cube	(+/−)WCST (+)perseverative errors (+)Porteus Mazes Q-scores (e.g., rule-breaking errors)	(+)Odour identification

(+) Deficits evident in at least one study; (−) no deficits evident in at least one study; BDAE indicates Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 1983); BNT, Boston Naming Test (Kaplan *et al.*, 1983); COWAT, Controlled Oral Word Association Test; CPT-IP, Continuous Performance Test, Independent Pairs; CVLT, California Verbal Learning Test (Delis *et al.*, 1987); CVMT, Continuous Visual Memory Test (Trahan and Larrabee, 1988); HRB, Halstead Reitan Battery (Reitan and Wolfson, 1985); MAE, Multilingual Aphasia Examination (Benton and Hamsher, 1976); WCST, Wisconsin Card Sorting Test (Heaton, 1981); WAIS-R, Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981b), WRAT-R, Wide Range Achievement Test-Revised (Jastak and Wilkinson, 1984); WMS-R, Wechsler Memory Scale-Revised (Wechsler, 1987); [a]Burgess (1992), mixed Cluster B 'dramatic' personality disorder.

(Voglmaier *et al.*, 1996). Overall, neuropsychological test results from our laboratory imply involvement of frontal and left temporal brain structures and suggest a specific deficit in the early processing stages of verbal learning in SPD. The results support hypotheses of a dysfunctional semantic language network in schizophrenia (Spitzer *et al.*, 1993). Dysfunction, inefficiency, or reduced inhibition of such a system could result in verbal learning deficits, deficient facilitation by semantic context, magical thinking, and thought disorder.

The paranoid and schizoid personality disorders are also thought to have some biological connection to schizophrenia, but neuropsychological test performance has not been studied independently in these groups. It is noteworthy that schizoid personality traits (e.g., limited social and emotional responses) are similar to those seen in social-emotional or 'right-hemisphere' learning disabilities (Weintraub and Mesulam, 1983), making this disorder worth a detailed study of neuropsychological test performance.

Methodological Concerns

There are several additional methodological concerns that are specific to the study of SPD. First, there is considerable overlap between SPD and major depression and borderline personality disorder (Siever, 1992) (Table XXVI-5.1), disorders that may be associated with cognitive deficits. Second, many studies have assessed neuropsychological function in populations thought to be at higher risk of developing schizophrenia, in an attempt to elucidate markers of vulnerability to the disease. Studying these groups is considered preferable because they do not include the confounding factors of chronic institutionalization and medications. These high-risk samples include the non-psychotic first-degree relatives of schizophrenic probands, and the so-called 'psychosis-prone' populations, non-clinical groups who have abnormally high scores on one or more measures thought to assess schizotypal symptoms (such as the Chapman scales [Chapman and Chapman, 1985]). These samples are often described as 'schizotypal' or their results as indicative of 'schizotypy'. However, it is not clear how such groups are related to clinically defined SPD or how many (if any) of the DSM-IV diagnostic criteria are actually met by each subject. Although the results of these studies also suggest similar deficits in information processing (Chen *et al.*, 1998), abstraction (e.g., Tallent and Gooding, 1999), and left-hemisphere function (Overby, 1992), it is possible that relatives of schizophrenics and 'psychosis-prone' samples differ from SPD in phenomenology as well as severity.

A related concern is the possibility of heterogeneous subgroups among individuals who meet diagnostic criteria for SPD. Those with a family history of schizophrenia have been found to have more 'negative' symptoms (e.g., constricted affect, anhedonia, and no close friends) than those without such a family history. Moreover, some SPD samples may share a genetic link with mood disorders. These 'familial' and 'clinical' samples may represent different SPD subtypes (Bergman *et al.*, 2000) with differing neuropsychological test profiles. Finally, it is unclear how subject groups should be matched in terms of general ability. As in schizophenic subjects, because education, IQ and verbal skills may be reduced in SPD groups as a result of the disorder, matching patient and control groups on these indices may artificially dilute the extent of any deficits (e.g., the 'matching fallacy' [Meehl, 1970], matching high-functioning patients with low-functioning controls). In our laboratory, we have found that matching groups on age, sex, handedness, parental socio-economic status, and non-verbal general ability (e.g., the WAIS-R Block Design subtest) yields the most consistent results without affecting the cognitive domains of particular interest.

DSM-IV CLUSTER B: THE DRAMATIC PERSONALITY DISORDERS

General Findings

This cluster includes the borderline, histrionic, narcissistic, and antisocial personality disorders. Burgess (1992) studied neuropsychological test performance in a mixed group of 37 subjects who met diagnostic criteria for at least one of the 'dramatic' personality disorders and 40 non-psychiatric control subjects. Of the 37 patients, 30 patients had borderline personality disorder and seven had another Cluster B disorder. On a 16-item neuropsychiatric scale, the dramatic patient group showed relative deficits on tasks requiring multistep or multielement associative procedures (Table XXVI-5.2).

Within Cluster B, only the borderline and antisocial personalities have been studied independently with regard to neuropsychological function. In patients with borderline personality disorder (BPD), the results of cognitive studies have been inconsistent, perhaps due to heterogeneous samples. Some studies have found deficits on memory and executive tasks, suggesting that the behaviours and interpersonal problems associated with BPD result from deficits in attention, impulsivity, memory, and processing complex information (e.g., O'Leary *et al.*, 1991), and have hypothesized frontotemporal brain involvement in at least a subgroup of patients (Swirsky-Sacchetti *et al.*, 1993). Performance deficits may be exacerbated by stress or increased emotional arousal (Farrell and Shaw, 1994). Stein *et al.* (1993) found increased left-sided neurological soft signs in a group of 28 patients with 'impulsive' personality disorders: all 28 subjects met criteria for BPD, and 10 subjects also met criteria for antisocial personality disorder (ASPD). Increased soft signs correlated with reduced performance on the Trail-Making Test and Matching Familiar Figures Test, and the authors suggested the possibility of right-hemisphere and/or frontal network dysfunction. However, these results are difficult to interpret given the heterogeneity of the patient group, which included males, females, inpatients, and outpatients. Eight subjects met current diagnostic criteria for major depression and 25 subjects had histories of substance abuse.

van Reekum *et al.* (1996) found evidence of a high proportion of cerebral insults among borderline patients (including developmentally based and acquired insults) and a neuropsychological profile reminiscent of traumatic brain injured patients. It is unclear whether borderline samples in earlier studies had been carefully screened for such insults. A recent study of females that carefully controlled for history of neurological insult and symptoms of depression found essentially no deficits on neuropsychological tests of frontal executive and memory tests. Sprock *et al.* (2000) studied a group of 18 women with BPD, and compared them to female depressed patients and a non-psychiatric control group. They employed a battery of tests thought to tap frontal-executive (e.g., the Trail-Making Test, Part B; Stroop Color-Word Test; Porteus Mazes; and Rey-Osterreith Complex Figure) and memory functions (e.g., Rey-Osterreith Figure immediate and delayed memory, Wechsler Memory Scale-Revised Logical Memory, Figural Memory, Digit Span subtests, Story Recall Task, and Verbal Recall with Interference). There were essentially no differences in test performance between the BPD and control groups, and only the depressed patients showed evidence of cognitive dysfunction. Similarly, in a study that revealed abnormal hippocampal volumes in females with BPD and history of trauma, neuropsychological performance was related to symptoms of depression, but not to hippocampal volume (Driessen *et al.*, 2000). The results suggest there may be considerable heterogeneity in the cognitive functioning of BPD patients. Moreover, evidence of abnormal neuropsychological test performance may be confounded by affective state and/or history of cerebral insult.

The neuropsychology of antisocial personality disorder (ASPD) is also unclear. The results of studies have been conflicting (Table XXVI-5.2), and this may also be related to sampling differences and comorbid disorders. Executive dysfunction has been associated with antisocial behaviour in adolescent females with conduct disorder (Giancola *et al.*, 1998). In another study, deficient language skills, but not executive functions, were associated with conduct disorder in a mixed group of male and female adolescents (Dery *et al.*, 1999). Some studies have indicated that ASPD may be characterized by deficits in self-modulation of executive functions and verbal skills, suggesting that frontal and left-hemisphere abnormalities may be related to impulsive aggression (Miller, 1987). A recent meta-analytical review of 39 studies supported the hypothesis that executive-function deficits (e.g., planning, organization, goal-formulation and follow-through, self-monitoring, and problem-solving) are associated with antisocial personality (Morgan and Lilienfeld, 2000), implying involvement of frontal networks and dorsolateral/prefrontal brain structures. In a community sample of adults with ASPD that were screened for neurological disorders, deficits were evident on measures sensitive to orbitofrontal function (e.g., object alternation and spatial working memory), but not on standard measures of frontal-executive function (Dinn and Harris, 2000).

Methodological Concerns

The study of neuropsychological function in BPD is complicated by the high incidence of developmental and/or acquired brain insults (van Reekum *et al.*, 1996), and comorbid disorders such as depression, bipolar illness (Atre-Vaidya and Hussain, 1999), post-traumatic stress, and substance use (McGlashan *et al.*, 2000). Many studies mix male and female subjects, and often include medicated and/or hospitalized patients or those with current mood disorders and substance abuse. Similarly, the study of ASPD is confounded by overlap with substance abuse, attention deficits, depression, and anxiety (see Widiger *et al.*, 1993). Moreover, the aggressive, violent, and impulsive lifestyle of these patients may also increase their risk of head trauma.

DSM-IV CLUSTER C: THE ANXIOUS PERSONALITY DISORDERS

This cluster comprises the avoidant, dependent, and obsessive-compulsive personality types. There has been little research on these disorders, which are often comorbid with anxiety disorders (Alpert *et al.*, 1997; Paris, 1998). Neuropsychological function has not been studied directly in this cluster, although these subjects have made up 'other' or 'mixed' personality groups for comparison. The relationship of obsessive-compulsive personality (OCP) and obsessive-compulsive disorder (OCD) is unclear, although one study found that 36% of its sample of OCD patients also had OCP (Bejerot *et al.*, 1998). Neuropsychological deficits have been found in OCD (e.g., Bolton *et al.*, 2000) and sub-clinical compulsive samples (Matai-Cols *et al.*, 1997; Roth and Baribeau, 1996). It is unclear whether similar deficits could be expected in OCP, but this represents another untapped area for neuropsychological research.

CONCLUSION: FUTURE NEEDS AND NEW RESEARCH

The DSM-IV personality disorders represent a fascinating area of study for the neuropsychologist, yet their comprehensive study is in its infancy. The available data imply involvement of frontal, temporal, and left-hemisphere structures in schizotypal personality disorder, and orbitofrontal, dorsolateral/prefrontal, and possibly left-hemisphere structures in ASPD. Findings in patients with BPD have been inconsistent, with some studies showing involvement of frontal networks. In particular, the schizoid and OCP disorders represent interesting untapped areas worthy of comprehensive neuropsychological evaluation.

Methodological concerns such as sampling criteria, gender differences, comorbid disorders, and neurological risk factors limit the interpretation of the neuropsychological performance of individuals with personality disorders, and there is evidence that some personality disorders may include heterogeneous subtypes. In future studies, detailed assessment of Axis I and Axis II disorders and careful screening for head trauma, substance abuse, and current mood disorders will help to clarify the specificity of any neuropsychological deficits. It is recommended that subject groups include unmedicated individuals who meet full diagnostic criteria for the personality disorder under study, and that multiple cognitive domains be evaluated, with attention to the possibility of gender effects in cognitive functioning. Matching study groups for age, handedness, and parental socio-economic status, rather than education or IQ, may reveal the most accurate picture of cognitive functioning. Careful evaluation of verbal ability, education, and IQ as possible areas of secondary deficit will also be informative. Use of statistical procedures such as analysis of covariance (ANCOVA) will help in evaluating the effect of these factors, as well as the variance associated with comorbid disorders. Whenever possible, use of psychiatric control groups is advised. It may be most informative to employ a comparison group of individuals who meet full diagnostic criteria for a specific personality disorder from a different diagnostic cluster than the one under study.

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XXVI-6

Functional Neuroanatomy and Brain Imaging of Personality and its Disorders

C. Robert Cloninger

People differ markedly in their personality traits, but there is a stable organizational structure of the brain circuits regulating personality traits. In this chapter, a clinical model of personality will be described that corresponds well with DSM-IV categories of personality disorder and also with available data about the functional neuroanatomy of temperament and character. Temperament refers to the emotional aspects of personality that are regulated by subdivisions of the limbic system and centrally integrated in the hypothalamus. Character refers to the higher cognitive aspects of personality that are regulated by subdivisions of the thalamo-neocortical system and centrally integrated in the frontal cortex. In this chapter, quantifiable personality traits will be related to a model of their functional neuroanatomy derived from classical and comparative neuroanatomy, along with supporting clinical data and experimental findings from brain imaging.

Temperament will be considered in terms of four dimensions of personality that are related to specific subdivisions of the limbic system. These traits are called 'harm avoidance' (anxiety-proneness versus libido, which is outgoing vigour and daring), 'novelty seeking' (exploratory impulsivity and irritable aggression versus frugality and stoicism), 'reward dependence' (social sensitivity attachment versus insensitivity and aloofness), and 'persistence' (industrious determination versus underachievement). A quantitative dimensional approach is used because this corresponds well with the functional neuroanatomy and because there is no evidence of discrete boundaries between traditional syndromes of personality disorder, as in DSM-IV. Instead, traditional syndromes emerge from the interaction of multiple dimensions, which give rise to specific configurations of temperament that distinguish developmentally stable subtypes (Cloninger, 1987; Cloninger and Svrakic, 2000).

Character will be considered in terms of higher cognitive functions that regulate an individual's goals and values. In other words, character refers to mental self-government, which includes executive, legislative, and judicial functions. These traits are called 'self-directedness' (purposeful and resourceful versus aimless and helpless in executive functions), 'cooperativeness' (helpful and principled versus hostile and opportunistic in legislative functions), and 'self-transcendence' (inventive and insightful versus unimaginative and undiscerning judgement) (Cloninger *et al.*, 1993).

Each of the seven dimensions of personality considered here has been shown to have genetic variability that is unique from all the others, indicating that a model with seven dimensions is needed to describe the organization of human personality (Gillespie *et al.*, in press). The correlations among the temperament dimensions are weak, as are the correlations among the character dimensions, but there are moderate non-linear relations between temperament and character. This is expected because character (i.e., higher cognitive functions) modulates temperament (i.e., emotionality). A well-integrated character allows coherence of personality by modulation of emotional conflicts. In contrast, to the degree that character is not well integrated, emotional conflicts arise from the interplay of competing urges from various temperament dimensions. Likewise, the dynamics of complex adaptive systems oscillates between multiple states as a result of small contextual influences on dissociable circuits whenever such circuits are coordinated by a self-organized interplay, as has been suggested as a model of brain systems (Bressler and Kelso, 2001). Consequently, different dimensions of temperament are correlated with differences in the activity of specific brain circuitry that is partially overlapping but functionally dissociated when character is not coherent in its guidance.

All individuals with personality disorders have immature character development, particularly low self-directedness and low cooperativeness (Svrakic *et al.*, 1993; Cloninger and Svrakic, 2000). Individuals with different DSM-IV categories of personality disorder are distinguished primarily by their temperament profiles. Descriptive labels for the personality subtypes defined by the temperament dimensions of novelty seeking, harm avoidance, and reward dependence are shown in Figure XXVI-6.1. For example, borderline personality disorder is associated with a temperament configuration comprising high novelty seeking, high harm avoidance, and low reward dependence. Individuals with antisocial personality disorder are also high in novelty seeking and low in reward dependence, but they are low in harm avoidance. Consequently, the brain images of individuals with antisocial and borderline personality disorder are similar in many, but not all, ways. Specifically, individuals with either borderline or antisocial personality disorder have brain images expected from low self-directedness (i.e., low activity in the medial prefrontal network) and low cooperativeness (i.e., low activity in the orbital prefrontal network) (Kuruoglu *et al.*, 1996; Raine *et al.*, 1998; London *et al.*, 2000; Soloff *et al.*, 2000). Likewise, both groups of individuals have brain images expected from high novelty seeking (e.g., high activity in the right insula) and low reward dependence (e.g., high activity in the right superior temporal gyrus) (Sugiura *et al.*, 2000; Soloff *et al.*, 2000). However, they differ in activity associated with harm avoidance, which is negatively correlated with activity in paralimbic regions such as the left parahippocampal gyrus, left orbitoinsular junction, and some neocortical regions such as the fusiform gyrus (Sugiura *et al.*, 2000). Borderline subjects have the brain correlates of high harm avoidance (Herpertz *et al.*, 2001), whereas antisocial subjects have the brain correlates of low harm avoidance (Raine *et al.*, 1998; Schneider *et al.*, 2000). Each of the dimensions of personality distinguished here has unique genetic variability and brain circuitry, so it is more informative to describe personality dimensionally than categorically. Studies of categorically defined groups of patients

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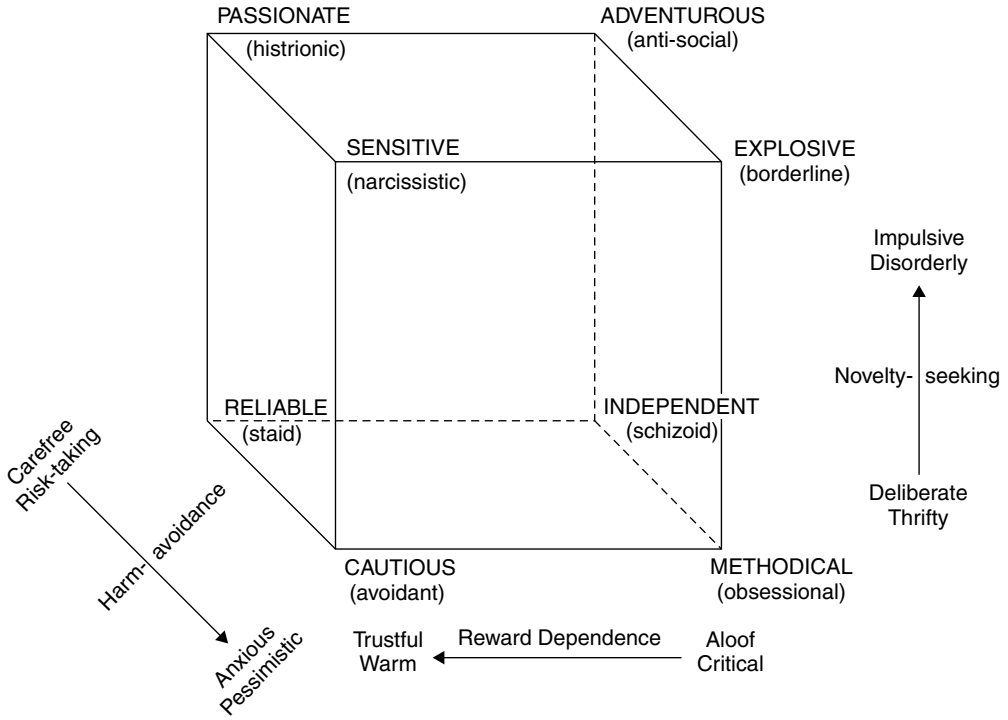

Figure XXVI-6.1 Subtypes of personality disorder defined by various combinations of novelty seeking, harm avoidance, and reward dependence (Reproduced by permission of Centre for Psychobiology of Personality, directed by C.R. Cloninger)

with personality disorder can be informative, nevertheless, and they support the findings based on dimensional studies of both clinical and general population samples.

TEMPERAMENT AND SUBDIVISIONS OF THE LIMBIC SYSTEM

Temperament is often defined as those aspects of personality that are emotion-based, heritable, and developmentally stable regardless of social and cultural influences. Four such dimensions of personality have been distinguished, and each has been shown to be moderately heritable and stable over time (Cloninger *et al.*, 1994). This same structure for temperament has been observed regardless of culture by studies throughout the world (Cloninger and Svrakic, 2000). Descriptors of individuals who score high versus low on each dimension are summarized in Table XXVI-6.1.

Activity of any or all of the brain networks involved in information processing, from sensory input to motor output, could vary with temperament. Sensory input into the primary sensory cortex is processed hierarchically, then is integrated within the multimodal association cortex, and finally reaches the limbic system via the paralimbic cortex. The limbic system is regarded as the anatomical basis for the regulation of emotion, motivation, and autonomic-endocrine functions. The paralimbic cortex plays a critical role in organizing motivated behaviour toward relevant intrapsychic and extrapersonal targets (Mesulam, 1998). Two partially dissociated networks have been distinguished in the paralimbic cortex by their connections with each another and other brain regions (Ongur and Price, 2000). The orbital network receives sensory inputs from several modalities, including olfaction, taste, visceral afferents, somatic sensation and vision. In contrast, the medial network provides the major cortical output to the visceromotor structures in the hypothalamus and the brainstem.

Table XXVI-6.1 Descriptors of individuals who score high and low on the four temperament dimensions

Temperament dimension	Descriptors of extreme variants	
	High	Low
Harm avoidance	Pessimistic Fearful Shy Fatigable	Optimistic Daring Outgoing Vigorous
Novelty seeking	Exploratory Impulsive Extravagant Irritable	Reserved Rigid Frugal Stoical
Reward dependence	Sentimental Open Warm Sympathetic	Critical Aloof Detached Independent
Persistence	Industrious Determined Ambitious Perfectionist	Apathetic Spoiled Underachiever Pragmatist

The limbic system itself has multiple subdivisions that are partly overlapping but functionally dissociable based on anatomical connections and physiology (MacLean, 1990). According to modern understanding, the limbic system is a distributed network of structures with synaptic proximity to the hypothalamus, which serves as the central integrator of the system (Nauta and Feirtag, 1986). MacLean distinguished three subdivisions of the limbic system with regulatory nodes in the septum, amygdala, and thalamus. More recent work has also distinguished a fourth subdivision involving

the ventral striatum. Here I propose the hypothesis that individual differences in harm avoidance, novelty seeking, reward dependence, and persistence are regulated by individual differences within the septal, amygdaloid, thalamo-cingulate, and striato-thalamic subdivisions, respectively.

The hypothalamus centrally integrates input from the limbic subdivisions and regulates the tonic opposition of the sympathetic and parasympathetic branches of the autonomic nervous system. The autonomic nervous system maintains homeostasis by the opposition of its parasympathetic functions (such as sexual arousal, feeding, digestion and storage of nutrients, elimination, and sleep) and its sympathetic functions (such as sexual orgasm, preparation for fighting or flight, and wakefulness). Accordingly, it is not surprising that each of the limbic subdivisions also regulates the tonic opposition of pairs of such psychodynamic drives, each of which has advantages and disadvantages depending on the context. Specifically, there are opposing drives for sexuality versus preservation of safety in the septal subdivision, feeding and aggression versus satiety and satisfaction in the amygdaloid subdivision, social attachment versus aloofness in the thalamo-cingulate subdivision, and industriousness versus impersistence in the striato-thalamic subdivision.

For clarity, I will describe each limbic subdivision individually, but it should be understood that they are partially overlapping and may be dissociated or integrated in function depending on the degree of coherence from higher cortical function. Then I will relate the functional neuroanatomy to available clinical and neurophysiological information.

THE SEPTAL SUBDIVISION AND HARM AVOIDANCE

The septal subdivision of the limbic system is hypothesized to regulate sexuality and the preservation of safety (MacLean, 1990). In humans and other mammals, stimulation of the septum, medial preoptic area, and anterior hypothalamus is pleasurable, with sexual arousal (Heath, 1963; Margules and Olds, 1962; MacLean, 1958, 1962, 1990; Mink *et al.*, 1983). Conversely, lesions in the anterior hypothalamus abolish sexual interest.

In addition, the septum projects to the entire hippocampal formation, but is strongly reciprocally connected with the giant pyramids of the CA3 and CA4 fields. The septal division also includes parts of the paralimbic cortex near the segments of the hippocampus to which it most heavily projects, namely, the caudal part of the entorhinal cortex (Brodmann's area 28) (BA 28), the postrhinal hippocampal gyrus, the presubicular part of the lingual gyrus (BA 27), and the retrosplenial cortex (BA 29) (MacLean, 1990). The septal subdivision also includes the pericallosal hippocampal rudiment and a narrow strip of preseptal cortex continuous with it. These areas are all closely interconnected with one another and the hippocampal formation in the medial aspects of the limbic system (Sanides, 1969; Reep, 1984). In turn, fibres from the hippocampal formation (primarily from the rostral subiculum) terminate in the medial prefrontal cortical network, including BA 24, 25, 32, and parts of 11 through 14 (Ongur and Price, 2000). This medial prefrontal network is the major source of cortical output to the visceromotor structures in the hypothalamus and brainstem. The hypothalamus also receives input directly from lateral septum and hippocampal formation.

Individual differences in harm avoidance have been observed to be significantly negatively correlated with individual differences in activity in the medial prefrontal network at rest (Sugiura *et al.*, 2000). This includes significant negative correlations between regional blood flow and the score for harm avoidance in paralimbic regions such as the left parahippocampal gyrus and the left orbitoinsular junction, and various neocortical regions in the frontal, parietal, and temporal cortex. Likewise, patients with familial pure depressive disorder, which is specifically associated with high harm avoidance, have a decrease in blood flow in the subgenual part of BA 24 (Drevets *et al.*, 1997). This decrease in blood flow is associated with a reduced volume of this area ventral to the genu of the corpus callosum in the medial prefrontal network. This decrease in volume is associated with a reduction in the number and density of glial cells in this area (Ongur and Price, 2000).

Gray (1982) has shown in detailed neurobehavioural studies that the septo-hippocampal-hypothalamic connections of the septal subdivision are essential in the inhibition of behaviour to preserve safety. According to Gray (1982), this circuit is essential in passive-avoidance learning, which is manifest as anticipatory anxiety in response to conditioned signals of punishment or frustrative non-reward. In particular, input to the lateral septum from the hippocampal CA3 field results in habituation and inhibition of unrewarded behaviour. Other aspects of Gray's model of anxiety involve interactions between the septal and thalamo-cingulate subdivisions, which are described later.

Key elements in the neuroanatomy of the septal subdivision include sensory input via the entorhinal cortex and internal sensory input from the midbrain limbic area, such as the dopaminergic ventral tegmental area and the serotonergic raphe nuclei. The septal connections with the dentate gyrus and hippocampal CA3 fields are involved in habituation of unpleasant or unrewarded behaviour. The connections of the subiculum and the lateral septum with the medial prefrontal network and the hypothalamus influence visceromotor output.

The significance of these neuroanatomical connections can be understood in behavioural and psychodynamic terms, which help to reveal their evolutionary and developmental significance. Individuals who are low in harm avoidance are described as outgoing, vigorous, and daring optimists, as shown in Table XXVI-6.1. Harm avoidance is hypothesized to be a behavioural manifestation of individual differences in the septal subdivision of the limbic system regulating the tonic opposition of sexual drive necessary for reproduction versus the preservation of personal safety (MacLean, 1958, 1962, 1990). Likewise, in his theory of libido, Freud (1938) described such vigorous and outgoing optimists as having a strong libido, which leads to anxiety when unsatisfied. Libido does not refer to sexual appetite or conscious sexual desires, although it does energize such desires. Rather, libido refers more broadly to quantitative differences between individuals in their vigour or energy derived from their procreative drive. Consequently, low harm avoidance is a valid measure of libido as originally conceived by Freud.

Freud noted that libido is essential for the preservation of the species by sexual reproduction. However, high harm avoidance is associated with anxiety-proneness, sensitivity to contextual stress, and concern about the preservation of personal safety. At the most basic reflex level, harm avoidance modulates the human startle reflex, which abruptly interrupts behaviour that is potentially harmful and causes withdrawal by widespread contraction of the flexor muscles (Graham, 1979; Corr *et al.*, 1995, 1997; Cloninger, 1998). When compared to the magnitude of the eye-blink startle to a loud noise while the subject is viewing a neutral foreground stimulus, high harm avoidance potentiates startle while viewing an unpleasant stimulus whereas low harm avoidance reduces startle while viewing a pleasant stimulus (Corr *et al.*, 1995, 1997). Other temperament dimensions are uncorrelated with the modulation of startle when subjects are viewing pleasant and unpleasant pictures (Corr *et al.*, 1995, 1997). Anxiety can also be induced in animals by prolonged exposure to bright light or intraventricular administration of the stress peptide corticotrophin-releasing hormone. These anxiety-producing effects depend on a brain area called the bed nucleus of the stria terminalis, rather than the central nucleus of the amygdala, which is involved in the fight or flight response (Davis *et al.*, 1999).

AMYGDALOID SUBDIVISION AND NOVELTY SEEKING

The amygdaloid subdivision of the limbic system regulates the tonic opposition of feeding and aggression versus satiety and satisfaction. Stimulation of the lateral hypothalamus is satisfying, but only if the human or other mammal is hungry (Heath, 1963; Margules and Olds, 1962; Hoebel and Teitelbaum, 1962). In contrast, stimulation of the septum is sexually arousing regardless of feeding status (Heath, 1963; Margules and Olds, 1962; Hoebel and Teitelbaum, 1962). Stimulation of the lateral hypothalamus leads to overeating, whereas stimulation of the ventromedial hypothalamus produces satiety. Conversely, lesions in these same areas have opposite results: lesions of the ventromedial hypothalamus produce hyperphagia whereas those in the lateral hypothalamus lead to aphagia and adipsia. The ventromedial hypothalamus seems to inhibit the activity of the lateral hypothalamus because destruction of both produces anorexia.

In cats and monkeys, activation of the lateral hypothalamus by stimulation of the basal amygdaloid nucleus also produces a defence reflex or rage reaction. This is characterized by pupillary dilation, snarling and aggressive gestures, piloerection, elevation of blood pressure with cardiac deceleration, generally lower amplitude and increased rate of breathing, and changes in gastric motility and acidity (Brodal, 1981; FitzGerald, 1985). This reflex prepares an individual for fight or flight by muscle vasodilation, increased muscle blood flow, and decreasing sensitivity to aversive stimulation (Kelly *et al.*, 1973; Graham, 1979; Deakin and Graeff, 1991). The defence reflex or its autonomic components are elicited from a neural circuit that extends from the basal amygdaloid nucleus, through the central nucleus and the lateral hypothalamus, and into the pontomedullary brainstem, ending approximately at the level of the dorsal vagal complex (Price and Amaral, 1981; Price *et al.*, 1987). Hypothalamic stimulation is ineffective if the amygdalas have been removed (FitzGerald, 1985). In humans, the defence response is usually more modulated, so stimulation of the amygdala most often produces a sensation of fear, occasionally anger, and rarely a full rage reaction (Brodal, 1981). However, in humans with frequent temper tantrums or severely violent rage reactions, surgical lesions in the amygdaloid or hypothalamic components of the defence reflex circuit produce clinical improvement with decreases in aggression, anxiety, restlessness, and sympathetic overactivity (Kelly *et al.*, 1973; Sano and Mayanagi, 1988).

The defence reflex circuit is also involved in fear-potentiated startle in which the startle reflex is potentiated by a conditioned aversive stimulus, as when a human subject is anticipating a painful shock. Fear-potentiated startle depends on the integrity of the central nucleus of the amygdala, and not on the bed nucleus of the stria terminalis (Davis *et al.*, 1999). Increasing the intensity of the threat of harm leads to a switch to active responses (which depend on the integrity of the dorsolateral periaqueductal grey) from more passive responses (which depend on the integrity of the ventral periaqueductal grey) (Davis *et al.*, 1999).

The amygdaloid subdivision of the limbic system receives highly processed sensory input through the orbital prefrontal network. The orbital prefrontal network in humans includes most of the areas on the posterior, central, and lateral orbital surface, including the agranular insular areas Ial, Iam, Iapl, and Iapm, and the orbital areas 11l, 12m, 12l, 13b, 13l, and 13m (Ongur and Price, 2000). Significant positive correlations are observed between scores on novelty seeking and cerebral blood flow in the orbital prefrontal network, particularly the right anterior and right posterior insula (Sugiuri *et al.*, 2000). Other temperament dimensions do not show such positive correlations.

Stimulation of the basal amygdaloid nucleus produces feeding, like the lateral hypothalamus, whereas stimulation of the central amygdaloid nucleus produces satiety, like the ventromedial hypothalamus. When hunger or hunting urges are unsatisfied, there is irritability and impulsive aggressive behaviour, so the amygdaloid subdivision is involved in both feeding and attack behaviour (Chi and Flynn, 1971; Siegel and Brutus, 1990). In cats, synaptic transmission within the amygdaloid subdivision has been experimentally manipulated to cause stable changes in fight or flight behaviour (Adamec and Stark-Adamec, 1983a; b).

The neuroanatomy of the amygdaloid subdivision of the limbic system can be considered functionally in relation to clinical and psychodynamic descriptions of novelty seeking. Individuals who are high in novelty seeking are supposed to have a strong psychodynamic drive for exploratory hunting and feeding, which leads to aggression rather than anxiety if unsatisfied (Freud, 1938). The novelty-seeking dimension is hypothesized to be regulated by the amygdaloid subdivision of the limbic system, which regulates the tonic opposition of drives for feeding and aggression versus satiety and satisfaction (MacLean, 1958, 1990; Kelly *et al.*, 1973; Kelly, 1980). Novelty seeking involves a tonic opposition of craving and desire for novel stimuli and avoidance of fear-inducing stimuli. Experimentally, the amygdaloid subdivision can be activated in humans by negative stimuli, such as exposure to faces expressing fear, or positive stimuli, such as pictures of erotic nudes (Davidson and Irwin, 1999; LaBar *et al.*, 1998). Likewise, individuals high in novelty seeking are particularly prone to thrill-seeking behaviour, overeating, and substance dependence, as well as to irritability and impulsive aggression when they are unsatisfied (Cloninger *et al.*, 1994). Individuals who are high in novelty seeking are quick-tempered, that is, they have a low threshold for the defence reflex.

THE THALAMO-CINGULATE SUBDIVISION AND REWARD DEPENDENCE

The thalamo-cingulate subdivision of the limbic system is also called the circuit of Papez in recognition of James W. Papez, who described it as the 'mechanism of emotion' (Papez, 1937). More specifically, the thalamo-cingulate subdivision is hypothesized here to regulate the tonic opposition of social attachment and aloofness by its role in selective attention to salient emotional events.

The thalamo-cingulate subdivision involves connections between the posterior hypothalamus, anterior thalamus, cingulate gyrus, and hippocampal subiculum. This subicular loop (subiculum to mammillary body to anterior thalamus to anterior cingulate and back to the subiculum) has been studied physiologically in detail (Parmeggiani *et al.*, 1971). For example in the curarized cat, conduction around the entire loop occurs within 50–60 ms and involves no other relay sites until there is transfer of information beyond this simple circuit (Parmeggiani *et al.*, 1974). The subiculum receives information from the outside world, as it is already processed when it reaches the entorhinal cortex or hippocampal CA3-CA1 fields. The subiculum compares the information it receives with what was expected, and then projects to the cingulate cortex and to the septal subdivision via the entorhinal cortex so as to select responses that should habituate and be inhibited (Gray, 1982) as well as what is useful to enter into long-term memory (Eccles, 1989). Information reaching the subicular circuit has already been extensively processed, and its potential importance or salience has already been tagged by processing in the septal or amygdaloid subdivisions (Gray, 1982). By acting as a comparator of observed and expected stimuli, the subicular circuit can modulate orienting responses.

Sensory input about the external context and internal milieu also enters this subdivision through the sensory association cortex and midbrain limbic area, respectively. Papez (1937) showed that connections among the posterior hypothalamus, anterior thalamus, cingulate gyrus, and hippocampus formed a two-way circuit by which emotions could be elicited either by ideational association in the neocortex or by visceral activation of the hypothalamus, which in turn provides central control of the autonomic nervous system.

MacLean (1990) showed that differentiation of the thalamo-cingulate subdivision in mammals is associated with the emergence of a behavioural triad of social interactions, including parental nursing, play, and audiovocal communication, to maintain communication between mother and offspring. This was a major advance in capacity for social attachment in mammals over reptiles and more primitive vertebrates.

Individuals who are high in reward dependence are sentimental and emotionally warm, whereas those who are low in reward dependence are aloof and emotionally cold. In psychodynamic terms, individuals who are high in reward dependence are sympathetic to emotional distress in others and attribute their own positive emotions onto others. However, individuals low in reward dependence do not form social attachments readily because they project or attribute their own negative emotions onto others and remain unresponsive to the positive emotions of others.

Accordingly, reward dependence is hypothesized to be regulated by the thalamo-cingulate subdivision of the limbic system (MacLean, 1990). More specifically, the subicular circuit is hypothesized to modulate the magnitude of the orienting reflex, which alerts and orients a person to information to which they need to pay selective attention (Graham, 1979; Gray, 1982). More specifically, it is hypothesized that the orienting reflex is potentiated in individuals who are high in reward dependence when they are stressed and responding to high-intensity stimuli, particularly stimuli of social interest, such as frowns and signs of distress in others. However, individuals low in reward dependence are expected to have greater orienting responses in the absence of stress when responding to low-intensity stimuli. Empirically, this expected interaction between intensity of stimulus and reward dependence is empirically supported by the results of personality measures with reward dependence as their major common correlate, such as high extraversion and low psychoticism, but not neuroticism (Fowles *et al.*, 1977; Wigglesworth and Smith, 1976; O'Gorman, 1983).

It should be noted that the initial magnitude and the rate of habituation of orienting responses are only weakly correlated (O'Gorman, 1983). Habituation of the orienting response may be more closely modulated by the septal subdivision, as proposed by Gray (1982), so harm avoidance would interact with reward dependence. Specifically, individuals with high reward dependence and low harm avoidance are expected to habituate slowly to unpleasant social cues, such as a frown, whereas individuals with low reward dependence and high harm avoidance are expected to habituate rapidly to pleasant social cues, such as a smile. Consequently, studies of the orienting reflex with extraversion as a measure may have had inconsistent results because extraversion confounds high reward dependence and low harm avoidance. More empirical research is therefore needed to test the tentative hypotheses about the orienting reflex suggested here.

Nevertheless, the hypothesis that reward dependence modulates the magnitude of the orienting reflex is suggested by the sentimentality and preference for social closeness of individuals who are high in reward dependence, in contrast to the preference for social distance in individuals who are low in reward dependence. It is supported empirically by the particular sensitivity of the thalamo-cingulate subdivision to facial cues and visceral (autonomic) variability through exteroceptive and interoceptive sensory inputs to the mesocortical ring. This concentric ring of mesocortex links the anterior cingulate (area 24) with the infralimbic (area 25), medial and ventrolateral orbital, insula, perirhinal (area 35), and retrosplenial (area 29) mesocortex (Reep, 1984). Individual differences in reward dependence are negatively correlated with cerebral blood flow in this mesocortical ring (Sugiura *et al.*, 2000).

THE STRIATO-THALAMIC SUBDIVISION AND PERSISTENCE

Persistence despite partial reinforcement has been poorly explained by the functional neuroanatomy of the preceding three subdivisions of the limbic system (Gray, 1982). Persistence can be measured by the partial reinforcement extinction effect (PREE), which means that behaviour is more persistent despite non-reward after an initial period of intermittent reinforcement than after an initial period of continuous reinforcement. This persistence effect is abolished after lesions of the nucleus accumbens, which interrupts an excitatory projection from the hippocampal subiculum to the ventral striatum (Tai *et al.*, 1991).

Phenomena that are closely related to PREE are the greater resistance to extinction after continuous small rewards than after continuous large rewards and the greater approach speeds during acquisition with intermittent rewards than with continuous rewards. The latter is called the partial reinforcement acquisition effect (PRAE) or eagerness reflex. Therefore, the temperament dimension of persistence is not simply a form of perseverative behaviour. Instead, it involves persistence in behaviour to achieve long-term goals with eagerness, enthusiasm, and industry, which may require flexible adaptation of routines. In fact, neurons in the ventral striatum selectively respond to motivationally significant stimuli that are rewarding or punishing (Rolls and Williams, 1987; Schneider and Lidsky, 1981; Schultz *et al.*, 1998). This reward-guided behaviour in animals involves activation in a circuit connecting the ventral striatum and the prefrontal cortex. In humans, this striatal-prefrontal circuit is known to include intermediate connections between the ventromedial striatum, pallidum, certain medial thalamic nuclei, and the prefrontal cortex (Knight, 1964; Kelly *et al.*, 1973; Modell *et al.*, 1989; Hay *et al.*, 1993; Joseph, 1999).

Individual differences in persistence in humans are strongly correlated with responses measured by fMRI in a circuit involving the ventral striatum, orbitofrontal cortex/rostral insula, and prefrontal/cingulate cortex (BA 32) (Gusnard *et al.*, 2001). Subjects low in persistence exhibited relative decreases in activity within this circuit whereas those high in persistence exhibited relative increases. Persistence scores also correlated with an apparent selection bias such that subjects with high persistence scores made relatively more pleasant judgements at the expense of neutral judgements when viewing pictures from the International Affective Picture System.

The neuroanatomy of this ventral striatal-prefrontal circuitry can be appreciated functionally in terms of its clinical and developmental significance for the temperament dimension of persistence. Individuals who are high in persistence are industrious overachievers. In contrast, those who are low in persistence are described as apathetic underachievers who are easily discouraged when their expectations are not quickly and consistently satisfied. More specifically, when individuals are intermittently reinforced, those who are high in persistence remain eager and industrious despite discouragement by others, whereas those low in persistence quit quickly despite encouragement by others. In terms of functional neuroanatomy, the ventral striato-thalamic-prefrontal circuit is likely to be involved in reward-guided choice behaviour (Schultz *et al.*, 1998). Striatal neurons are almost silent at rest, whereas nigro-striatal and pallido-thalamic projections are tonically active and inhibitory. The motivation to initiate behaviour is associated with increases in both striatal and pallidal activity, which selectively disinhibits thalamo-frontal activity (Penney and Young, 1983). In turn, the increased thalamo-frontal activity completes the fronto-striato-pallido-thalamo-frontal circuit, so that a positive fronto-striatal feedback loop can maintain activity in a persistent goal-directed manner. However, decreased nigro-striatal activity may lead to tonic inhibition of the pallidum, which produces akinesia, rigidity, and tremor in the dorsolateral striatum. It is hypothesized that the analogous effect of tonic

inhibition of the ventral pallidum on motivation mediated by the ventromedial striatum is low persistence, characterized by amotivation (apathy, underachievement, and discouragement), inflexibility (spoiled behaviour — wanting your way regardless of consequences and subjectively complaining without adapting efforts), and lability (mood swings).

Patients with bipolar mood swings are often low in persistence, even when euthymic (Osher *et al.*, 1996; 1999). Moreover, subcaudate tractotomy is frequently beneficial in such patients when they are chronically or recurrently depressed (Mindus and Jenike, 1992). The target of this surgery is the substantia innominata below the head of the caudate, which is the location of the ventral pallidum. The treatment produces a relief of amotivation, that is, an increase in industry with improved mood and work capacity (Strom-Olsen and Carlisle, 1971; Goktepe *et al.*, 1975; Lovett and Shaw, 1987).

HIGHER COGNITIVE FUNCTIONS OF FRONTAL CORTEX

Character has long been regarded as what human beings make of themselves intentionally (Cloninger, 1994). In contrast to temperament, which involves basic emotional responses, character involves our concepts of self and our relations with other people and the world as a whole. In other words, character involves our conceptual or propositional learning about goals and values that allow us to organize and integrate the behavioural drives motivated by temperament. The subdivisions of the limbic system produce a variety of responses, often producing conflict. For example, individuals may be high in both harm avoidance and novelty seeking, so when they encounter unfamiliar situations or strangers, they will have conflicting impulses to experience something new (thereby producing a thrill or relieving boredom) and to avoid the unfamiliar (thereby playing it safe). The way such emotional conflicts are organized and resolved defines a person's character or style of higher cognitive processing.

Empirically, individual differences in temperament do not tell us whether or not a person is integrated in character (that is, mature as opposed to having a character disorder). However, individual differences in maturity can be well described by three dimensions of character called self-directedness, cooperativeness, and self-transcendence (Cloninger *et al.*, 1993; Svrakic *et al.*, 1993). Descriptors of individuals who are high or low on each of these character dimensions are summarized in Table XXVI-6.2.

Table XXVI-6.2 Descriptors of individuals who score high and low on the three character dimensions

Character dimension	Descriptors of extreme variants	
	High	Low
Self-directed	Responsible	Blaming
	Purposeful	Aimless
	Resourceful	Inept
	Self-accepting	Vain
	Generative	Unproductive
Cooperative	Reasonable	Prejudiced
	Empathic	Insensitive
	Helpful	Hostile
	Compassionate	Revengeful
	Principled	Opportunistic
Self-transcendent	Judicious	Undiscerning
	Insightful	Superficial
	Intuitive	Dualistic
	Inventive	Unimaginative
	Spiritual	Materialistic

These dimensions allow quantitative ratings of the degree of maturity as well as the severity of personality disorder. The presence of personality disorder can be defined by those individuals who are in about the lowest 10% of the distribution of the sum of self-directedness and cooperativeness (Svrakic *et al.*, 1993). Even those in the bottom third of this distribution are noticeably immature and disorganized. However, there is more clinical information in considering the full distribution of scores. Likewise, consideration of quantitative variation in these dimensions is more helpful as a way of understanding the functional neuroanatomy of personality.

Empirical findings showed temperament and character to be interactive aspects of personality in which each influences the other. Specifically, the relations are non-linear, as expected in a self-organizing adaptive system in which temperament influences the emotional salience of events, and character influences the higher cognitive processing of the meaning of events (Cloninger *et al.*, 1997). Furthermore, character, but not temperament, was observed to be correlated with individual differences in event-related potentials of healthy adults that are characteristic of higher cognitive processes subserved by the prefrontal cortex and sensory association cortex (Fuster, 1984, 1997). Specifically, P300 is positively correlated with self-directedness and no other temperament or character dimension; in parietal leads, the correlations are $+0.3$ to $+0.4$ in healthy adults (Cloninger, 1998; Vedeniapin *et al.*, 2001). The P300 is a positive potential recorded at the scalp surface and elicited by an unexpected event that has been awaited while a subject deals with another repetitive, highly structured task. Likewise, contingent negative variation (CNV) is negatively correlated with cooperativeness and self-transcendence, and not with temperament dimensions (Cloninger, 1998; Vedeniapin *et al.*, 2001). CNV is a scalp-recorded, slow negative potential recorded at the scalp in the interval between two events that lead to a motor act. In recordings from single neurons in the prefrontal cortex, Fuster (1984, 1997) has shown that CNV originates in the prefrontal cortex. In delayed response tasks, large numbers of prefrontal neurons increase in their discharge rate, and this discharge is measured as the CNV surface potential.

It has long been recognized that higher cognitive functions depend on the integrity of the thalamo-neocortical system, and are centrally integrated in the prefrontal cortex (Freeman and Watts, 1942; Nauta, 1971; Fuster, 1984, 1997; Cloninger, 1994; Joseph, 1999). The prefrontal cortex is seen as the peak in a hierarchy of structures modulating goal-directed behaviour. These higher cognitive functions have a delayed experience-dependent maturation, just as the prefrontal cortex and sensory association areas have a delayed onset of myelination, not maturing until puberty (Sanides, 1969; Stuss, 1992). Likewise, social deprivation and psychological trauma during early childhood can disrupt normal neocortical development and higher cognitive processing (Meares *et al.*, 1999). These observations suggest the hypothesis that the maturation of higher cognitive functions, which I will call character development, involves the integration of the interactions among the areas of neocortex that supervise the individual limbic subdivisions.

A useful metaphor for the integrative activity of the thalamo-neocortical system is the government of a large and complex country. To manage a country effectively, it is useful to have executive, legislative, and judicial branches of government (Sternberg, 1990). Likewise, as previously described on empirical clinical grounds, higher cognitive functions can be measured as three dimensions of character that have been shown to distinguish individuals with personality disorder from those who are mature (Cloninger *et al.*, 1993; Svrakic *et al.*, 1993; Cloninger and Svrakic, 2000). These dimensions involve executive functions (self-directedness), legislative functions (cooperativeness), and judicial functions (self-transcendence).

Alterations in personality and cognitive processing caused by frontal lobe lesions have been described in detail (Freeman and Watts, 1942; Damasio, 1985; Milner and Petrides, 1984; Stuss and Benson, 1986; Joseph, 1999). Three syndromes of frontal lobe dysfunction have been described that can be systematically related to the three character dimensions of self-directedness, cooperativeness, and self-transcendence, as shown in what follows.

The prefrontal cortex is not homogeneous in its connectivity, cytoarchitecture, ontogeny, and phylogeny, or its function (Sanides, 1969; Nauta, 1971; Reep, 1984; Joseph, 1999; Ongur and Price, 2000). Most recent research on the prefrontal cortex distinguishes regions including the medial, orbital, dorsolateral, and polar areas (Ongur and Price, 2000; Joseph, 1999; Bechara *et al.*, 1998; Roland, 1984). The medial prefrontal network includes all the areas on the medial wall of the cerebrum (areas 25, 32, 14r, 14c, 24a, and 24b) and related areas on the orbital surface (areas 11m, 13a, Iai, and 12o). The orbital prefrontal network includes most of the areas on the posterior, central, and lateral orbital surface (agranular insular areas Ial, Iam, Iapl, and Iapm, and orbital areas 13b, 13l, 13m, 11l, 12r, 12m, and 12l) (Ongur and Price, 2000). The frontal poles and dorsolateral prefrontal cortex are designated as areas 10 and 11, respectively.

Furthermore, there are prominent functional differences related to hemispheric specialization or laterality. Whereas the left side is usually dominant for verbal analysis and speech, some observations suggest that the right side is usually dominant for the integrative and modulatory functions described here as character (Joseph, 1999). It is suggested that the right frontal lobe has a greater attentional capacity than the left and exerts bilateral inhibitory influences on arousal (Joseph, 1999; Pardo *et al.*, 1991). In contrast, the left frontal lobe appears to exert unilateral excitatory influences on arousal and attention (Davidson and Irwin, 1999). Finally, it is useful to distinguish the frontal pole (area 10) from other prefrontal regions since it has distinct patterns of development, connectivity, and coactivation (Roland, 1984), such as receiving projections from the mediodorsal thalamus, but not the amygdala (Reep, 1984).

Patients with left-sided or bilateral lesions in the medial prefrontal network show deficits suggestive of the executive functions associated with self-directedness, as indicated by apathy, abulia, or lack of initiative, and inability to organize or prioritize activities in their life (Damasio, 1985; Joseph, 1999). In recent brain-imaging research, individual differences in Self-directedness, as measured by the Temperament and Character Inventory, were strongly correlated with fMRI response magnitudes in BA 9/10 when subjects were carrying out simple executive tasks (Gusnard *et al.*, 2001). Self-directedness was also negatively correlated with reaction times in the same tasks.

Likewise, Bechara *et al.* (1994, 1996, 1997, 1998) have found that patients with bilateral medial prefrontal lesions cannot anticipate future positive or negative consequences of their actions, although their behaviour is influenced appropriately by immediately available contingencies. The same patients fail to show anticipatory electrodermal responses when confronted by a risky choice, whereas normal controls generate such anticipatory autonomic responses even before they are able to declare this explicitly. Patients with medial prefrontal lesions who were unable to anticipate future positive or negative consequences had normal working memories, whereas other patients with dorsolateral prefrontal lesions had impaired working memories but were able to anticipate future positive or negative consequences on a gambling task (Bechara *et al.*, 1998).

Right-sided or bilateral medial prefrontal lesions usually produce a deficit in a subjects' concept of temporal self-continuity in which they have difficulty thinking about themselves as having a continuity from the past to the future (Freeman and Watts, 1942). This leads to severe impairment of self-directedness and sometimes to bizarre ideation. This has led some to suggest that the right prefrontal cortex is dominant over the left in character functions (Joseph, 1999). The right-sided dominance in self-directedness is also shown by the release of inhibitory control over limbic impulses, as manifest by the emergence of impulsivity, distractibility, hypertalkativeness, and overactivity. The mood is usually hyperthymic and labile, with an increased risk of hypomania and manic episodes (Joseph, 1999). However, self-directedness, as measured by the Temperament and Character Inventory, has been correlated with medial prefrontal activity in both hemispheres (Gusnard *et al.*, 2001).

In contrast, lesions in the orbital network cause deficits in legislative function indicative of low cooperativeness. Low cooperativeness is indicated by reduced empathy and social sensitivity, ethical unreliability, and poor social judgement in which patients seem to lack principles and compassion regarding the effects of their behaviour on others (Damasio, 1985; Stuss and Benson, 1986; Joseph, 1999). This can lead to criminality, promiscuity, profane speech, and a crude, immature social manner that is inconsiderate of the feelings of others. In addition, as in right medial lesions, there is often excessive eating, drinking, and hypersexuality (Joseph, 1999).

Although right-sided lesions of either the medial or orbitofrontal cortex produce behavioural disinhibition, there is still a difference in their impact on the capacity for self-directedness (which is impaired by medial lesions) and cooperativeness (which is impaired by orbital lesions), a finding which illustrates the distinction between executive and legislative functions. Patients with low cooperativeness as a result of orbital lesions do not make rules to moderate their social interactions; they are unprincipled and opportunistic, and show pervasive impairment in social judgement according to the standards of others because they make no standards for themselves (a legislative function). In contrast, patients with only right medial lesions do have standards and principles of which they are consciously aware, but are undisciplined, have difficulty in conforming to rules (an executive function), and tend to be hyperthymic and impulsive (Joseph, 1999). Thus, there are interactions between mood and character effects.

The frontal poles are uniquely increased in human beings compared to other primates, and are the latest to myelinate of all parts of neocortex (Sanides, 1969; Eccles, 1989). Lesions to the frontal pole (BA 10 or the part of the mediodorsal thalamus that projects to area 10) may possibly impair Self-transcendence, but relevant observations are old, limited, and no studies have used the Temperament and Character Inventory (Cloninger *et al.*, 1993) to test this. Nevertheless, low self-transcendence may be indicated by early reports of impairment in creativity, spirituality, and the capacity for abstract fantasy after surgery to the frontal poles (Freeman and Watts, 1942). These are characteristics that distinguish human beings from all other primates (Eccles, 1989; Mithen, 1996). Consequently various artists, theatrical performers, writers, musicians or scientists lost their capacity for creative insight and judgement after bilateral lesions of the frontal pole, even though they remained self-directed and cooperative (Freeman and Watts, 1942). This impairment can be understood as the loss of judicial function, that is, the ability to know when a particular rule should be applied. Such judgement is intuitive in the sense that it cannot be taught algorithmically.

CONCLUSIONS

Substantial progress has been made toward the development of a functional neuroanatomy of personality and its disorders based

on classical neuroanatomy, neurophysiology, and recent brain-imaging studies using reliable quantitative measures of personality or related cognitive and emotional functions. Reliable clinical measures of temperament are available to quantify personality traits that correspond well to functional subdivisions of the limbic system. Likewise, dimensions of character may distinguish the executive, legislative, and judicial functions of different regions of the frontal cortex.

The temperament and character dimensions are now being investigated by brain imaging, but much more work is needed. Modern methods of brain imaging and psychophysiology offer excellent opportunities to relate individual differences in personality to regional brain activity. These methods can provide rigorous tests of the hypotheses and findings described here, which are often based on limited data or the synthesis of diverse observations from animals and humans. Nevertheless, the multidimensional model of temperament and character is well tested clinically and psychometrically. Accordingly, the remarkable correspondences described here between personality and available data on functional neuroanatomy are much too extensive and systematic to be coincidental.

The functional neuroanatomy of personality described here offers hope that psychiatry can progress beyond a purely descriptive approach to diagnosis and treatment. Psychiatry can become a science of functional psychobiology only when it approaches individual patients in terms of assessments based on the psychodynamics and neurodynamics of specific brain systems as described here for personality and its disorders.

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XXVI-7

Neurogenetics of Personality Disorders

Andreas Reif and Klaus-Peter Lesch

INTRODUCTION

The behavioural predisposition of an individual is commonly referred to as temperament or personality. In genetics, personality is generally defined as the characteristic manner and style of an individual's behaviour and encompasses the emotional expression, such as fearfulness, exuberance, aggressiveness, and self-restraint, as well as the vigour, temper, and persistence of the resulting behaviour (Benjamin *et al.*, 1998; Ebstein *et al.*, 2000). Personality disorder (PD) is an umbrella term covering a plethora of conditions characterized by a persistent pattern of abnormal behaviour, dysfunctioning in social contexts, and suffering of either the individual or the environment. Despite problems of classification, epidemiological research involving family, twin, and adoptee studies has gathered persuasive evidence that several categories of PD including antisocial, anxious/avoidant, and schizoid-schizotypal personalities are influenced by genetic factors, and that the genetic component is highly complex, polygenic, and epistatic (Coolidge *et al.*, 2001; Eley *et al.*, 1999; Jang *et al.*, 1996; Kendler *et al.*, 1991; McGuffin and Thapar, 1992; O'Connor *et al.*, 1998; Vernon *et al.*, 1999).

The analysis of genetic contributions to normal personality and behaviour as well as to PDs is both conceptually and methodologically difficult, so that consistent findings remain sparse. The documented heterogeneity of both genetic and environmental determinants suggests the ineffectiveness of searching for unitary causes. This vista has therefore increasingly encouraged the pursuit of dimensional and quantitative approaches to personality and behavioural genetics, in addition to the traditional strategy of studying individuals with categorically defined psychiatric entities (Plomin *et al.*, 1994). While quantitative genetics has focused on complex, quantitatively distributed traits and their origins in naturally occurring variation caused by multiple genetic and environmental factors, molecular genetics has begun to identify specific genes for quantitative traits, called quantitative trait loci (QTLs) (Eley and Plomin, 1997). In this polygenic model, behavioural disorders are extremes of continua in which various genes interact additively or nonadditively. The QTL concept thus implies that there may not be genes for psychiatric disorders, just genes for behavioural dimensions. In line with this notion, Livesley *et al.* (1998) have recently suggested that personality disorders are best classified by dimensional scales and are not qualitatively distinct from normal personality traits. Underlying genes of extremes of normal personality traits may therefore contribute to a range of psychopathology including psychoses (Tien *et al.*, 1992).

Any attempt to provide a comprehensive overview would be futile; however, an appraisal of the genetics of PDs requires the consideration of several critical caveats. This chapter therefore describes fundamental aspects of the genetics of personality and focuses on topics undergoing particularly rapid progress. Conceptual and methodological issues in the search for candidate genes of personality traits and PDs as well as for the development of mouse models of human PDs will also be reviewed.

PERSONALITY DISORDERS — A DIAGNOSTIC DILEMMA

The diagnosis of PD is evidently not as clear-cut as is desirable. In a continuum ranging from reasonably well-characterized brain disorders with a defined genetic cause, such as Huntington's disease or fragile-X syndrome, to wider and more unspecific diagnoses such as bipolar affective or schizophrenic disorders, extreme variants of personality represent disorder entities which are the least well defined and accessible to psychobiological research. DSM-IV (American Psychiatric Association, 1994) lists the following criteria of PD: a persistent pattern of abnormal behaviour, dysfunctioning in social contexts, suffering of either the patient or the environment, and early onset in childhood or adolescence. When the first three DSM-IV criteria are inverted, (pattern of normal behaviour, functioning, and no suffering), and the last is kept, one might see this as a definition of 'normal' personality, or behaviour, as human personality is mainly what is constructed from an individual's actions. It is conceivable that the DSM-IV definition of PD covers a wide range of disorders; may be subdivided in almost infinite different ways, just as human personality; and depends not only on the patient, but also the environment. The social context is of substantial importance when the diagnosis of PD is applied; individuals functioning perfectly in some societies may fail to do so in others. Thus, we have the media stereotype of a workaholic German manager, who could be indexed as neurotic and anankastic in other socio-economic models; the temperamental stereotype of a young lady from Sicily, readily regarded as highly histrionic in Norway; and that revered eccentric English gentlemen, in DSM-IV euphemisms the archetype of a schizotype personality. Accordingly, PD, in part, lies in the eye of the beholder.

What leads to the term 'disorder', is the suffering of the individual or — as is sometimes more common in PDs — the environment. Otherwise, this 'disorder' could easily be described as 'personality variation', the outer limits of a Gaussian distribution of personality traits. Thus, the presence of PDs could be viewed as an evolutionary strategy to adapt to different environmental conditions. Dysfunctioning and abnormal behaviour might be appropriate attributes for a certain kind of personality structure in a given culture; but cultures and societies vary and, even more meaningfully, constantly change, and what was abnormal or dysfunctional yesterday might be successful and perfectly normal today. PDs therefore may be the price a given society has to pay for the flexibility to adapt to different environments and for the variation of behaviour which helps it to maintain reproductive success and to ensure survival. Furthermore, the genes underlying behavioural traits have been shaped

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throughout evolution for success in primordial societies, and not in contemporary civilization. Humans still bear the constraints of this genetic background, and it has to be kept in mind that some of the features now regarded as 'disordered', such as exaggerated anxiety, novelty seeking and risk-taking hyperactivity, or straightforward aggression, might represent behavioural strategies that were, in the evolutionarily perspective, highly successful in ancient times. For further reading, several monographs are recommended to the interested reader (Allman, 1994; Hamer and Copeland, 1998; Nesse and Williams, 1995; Weiner, 1999; Wilson, 1998).

The current classification systems of DSM-IV (American Psychiatric Association 1994) and ICD-10 (World Health Organization 1992) distinguish three distinct subgroups of PD: paranoid, histrionic, and avoidant. While an operationalized diagnostic procedure is laborious to obtain, these subgroups are familiar and intuitively comprehensible in the clinical setting. However, rating scales often fail to identify a specific PD, or the results are contrary to the experienced clinician's view. This is likely to be due to the fact that PDs arise in social contexts hardly to be reflected in standardized interviews.

Furthermore, a distinct PD, according to DSM-IV or ICD-10, does not represent a categorical unit of human behaviour but rather a blend of temperamental traits, cognitive plans, mental 'master' attitudes, patterns of behaviour, and so on. Some of these features have a substantial genetic component, while others do not. Biological background mingles with environmental factors and education — both nature and nurture shape personality and PDs alike. Due to the heterogeneity of PD diagnoses, it appears to be naive and futile to search for a single genetic cause resulting in exactly one specific PD. In DSM-IV, PDs have been grouped in three clusters (withdrawn, flamboyant, and fearful), which might be a reasonable starting point in the search for genetic influences. Several authors assign PDs of the so-called Cluster A (paranoid, schizoid, and schizotype) to the schizophrenia spectrum disorders, with an overlapping genetic background; Cluster B, the flamboyant group, appears to be influenced more by environmental than genetic factors (Kendler and Eaves, 1986). Cluster C, comprising obsessive-compulsive, avoidant, and dependent traits, seems to share genetic and environmental causes in equal parts.

In order to avoid the inherent problems of multidimensional diagnosis of PDs, it is sensible to identify the temperamental and behavioural traits, such as anxiety, novelty seeking, impulsivity, aggressiveness, and antisocial behaviour, that underlie PDs. These traits can be assessed by rating scales for humans (e.g., the Revised NEO Personality Inventory NEO-PI-R [Costa and McCrae, 1992], the Tridimensional Personality Questionnaire/Tridimensional Character Inventory TPQ/TCI [Cloninger *et al.*, 1993], and the Karolinska Scales of Personality [KSP] [Schalling *et al.*, 1987] questionnaires), as well as by specific behavioural paradigms in animal models, making it possible to investigate specific genetic influences. A specific PD could then be dissected into behavioural features, with known genetic components and non-genetic contributions, by cognitive schemes and strategies. The search for the genetics of PD thus closely resembles the search of the genetics of behaviour.

TOWARDS GENETIC DISSECTION OF PDs

It is now widely accepted that human temperament and personality is a multigene product. There is no single gene or major gene effect, respectively, for a specific behavioural trait, and, consequently, there is no single gene for a PD subtype. Rather these characteristics are polygenic, a fact which results in the failure of classical genome-wide linkage studies to detect the genes accounting for personality traits or PDs. Many genes, with only modest phenotypic effects, add up to account for the full variance of a particular personality dimension. This implies also that single variation in a given gene might lead to only a minimal change in the phenotype, but another variation in the same gene might have deleterious effects, resulting in an apparent 'one gene, one disorder (OGOD)' type of disease. This notion becomes even more complicated when gene–environment interactions and epistatic phenomena are taken into account.

A gene variant which is associated with a PD in one population may not be so associated in another ethnic group — a problem which is increasingly being encountered in behavioural genetics. Since a given gene influences distinct personality traits only to a small extent — for instance, allelic variation in 5HTT function, which is described in more detail below, accounts for approximately 8% of the genetic variance of traits related to anxiety and aggression — and since the genetic component of PD is polygenic, classical linkage analysis has failed to identify relevant genomic variation. Because the power of linkage analysis to detect small gene effects is quite limited, at least with realistic samples, QTL research relies on association analysis using DNA variants in or near candidate genes that are presumably functional. The first QTL analysis was conducted 1988 by Paterson *et al.*, who searched for the genes responsible for the phenotypes of tomato fruits (Paterson *et al.*, 1988). While this study demonstrated that a set of six genes controls fruit mass, it highly likely that personality traits are defined by sets of at least 10–20 different genes, and that both pleiotropy and epistasis play an critical role in the molecular basis of personality.

The pace of integration of quantitative and molecular genetics has been accelerated remarkably as a result of the *Drosophila*, mouse, and human genome projects, which have brought neuroscience to the threshold of the postgenomic era in which the human genome and those of other species are known. While several million DNA variations (polymorphisms) have been identified in the genomic sequence, most importantly for the analysis of complex traits, roughly 30 000–60 000 common polymorphisms are located in coding and regulatory regions of genes that are the ultimate causes of the heritability of complex traits (McPherson *et al.*, 2001; Sachidanandam *et al.*, 2001). These advances have generated new technologies, such as DNA microarrays, that make it possible to investigate the role of thousands of DNA variants in complex traits. The rate of discovery of genes associated with complex traits, such as personality and behaviour, will also increase, as systematic QTL scans can be conducted using functional DNA variants in the brain that affect coding regions or gene regulation. Of the nearly 40 000 genes, approximately 50% are expressed in the brain, but a substantial proportion of these are housekeeping genes. Thus, fewer than 15 000 tissue-specific genes may be expressed in the brain. Identifying the estimated 8000–16 000 functional DNA variants in coding regions of these genes and the DNA variants that regulate the expression of these genes is now a high priority for behavioural genetic research. Several classes of genomic variations in coding and regulatory regions of genes, commonly referred to as candidate genes, are of particular interest. The variety of these functionally relevant variations ranges from single nucleotide polymorphisms (SNPs) to microdeletions (or insertions), and polymorphic simple sequence repeats (SSRs) of 2–50+ nucleotides in length.

Quantitative genetic research on animal models consists primarily of inbred strain and selection studies. While comparisons between different inbred strains of mice expose remarkable differences in measures of anxiety-related behaviour, such as performance in the Open Field or Elevated Plus Maze paradigm, differences within strains can be attributed to environmental influences. Inbred and recombinant inbred strain studies are highly efficient in dissecting genetic influences, for investigating interactions between genotype and environment, and for testing the disposition-stress model. Selective breeding of mice for many

generations produces differences between high and low anxiety lines that steadily increase from generation to generation. Selection studies of behavioural traits strongly suggest a genetic influence and that many genes contribute to variation in behaviour. Mice strains that have been selectively bred to display a phenotype of interest are currently being used to identify genetic loci that contribute to behavioural traits. The QTL approach has been applied with some success to a trait in mice called 'emotionality' (Flint *et al.*, 1995). Crosses between the high- and low-activity selected mouse lines yielded three QTL regions that appear to be related to various measures of fearfulness. A modified QTL strategy that uses recombinant inbred mouse strains produced candidate QTLs for Open Field fearfulness (Phillips *et al.*, 1995).

Regrettably, such linkage analyses provide only a rough chromosomal localization, whereas the next step, identifying the relevant genes by positional cloning, remains a challenging task (Tecott and Barondes, 1996). Since mice and humans share many orthologous genes mapped to synthenic chromosomal regions, it is conceivable that individual genes identified for behaviour may be developed as animal models. After chromosomal mapping of polymorphic genes and evaluation of gene function with knockout mutants, behavioural parameters are investigated. Thus, the combination of elaborate genetic and behavioural analyses results in the identification of many genes with effects on the variation and development of murine behaviour; ultimately, mouse QTL research is likely to generate candidate genes for human behavioural disorders.

CANDIDATE GENES

A complementary approach to quantitative genetic studies is investigation of whether certain candidate genes are involved in the manifestation of a specific phenotype, and whether such genes are involved in the pathogenesis of disease states. While this approach was restricted until recently to a relatively small number of genes, identification of new candidates will be facilitated by the new tools of the postgenomic era. Because it is hypothesis-driven, the investigation of candidate genes is a powerful means to examine the biological basis of personality and behaviour. In this context, psychobiology accumulated a thorough knowledge of neurotransmitter networks; consequently, the first candidate genes investigated were components of the monoamine neurotransmitter pathways. Interest has primarily been focused on the serotonergic, dopaminergic, and — to a lesser extent — noradrenergic systems. Almost no information is available on the role of genetic variations of the constituents of second and third messenger pathways, transcription factors, or other morphoregulators.

Nevertheless, several caveats have to be kept in mind with respect to candidate gene studies. First of all, negative studies might be underrepresented due to publication bias, an error with declining impact, since this problem is increasingly acknowledged by scientists, reviewers, and editors. Also of importance seems to be the problem that initial false-negative association studies impede further research, as they are unlikely to be replicated (Cardon, 2001a; b). This emphasizes the importance of further research on known or hypothesized candidate genes. Further issues are the artefacts due to population stratification as well as to gene–gene and gene–environment interactions. However, as QTL loci mostly require very large sets of sib-pairs to gain enough statistical power in linkage analyses (Speer, 1998), candidate gene studies are currently more feasible despite these limitations.

Serotonergic Gene Pathway

In view of the converging lines of evidence that 5-HT and serotonergic gene expression is involved in a myriad of processes during brain development as well as synaptic plasticity in adulthood, temperamental predisposition and complex behaviour are likely to be influenced by genetically driven variability of 5-HT function. Originating from the raphe nuclei in the brainstem, the serotonergic system innervates multiple regions throughout the brain. For instance, serotonergic neurons are found in the basal ganglia, amygdala, hippocampus, nucleus accumbens, cingulum, thalamus, cortex, and many other areas. The central 5-HT system is thought to function as a behavioural inhibition system, and to be involved in the regulation of motor activity, food intake, sleep and other circadian rhythms, cognition, mood, anxiety, aggression, and impulsivity. It exerts its actions by the modulation of various other transmitter systems, such as the noradrenergic, dopaminergic, glutamatergic, GABAergic, and cholinergic systems. The 5-HT system integrates and connects these spatially separated systems and thus forms a global meta-network of distinct local networks, thereby coordinating and balancing common brain functions. Moreover, 5-HT not only acts as a neurotransmitter but is also involved in the regulation of neurogenesis and synaptic plasticity in the pre- and post-natal period, as well as in the adult brain.

Serotonergic system dysfunction has been implicated in various psychiatric disorders, including depression, bipolar disorder, panic and generalized anxiety disorder (GAD), seasonal affective disorder (SAD), obsessive-compulsive disorder (OCD), eating disorders, and possibly also schizophrenic and neurodegenerative disorders (Lucki, 1998). Regarding PDs, a pivotal role of the serotonergic system has been hypothesized in the pathogenesis of emotional instability syndrome, commonly referred to as borderline personality disorder (BPD) (Goodman and New, 2000; Gurvits *et al.*, 2000). Consequently, psychoactive drugs targetting the serotonergic neurocircuitries, such as selective 5-HT reuptake inhibitors (SSRIs) and 5-HT (ant)agonists, including atypical neuroleptics, have been proven to be beneficial in most of these diseases. Low 5-HT concentrations or turnover — as measured by the metabolite 5-hydroxyindole acetic acid (5-HIAA) in cerebrospinal fluid (CSF) — are correlated with suicide, impulsivity, aggression (Asberg *et al.*, 1986; Higley *et al.*, 1996; Higley *et al.*, 1992a), and — in neonates — a family history of antisocial PD (Constantino *et al.*, 1997). In line with these findings, patients suffering from severe PDs of the high aggression/low impulse-control type (e.g., antisocial PD) exhibit decreased 5-HIAA levels (Brown *et al.*, 1982; Brown *et al.*, 1979; Coccaro *et al.*, 1992). A landmark study by Raleigh *et al.* (1991) elegantly demonstrated that male vervet monkeys with low social status also have low CSF 5-HIAA concentrations. Correspondingly, high 5-HIAA levels are associated with high social status. Intriguingly, pharmacological alterations of serotonergic function influence social rank, a fact which further underscores the critical role 5-HT plays in controlling temperament and behavioural traits. Hence, 5-HT appears to be a stabilizer of neural circuits as well as of social networks.

In a two-step reaction, 5-HT is synthesized from the essential amino acid, tryptophan. The first and rate-limiting step, the hydroxylation at the 5-position, is catalysed by the enzyme tryptophan hydroxylase (TPH) and is restricted to raphe neurons. Decarboxylation of 5-hydroxytryptophan represents the second step, which is catalysed by an amino acid decarboxylase. This non-specific enzyme is expressed not only in serotonergic neurons but also in catecholamine-synthesizing cells. Once formed, 5-HT is transported into synaptic vesicles by a nonselective monoamine transporter (VMAT). When intracellular free calcium levels reach a certain threshold, 5-HT is released into the synaptic cleft by fusion of the vesicle with the cell membrane. It may act thereafter as a paracrine hormone with effects also on non-excitable cells, as well as a classical neurotransmitter; up to now, 14 receptor subtypes are known. The reuptake of extracellular 5-HT is performed by the selective 5-HT transporter (5-HTT). 5-HT is thereafter recycled for repetitive

release or degraded to 5-HIAA by the enzyme monoamine oxidase A (MAO-A), which is localized at the outer membrane of mitochondria. For several of the genes involved in the regulation and function of the serotonergic pathway, various polymorphisms have been identified that may influence personality traits and related disorders.

Tryptophan Hydroxylase

Tryptophan hydroxylase (TPH), the rate-limiting enzyme in the synthesis of 5-HT, is a tetrahydrobiopterin (H_4Bip)-dependent mono-oxygenase which, in the CNS, is localized exclusively in raphe neurons. It is a member of the superfamily of aromatic amino acid hydroxylases, which also comprises the tyrosine and phenylalanine hydroxylases (Ledley *et al.*, 1987). The TPH gene has been assigned to human chromosome 11p15.3-p14, comprises 11 exons, and is subject to alternative splicing (Boularand *et al.*, 1995; Craig *et al.*, 1991). Besides polymorphisms in the upstream regulatory region, intron 7 is the site of two variations, although their functional relevance remains elusive (Nielsen *et al.*, 1997). Both are A to C transversions at the base pairs 218 and 779, respectively. 779A is termed allele U, and 779 C allele L (Nielsen *et al.*, 1992). Since low 5-HT system function is known to correlate with (violent) suicide, aggression, and impulsivity, a link between TPH gene variants and these traits had been predicted.

In a pioneering study by Nielsen *et al.* (1994), it was shown in a Finnish sample that the L variant of intron 7 of the TPH gene is associated with decreased CSF 5-HIAA concentrations, and that this polymorphism may predispose to suicidal behaviour. However, no association between this genetic variation and impulsivity was found. In a replication study by the same group, the TPH L allele was again linked to suicidality, history of suicide attempts, and alcohol dependence (Nielsen *et al.*, 1998). Siever and associates (New *et al.*, 1998) reported an association of the TPH gene L allele with measures of impulsivity and aggression with the Buss-Durkee Hostility Inventory (BDHI). However, it was demonstrated that in a non-patient sample 'outward bound' aggression is correlated with the U allele of TPH (Manuck *et al.*, 1999) and low CSF 5-HIAA levels (Jonsson *et al.*, 1997a). In depressed patients, the U allele was shown to be linked to a history of suicide attempt (Mann *et al.*, 1997); in schizophrenic patients, the L allele was more frequent in male subjects with a history of violent behaviour (Nolan *et al.*, 2000b). Regarding the A218 C polymorphism, the results are similarly equivocal, with both positive (Tsai *et al.*, 1999) and negative (Geijer *et al.*, 2000) findings. Recently, a negative report on the relation between both TPH polymorphisms and suicidality was published (Bennett *et al.*, 2000) in line with the findings by Abbar *et al.* (1995) and Bellivier *et al.* (1998), who argued that TPH polymorphisms are not causally linked to suicide, but that the reported associations are due to ethnic stratification. As both investigated polymorphisms are non-coding, it seems likely that they are linked with functional polymorphisms in some, but not all, populations. This possibility may explain the observed inconsistencies and thus warrants further research. In conclusion, it seems that at least in some populations, or in connection with psychiatric disease states, TPH polymorphism may play a role in the predisposition to suicidality, aggression, and possibly alcoholism. To resolve these apparent discrepancies, studies with large and ethnically matched samples need to be conducted to clarify the role of the TPH gene in personality traits and related disorders.

5-HT Receptors

Fourteen 5-HT receptor subtypes are known and are distinguished pharmacologically, structurally, functionally, and/or by molecular biologic means (for reviews, see Hoyer and Martin, 1996; Saxena, 1995). They probably derive from a common primordial 5-HT receptor, which developed in the course of evolution more than a billion years ago. The 5-HT receptors are subdivided in seven families, the largest being the 5-HT_1 family with five 5-HT receptor subtypes termed 5-HT_{1A-F} (5-HT_{1C} has been reassigned as 5-HT_{2C}). The 5-HT_2 family has three members, the 5-HT_5 family two subtypes; with respect to 5-$HT_{4,6,7}$, only a single member is known. 5-HT_3 comprises two subunits, A and B. The diversity of 5-HT receptors is probably due to the ontogenetically old role of 5-HT in neuromodulation; the apparent complexity of serotonergic subsystems allows fine tuning. Regarding personality and behaviour, a large body of evidence suggests that 5-HT receptors are involved in anxiety, impulsivity, and aggression (Goldman *et al.*, 1996; Roth, 1994). While 5-HT_{1A} and 5-$HT_{1B/D}$ (which share a high degree of similarity), 5-HT_{2A}, and 5-HT_{2C} have been extensively investigated, little is known about subtypes 5-HT_{4-7}.

5-HT_{1A} receptors are expressed on neurons as well as on astrocytes. They act presynaptically as somatodendritic autoreceptors in the raphe complex and postsynaptically in the hippocampus, septum, and the amygdala, where they initiate neuronal inhibition by hyperpolarization. The 5-HT_{1A} receptor gene is located on human chromosome 5q11.2-q13. Studies employing pharmacological techniques suggested that 5-HT_{1A} activation decreases aggressive behaviour in humans as well as rodents (Coccaro *et al.*, 1990; De Vry, 1995; Kavoussi *et al.*, 1997). In line with these findings, mice with a targetted inactivation of the 5-HT_{1A} receptor gene display increased anxiety-related behaviours in three independent studies (Heisler *et al.*, 1998; Parks *et al.*, 1998; Ramboz *et al.*, 1998). While other mechanisms for the observed behavioural changes could be excluded, a link between serotonergic and GABAergic transmission was recently reported. Mice with a targetted inactivation of the 5-HT_{1A} receptor consistently display a spontaneous phenotype that is associated with a gender-modulated and gene/dose-dependent increase of anxiety-related behaviours. With the exception of an enhanced sensitivity of terminal 5-HT_{1B} receptors and downregulation of GABA-A receptors (Sibille *et al.*, 2000), no major neuroadaptational changes were detected. Activation of presynaptic 5-HT_{1A} receptors provide the brain with an autoinhibitory feedback system controlling 5-HT neurotransmission. Thus, enhanced anxiety-related behaviour probably represents a consequence of increased terminal 5-HT availability resulting from the lack or reduction in presynaptic somatodendritic 5-HT_{1A} autoreceptor negative feedback function. Indirect evidence for increased presynaptic serotonergic activity is provided by the compensatory upregulation of terminal 5-HT_{1B} receptors. This mechanism is also consistent with recent theoretical models of fear and anxiety that are primarily based upon pharmacologically derived data. The cumulative reduction in serotonergic impulse flow to septohippocampal and other limbic and cortical areas involved in the control of anxiety is believed to explain the anxiolytic effects of ligands with selective affinity for the 5-HT_{1A} receptor in some animal models of anxiety-related behaviour. This notion is based, in part, on evidence that 5-HT_{1A} agonists (e.g., 8-OH-DPAT) and antagonists (e.g., WAY 100635) have anxiolytic or anxiogenic effects, respectively. However, to complicate matters further, 8-OH-DPAT has anxiolytic effects when injected in the raphe nucleus, whereas it is anxiogenic when applied to the hippocampus. Thus, stimulation of postsynaptic 5-HT_{1A} receptors has been proposed to elicit anxiogenic effects, while activation of 5-HT_{1A} autoreceptors is thought to induce anxiolytic effects via suppression of serotonergic neuronal firing, resulting in attenuated 5-HT release in limbic terminal fields.

Taken together, these findings lead to the conclusion that the 5-HT_{1A} receptor is a key player in the modulation of anxiety-related behaviour and thus behavioural inhibition. Anxiety-related behaviour in 5-HT_{1A}-deficient mice seems to be caused by an

increased serotonergic tone due to the absent negative feedback of the 5-HT_{1A} autoreceptor. Interestingly, when wild house mice were divided into those with high and those with low-aggression scores, the aggressive mice showed high levels of 5-HT_{1A} receptor expression in the dorsal hippocampus compared with low-aggression mice (Korte *et al.*, 1996). This finding supports the notion of a contraposition of aggression and anxiety and the critical role of the serotonergic system in balancing both.

The 5-HT_{1B} receptor (like the structurally similar 5-HT_{1D} receptor) serves as an inhibitory autoreceptor on serotonergic terminals but also as a postsynaptic heteroceptor on non-5-HT neurons. It is expressed in the basal ganglia, striatum, hippocampus, amygdala, and frontal cortex. 5-HT_{1B} has been assigned to human chromosome 6q13 (Demchyshyn *et al.*, 1992). Based on rather indirect pharmacological evidence, it is thought to play a role in aggressive as well as reproductive behaviour. Accordingly, 5-HT_{1B} knockout mice show increased exploratory behaviour and are more aggressive in the resident-intruder paradigm (Brunner *et al.*, 1999; Malleret *et al.*, 1999; Ramboz *et al.*, 1996; Saudou *et al.*, 1994). As for 5-HT_{1A} knockout mice, this was not due to changes in locomotor activity. Mice lacking the 5-HT_{1B} receptor were also more prone to develop cocaine (Rocha *et al.*, 1998) and alcohol dependence (Brunner and Hen, 1997), a finding which suggests a role of this receptor in the pathophysiology of substance abuse. Two polymorphisms in the human 5-HT_{1B} gene, C129T and G861C, were not associated with depression, suicidality, or alcoholism (Huang *et al.*, 1999). However, in Finnish antisocial alcoholics, as well as a Southwest Native American tribe with a high rate of alcoholism, a significant association with G861C was demonstrated (Lappalainen *et al.*, 1998).

The human 5-HT_{2A} receptor gene is located on chromosome 13q14-q21 near the genes for retinoblastoma-1 and esterase D (Hsieh *et al.*, 1990) and consists of three exons and two introns (Chen *et al.*, 1992). There is reason to assume that 5-HT_{2A} is subject to genomic imprinting; only the maternal allele is expressed (Kato *et al.*, 1996). The hallucinogenic effects of LSD have been attributed to its 5-HT_{2A} agonism (Aghajanian and Marek, 1999); consequently, an association between the T102C polymorphism of the 5-HT_{2A} receptor and schizophrenia has been suggested by several, but not all, studies (He *et al.*, 1999; Spurlock *et al.*, 1998; Williams *et al.*, 1996; 1997). However, Holmes *et al.* (1998) reported that the 102 C allele is linked to the presence of hallucinations in late-onset Alzheimer's disease. No association between suicide attempts and 5-HT_{2A} polymorphism was detected (Geijer *et al.*, 2000) when suicide attempters were split into different categories including Cluster B, but not Cluster C, PD. In a study with patients suffering from bipolar affective disorder and healthy controls, no major effect of the T102C polymorphism on the TPQ personality dimension 'harm avoidance' was found (Blairy *et al.*, 2000). All in all, there is, as yet, no clear-cut evidence for a role of this polymorphism in PDs.

The physiological function of the 5-HT_{2C} receptor, which is located in the cortex, hippocampus and striatum, remains elusive. It has been mapped to Xq24 (Milatovich *et al.*, 1992). A functional knockout mouse model, in which the carboxy-terminal half of the receptor is removed, has been generated by Tecott *et al.* (1995). In addition to increased vulnerability of these mice to die from seizures, they were also overweight, hinting at a role for 5-HT_{2C} in the regulation of food intake (Tecott *et al.*, 1995). In humans, the Cys23Ser polymorphism in the N-terminal end of the 5-HT_{2C} receptor protein has attracted considerable attention (Lappalainen *et al.*, 1995). The 23Ser allele was associated with hallucinations and, interestingly, hyperphagia in Alzheimer's disease (Holmes *et al.*, 1998). Furthermore, an association between the Cys23Ser polymorphism and violent alcoholism was reported (Virkkunen *et al.*, 1996), although failure of replication casts some doubt on the initial finding (Himei *et al.*, 2000; Lappalainen *et al.*, 1999; Schuckit *et al.*, 1999).

Based on a personality assessment with the TPQ questionnaire, an interesting gene–gene interaction between a polymorphism of the dopamine receptor DRD4 (also see below) and the 5-HT_{2C} Cys23Ser polymorphism was demonstrated. When both polymorphisms were present, they accounted for 13% of the variance of TPQ 'reward dependence' and 30% of the variance for 'persistence' — a subscore of 'reward dependence' (Ebstein *et al.*, 2000; Ebstein *et al.*, 1997c; Kuhn *et al.*, 1999). No influence of the Cys23Ser polymorphism alone was found (Kuhn *et al.*, 1999); no replication studies have been performed to date.

With respect to 5-HT_{3-7} receptors, little evidence for involvement of these molecules in behaviour has been presented to date. Clearly, more research on these, in part, poorly understood and characterized receptors is needed. An interesting finding has been presented by Grailhe *et al.* (1999), who demonstrated that 5-HT_{5A} knockout mice, just like 5-HT_{1B}-deficient mice, display increased explorative behaviour, as well as altered response to hallucinogens, such as LSD. In a Finnish group of antisocial alcoholics, an association with a polymorphism of the 5-HT_7 receptor gene has been suggested, but this finding still awaits confirmation (Pesonen *et al.*, 1998).

5-HT Transporter

In contrast to the remarkable diversity of 5-HT receptors, only a single protein, the 5-HT transporter (5-HTT), mediates the reuptake of 5-HT from the synaptic cleft and thus the termination of its action. The 5-HTT is structurally and functionally similar to the dopamine (DAT) and norepinephrine (NET) transporter proteins (Blakely *et al.*, 1994; Borowsky and Hoffman, 1995). The exchange of intracellular sodium against extracellular potassium generates the energy necessary for transport of 5-HT against the extra-intracellular gradient. When intracellular 5-HT levels are very high — for instance, as a consequence of VMAT2 inhibition following MDMA ('ecstasy') intake — the 5-HTT reverses its action by releasing 5-HT into the synaptic cleft. 5-HTT is an initial target for antidepressant drugs, either with relatively low specificity, such as the TCAs (e.g., imipramine and clomipramine) or high selectivity, such as the SRIs (e.g., fluoxetine, sertraline, and citalopram), as well as for psychostimulants, including amphetamines and cocaine (Schloss and Williams, 1998). The efficacy and versatility of 5-HTT-inhibiting drugs underscores the outstanding role of the 5-HTT in the fine regulation of the serotonergic system.

The human 5-HTT is encoded by a single gene on chromosome 17q11 adjacent to the CPD and NF1 genes (Shen *et al.*, 2000). It is composed of 14–15 exons spanning approximately 35 kb (Lesch *et al.*, 1994). In humans, the transcriptional activity of the 5-HTT gene, 5-HTT availability, and ultimately 5-HT reuptake are modulated by a polymorphic repetitive element, the 5-HTT gene-linked polymorphic region (5-HTTLPR), located upstream of the transcription start site (for review, see Lesch, 1997). Additional variations have been described in the 5′ untranslated region (5′UTR) due to alternative splicing of exon 1B (Bradley and Blakely, 1997), and in intron 2 (variable number of a 16/17-base-pair tandem repeat, VNTR-17) (Lesch *et al.*, 1994), and 3′UTR (Battersby *et al.*, 1999). Comparison of different mammalian species confirmed the presence of the 5-HTTLPR in Platyrrhini and Catarrhini (hominoids, cercopithecoids), but not in prosimian primates and other mammals (Lesch *et al.*, 1997). The 5-HTTLPR is unique to humans and simian primates. The majority of alleles are composed of either 14-or 16-repeat elements in humans (short and long allele, respectively), while alleles with 15, 18, 19, 20, or 22 repeat elements, and variants with single-base insertions/deletions or substitutions within

individual repeat elements are rare. A predominantly Caucasian population displayed allele frequencies of 57% for the long (*l*) allele and 43% for the short (*s*) allele with a 5-HTTLPR genotype distribution of 32% *l*/*l*, 49% *l*/*s*, and 19% *s*/*s* (Lesch *et al.*, 1996); different allele and genotype distributions are found in other populations (Gelernter *et al.*, 1998). Consequently, the contribution of the 5-HTTLPR to individual phenotypic differences in personality traits was explored in independent population- and family-based genetic studies (for review, see Lesch, 2001a). Anxiety-related and other personality traits were assessed by the NEO Personality Inventory — Revised (NEO-PI-R), a self-report inventory based on the five-factor model of personality ('Big Five') (Costa and McCrae, 1992), and by the 16PF Personality Inventory (Cattell, 1989). The five factors assessed by the NEO are 'neuroticism' (emotional instability), 'extraversion', 'agreeableness' (cooperation and reciprocal alliance formation), 'openess' (intellect and problem-solving), and 'conscientiousness' (will to achieve).

In an initial study, population and within-family associations were discovered between the low-expressing s allele and 'neuroticism', a trait related to anxiety, hostility, and depression, on the NEO-PI-R in a primarily male population ($n = 505$) (Lesch *et al.*, 1996). Individuals with either one or two copies of the short 5-HTTLPR variant (group S) had significantly greater levels of neuroticism, defined as proneness to negative emotionality, including anxiety, hostility, and depression, than those homozygous for the long genotype (group L) in the sample as a whole and also within sibships. Individuals with 5-HTTLPR S genotypes also had significantly decreased NEO 'agreeableness'. In addition, the group S individuals had increased scores on 'anxiety' on the separate 16PF personality inventory, a trait related to NEO 'neuroticism'. In a more recent investigation, this association was tested in an independent sample ($n = 397$, 84% female, primarily sib-pairs) (Greenberg *et al.*, 2000). The findings replicated the association between the 5-HTTLPR and NEO 'neuroticism'. Combined data from the two studies ($n = 902$), which were corrected for ethnicity and age, gave a highly significant association between the *s* allele and anxiety-related traits both across individuals and within families, reflecting a genuine genetic influence rather than an artefact of ethnic admixture.

Another association encountered in the original study between the *s* allele and lower scores of NEO 'agreeableness' was also replicated and was even more robust in the primarily female replication sample. Gender-related differences in 5-HTTLPR-personality trait associations are possible, since several lines of evidence demonstrate gender-related differences in central 5-HT system function in humans and in animals. These findings include the effects of gonadal steroids on 5-HTT expression in rodent brain and differences in anxiety-related behaviours in male and female 5-HTT knockout mice (Li *et al.*, 2000).

Analysis of NEO subscales further defined the specific aspects of personality that were reproducibly associated with the 5-HTTLPR genotype. For the NEO 'neuroticism' subscales, the combined samples from Lesch *et al.* (1996) and Greenberg *et al.* (2000) demonstrated significant associations between 5-HTTLPR S group genotypes and the facets of 'depression' and 'angry hostility', two of the three facets that showed the most significant associations in the initial sample (Lesch *et al.*, 1996). In contrast, the NEO 'neuroticism anxiety' facet, which was associated with genotype in the first study at a weaker significance level, was not significantly associated with the 5-HTTLPR genotype in the new cohort. With regard to the NEO 'agreeableness' subscales, the previously observed associations of 5-HTTLPR S group genotypes with decreased 'straightforwardness' and 'compliance' were consistently replicated. It therefore appears more accurate to state that the 5-HTTLPR is associated with traits of negative emotionality related to interpersonal hostility and depression. The relationship between these two aspects of negative emotionality is not unexpected in view of the previously observed negative correlation between 'angry hostility', a facet of 'neuroticism', and 'agreeableness' (Costa and McCrae, 1992), indicating that both measures assess a behavioural predisposition to uncooperative interpersonal behaviour (Greenberg *et al.*, 2000).

The effect sizes for the 5-HTTLPR-personality associations, which were comparable in the two samples, indicate that this gene variation has only a modest influence on the behavioural predisposition of approximately 0.30 standard deviation units. This corresponds to 3–4% of the total variance and 7–9% of the genetic variance, based on estimates from twin studies using these and related measures that have consistently demonstrated that genetic factors contribute 40–60% of the variance in neuroticism and other related personality traits. Although the genetic effect suggests that the 5-HTTLPR represents an above-average size QTL, the associations represent only a small portion of the genetic contribution to anxiety-related personality traits. If additional genes were hypothesized to contribute similar gene-dose effects to anxiety, at least 10–15 genes are predicted to be involved. Additive contributions of comparable size based on epistatic epigenetic interactions have, in fact, been found in studies of other quantitative traits. Thus, the results are consistent with the view that the influence of a single, common polymorphism on continuously distributed traits is likely to be small in humans, and possibly to influence different quantitative characteristics in other species (Plomin *et al.*, 1994).

At first glance, association between the high-activity 5-HTTLPR *l* allele and lower 'neuroticism' and related traits seems to be counter-intuitive with regard to the known antidepressant and anxiolytic effects of 5-HTT inhibitors (SRIs). Likewise, Knutson *et al.* (1998) reported that long-term inhibition of the 5-HTT by the SRI paroxetine reduced indices of hostility through a more general decrease in negative affect, a personality dimension related to neuroticism. The same individuals also demonstrated an increase in directly measured social cooperation after paroxetine treatment, an interesting finding in view of the replicated association between 5-HTTLPR genotype and agreeableness. That a drug which inhibits 5-HTT lessened negative emotionality and increased social cooperation appears to conflict with findings that the 5-HTTLPR long allele, which confers higher 5-HTT expression, is associated with lower NEO 'neuroticism' and higher NEO 'agreeableness'. However, this apparent contradiction may be due to the fact that both 5-HT and 5-HTT play critical roles in brain development that differ from their functions in regulating neurotransmission in the adult.

The conclusion that the 5-HTT may affect personality traits via an influence on brain development and synaptic plasticity is strongly supported by recent findings in rodents and nonhuman primates. Studies in rats confirmed that the 5-HTT gene is expressed in brain regions central to emotional behaviour during foetal development, but not later in life (Hansson *et al.*, 1998), hence enduring individual differences in personality could result from 5-HTTLPR-driven differential 5-HTT expression during pre- and perinatal life. In support of this notion, mice with a targetted disruption of 5-HTT display enhanced anxiety-related behaviours (also see section on mouse models of anxiety-related traits) in models of avoidance and anxiety (Wichems *et al.*, 2000).

Together with DRD4, the 5-HTTLPR is the best-investigated candidate gene polymorphism with regard to personality dimensions, with 22 studies directly or indirectly targetting this subject. The results are controversial, for manifold reasons. The limiting factor in QTL analysis often is sample size, because studies including small samples lack the statistical power to detect QTL gene effects, which account typically for 2–10% of the genetic variance. In subsequent investigations, only two attempts have been reported to replicate the original finding using large

Table XXVI-7.1 Overview of published data on the association between the 5-HT transporter gene-linked polymorphic region (5HTTLPR) and anxiety-related traits in general populations and patient samples

	n	Population and study design	Trait assessment/inventory	Association of *s* allele, *P*	Family-based studies, *P*
Lesch *et al.*, 1996	505	General, USA, males, two subsamples	NEO-PI-R, 16PF	0.02–0.002	0.03–0.004
Ebstein *et al.*, 1997	120	General, Israeli	TPQ	0.75, n.s.	—
Ball *et al.*, 1997	106	General, German, 5% of highest vs. lowest N scores	NEO-FFI	0.89, n.s.	—
Nakamura *et al.*, 1997	203	General, Japanese, females	NEO-PI, TCI	*s* increased, *l* rare	—
Mazzanti *et al.*, 1998	655	Controls, alcoholic violent offenders, Finnish	TPQ	n.s.	0.45, but 0.003 for HA1 + HA2
Ebstein *et al.*, 1998	81	General, Israeli, neonates (2 weeks old)	NBAS	interaction with DRD4[#]	—
Ricketts *et al.*, 1998	84	Controls, Parkinson's disease, USA	TPQ	0.04–0.003	—
Gelernter *et al.*, 1998	185/322	Controls, substance dependence, personality disorder, USA	NEO-PI TPQ	0.47, n.s. 0.87, n.s.	—
Jorm *et al.*, 1998	759	General, European Australian	EPQ-R	n.s.	—
Seretti *et al.*, 1999	132	Depression, bipolar disorder	HAMD, anxiety	0.01	—
Murakami *et al.*, 1999	189	General, Japanese	SRQ-AD	0.05	—
Flory *et al.*, 1999	225	General, USA	NEO-PI-R	0.97	—
Kamakiri *et al.*, 1999	144	General, Japanese	NEO-PI-R, TPQ	n.s.	—
Katsuragi *et al.*, 1999	101	General, Japanese	TPQ	0.007	—
Auerbach *et al.*, 1999	76	General, Israeli, infants (2 months old), follow-up of Ebstein *et al.*, 1998	IBQ	0.05–0.005, interaction with DRD4[#]	—
Menza *et al.*, 1999	32	Parkinson's disease	HAMD	0.05	—
Dreary *et al.*, 1999	204	General, Scottish, 20% of highest vs. lowest N scores	NEO-FFI	n.s.	—
Gustavsson *et al.*, 1999	305	General, Swedish	KSP	n.s.	—
Osher *et al.*, 2000	148	General, Israeli	NEO-PI-R TPQ	—	0.06 0.07
Greenberg *et al.*, 2000	397	General, USA, females	NEO-PI-R, 16PF	0.03–0.01	0.01
Melke *et al.*, 2001	251	General, Swedish, females, same age	KSP	0.002–0.04	—
Du *et al.*, 2000	186	General, Canadian, males	NEO-PI-R	0.018	—

NEO-PI-R and NEO-FFI: Costa and McCrae's NEO Personality Inventory; TPQ/TCI: Cloninger's Tridimensional Personality Questionnaire/Temperament and Character Inventory; 16PF: Catell's 16 Personality Factor Inventory; EPQ-R: Eysenck Personality Questionnaire; KSP: Karolinska Scales of Personality; SRQ-AD: Self-Rating Questionnaire for anxiety and depression; HAMD: Hamilton Depression Rating Scale; IBQ: Rothbart's Infant Behaviour Questionnaire; NBAS: Brazelton's Neonatal Assessment Scale.
[#]Dopamine receptor D4.
n.s.: not significant; —: not determined.

populations with samples sizes of >400 individuals (Jorm *et al.*, 1998; Mazzanti *et al.*, 1998) (Table XXVI-7.1). Two of the three available large studies found evidence congruent with an influence of the 5-HTTLPR on 'neuroticism' and related traits (Lesch *et al.*, 1996; Mazzanti *et al.*, 1998), whereas a large population study not employing a within-family design (Jorm *et al.*, 1998) did not. Smaller population-based studies have had variable, but generally negative results (Table XXVI-7.1). Interpretation of these studies is complicated by their use of relatively small or unusual samples, and the lack of within-family designs that minimize population stratification artefacts. Other efforts to detect associations between the 5-HTTLPR and personality traits have been complicated by the use of small sample sizes, heterogeneous subject populations, and differing methods of personality assessment, that is, the use of other questionnaires as the original report, rendering between-study comparison difficult or even impossible. This may explain the conflicting results of Jorm *et al.* (1998), since this group utilized the EPQ-R questionnaire, which was not used in any of the other studies (Table XXVI-7.1). Furthermore, the subject selection in two studies (Ball *et al.*, 1997; Deary *et al.*, 1999) was unusual in that subjects at the high or low ends of the distribution for 'neuroticism' were selected on the assumption that the 5-HTTLPR affects the trait uniformly across its distribution. However, reanalysis of the data from the initial study revealed that the contribution of the 5-HTTLPR to NEO 'neuroticism' is greatest in the central range of the distribution and actually decreases at the extremes (Sirota *et al.*, 1999). This illustrates the need to obtain genotypes from individuals across the distribution of a trait, and suggests caution in the use of extreme populations in attempting to establish genetic influences on traits that are continuously distributed in the population.

Finally, difficulties in interpretation of population-based association studies due to ethnic differences in 5-HTTLPR allele frequencies have also been raised by recent studies. One found no association between the 5-HTTLPR and NEO 'neuroticism' in a sample of 191 Japanese, or between 5-HTTLPR genotype and TCI 'harm avoidance' in a subsample (Kumakiri *et al.*, 1999). In addition to its relatively small sample size, a major difficulty with this study is that the frequency of the *l*/*l* genotype (corresponding to group L) was only 6% of the total population,

giving very low statistical power to detect a genotype-related difference. Another study of association between the 5-HTTLPR and TPQ 'harm avoidance' in a Japanese population is also difficult to interpret due to differences in genotypes related to ethnicity (Katsuragi *et al.*, 1999b). In that case, the small sample ($n = 101$) had a frequency of l/l genotypes of only 4%. From an evolutionary psychological perspective, anxiety is a pervasive and innately driven form of distress that arises in response to actual or threatened exclusion from social groups (Baumeister and Tice, 1990; Buss, 1995). Notably, Nakamura *et al.* (1997) have discussed the higher prevalence of the anxiety and depression-related l/s and s/s genotypes in the context of the extraordinary emotional restraint and interpersonal sensitivity in the Japanese as a possible population-typical adaptation to prevent social exclusion (Ono *et al.*, 1996).

Nevertheless, spurious results due to population stratification cannot be ruled out entirely. It is, however, important to note that, while population stratification may lead to false-positive results, false-negative findings occur due to background factors masking QTL effects (Cardon, 2001a; Pericak-Vance, 1998). Studies which incorporate large samples of different population groups, the assessment of genetic background markers, and within-family study designs, such as the Transmission Disequilibrium Test (TDT), might help to minimize the bidirectional effects of stratification (Cardon, 2001b). In addition to regional or ethnic variations of allele frequency, epistatic and gene–environment interactions may well influence the possible phenotypic effect of a given genotype, especially in behavioural genetics, where roughly equal proportions of genetic effects and non-genetic effects contribute to trait variance. With respect to the 5-HTTLPR, further studies aiming at these questions are clearly needed to resolve these issues, which should include large, community-based cohorts of different populations. Of course, the same is true for other target genes such as DRD4.

The 5-HTTLPR has also been extensively studied in other psychiatric conditions, with both positive and negative findings (for review, see Lesch, 2001b). Especially the link between depression, suicidality, violent suicide attempts, and the 5-HTTLPR remains controversial. Seasonality and seasonal affective disorder (SAD) also seem to be associated with the 5-HTTLPR *s* allele (Rosenthal *et al.*, 1998; Sher *et al.*, 1999). This effect was shown to be independent of the gene effect on neuroticism, thus arguing for genetic pleiotropy of the 5-HTTLPR gene (Sher *et al.*, 2000). Furthermore, the 5-HTTLPR is thought to be associated with an increased vulnerability to develop nicotine (Hu *et al.*, 2000; Lerman *et al.*, 2000) and alcohol (Sander *et al.*, 1997b) dependence, the latter being comorbid with antisocial PD (Hallikainen *et al.*, 1999; Sander *et al.*, 1998).

As in the 5-HT_{1A} knockout mouse, exaggerated serotonergic neurotransmission has been implicated in the increased anxiety-related behaviours recently reported in 5-HT transporter-deficient mice (Wichems *et al.*, 2000). These findings are consistent with other evidence suggesting that increased 5-HT availability may contribute to increased anxiety in rodents, and the studies reporting that anxiety-related traits in humans are associated with allelic variation of 5-HTT function (Lesch *et al.*, 1996). Mice with a disrupted 5-HTT gene have been suggested as an alternative model to pharmacological studies of SSRI-evoked antidepressant and anxiolytic mechanisms to assess the hypothesized association between 5-HT uptake function and 5-HT_{1A} receptor desensitization (Bengel *et al.*, 1998). Excess serotonergic neurotransmission in mice lacking 5-HT transport results in desensitized and — unlike observations following SSRI administration — downregulated 5-HT_{1A} receptors in the midbrain raphe complex, but not in the hippocampus (Li *et al.*, 2000), and is suspected to play a role in the increased anxiety-related and antidepressant-like behaviours in these mice shown by the Light-Dark and Elevated Zero Maze paradigms, and the Tail Suspension paradigms. In contrast to that of 5-HT_{1A}-deficient mice, the anxiety-related behaviour, which can be reversed by anxiolytics of the benzodiazepine type, is more pronounced in female 5-HTT null mutants.

Dopaminergic Gene Pathway

While the 5-HT system exerts global, brain-wide modulatory functions, the action of the dopamine (DA) system is mediated via four distinct tracts, namely, the nigro-striatal, tubero-infundibular, meso-limbic, and fronto-cortical tract. The nigro-striatal tract, which originates in the substantia nigra and projects to the nucleus caudatus, is involved in motor control and has been implicated in the pathophysiology of Parkinson's disease; the tubero-infundibular system regulates prolactin secretion. While both systems participate in the common side effects of DA receptor antagonists, such as the classic neuroleptic drugs (e.g., dyskinesia, akathisia, and hyperprolactinaemia), they do not appear to be particularly relevant to the psychobiological aspects of dopaminergic neurotransmission. More outstanding in this regard are both the meso-limbic and fronto-cortical tracts, formerly termed the meso-cortico-limbic system. The two systems are thought to play important roles in a variety of psychiatric disease states. According to the DA hypothesis of schizophrenic disorders, functional alterations in the meso-limbic and fronto-cortical tracts underlie specific symptoms observed in schizophrenic psychoses. The meso-limbic system appears to be involved in 'positive' or 'productive' symptoms, such as hallucinations, paranoia, and delusions, whereas the fronto-cortical tract may cause 'negative' symptoms such as disorganization and lack of initiative. Furthermore, the DA system is involved in the brain's reward system and consequently in the pathogenesis of addictive behaviour. Pharmacological disinhibition of dopaminergic neurons in the ventral tegmental area, which project to the nucleus accumbens, results in the activation of the reward mechanism, while blockade of the reward system provokes symptoms similar to drug withdrawal.

Based on its functional neuroanatomy, the DA system seems to be involved in Cluster A PDs, associated in some cases with 'soft signs' of schizophrenic disorders and hence they are labelled by some authors as 'schizophrenia spectrum disorders' (Kety *et al.*, 1971), 'schizoid disease' (Heston, 1970), or 'schizotaxia'. A dysfunctional reward system in conjunction with dopaminergic abnormalities, as reflected by high novelty-seeking behaviour seems to play a role in PDs.

DA is synthesized from tyrosine in two steps. The first step is catalysed by the rate-limiting enzyme tyrosine hydroxylase, and the resulting L-DOPA is then decarboxylated by an aromatic amino acid decarboxylase (DOPA decarboxylase). Dopamine acts on at least five subtypes of receptors termed D1 to D5; the reuptake is performed by a specific DA transporter. Metabolic pathways involve two non-specific enzymes, monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT). A critical appraisal of the key components of dopaminergic transmission implicated in PDs follows.

Tyrosine Hydroxylase

Tyrosine hydroxylase (TH) is central to the function of the dopaminergic as well as noradrenergic neurotransmission. In a series of elegant experiments, Zhou *et al.* (1995) showed that targetted inactivation of both TH genes results in an embryonic lethality that was prevented by prenatal administration of L-DOPA. It was also demonstrated that TH-deficient mice pups may be rescued with restored with wild-type noradrenergic cells, although marked behavioural abnormalities persist (Zhou and Palmiter, 1995): TH knockout mice remained hypoactive and refused to eat

and drink. Upon acute L-DOPA injection, the behavioural deficits were reversed, and by continuous L-DOPA administration normal growth was restored.

The human TH gene has been assigned to chromosome 11p15 (Craig *et al.*, 1986) near the insulin and IGF1 gene. Several polymorphisms have been identified in the TH gene which cause different forms of Segawa syndrome, an inborn, autosomal, recessive, parkinsonism-like disorder starting in early childhood. Linkage of TH polymorphisms to several psychiatric Axis 1 disorders, including bipolar illness or schizophrenia, has been hypothesized, but the results to date are equivocal. Recently, a seemingly functional tetranucleotide repeat polymorphism ($TCAT_n$) in intron X of the TH gene (M) has been investigated for association with personality traits as assessed by the NEO-PI-R questionnaire in a Swedish population (Persson *et al.*, 2000b). Carriers of the T8 allele of $TCAT_n$ displayed significantly elevated 'neuroticism' scores, which were most prominent in the 'neuroticism' subitems 'angry hostility' and 'vulnerability'. Furthermore, a correlation of the T8 allele with both suicide attempts among subjects suffering from adjustment disorders (Persson *et al.*, 1997) and late-onset alcoholism among other patients was reported (Geijer *et al.*, 1997). It was concluded that the $TCAT_n$ polymorphism is linked to anxiety-related traits, and that the search for gene–gene interactions between $TCAT_n$ and 5-HTTLPR could be promising.

Dopamine (DA) Receptors

Although genomic variations have been identified in all five DA receptor genes, only the role of the D_2, D_3, and D_4 receptor genes (DRD2, DRD3, and DRD4, respectively) has been studied in normal personality and PDs. However, each receptor subtype has been investigated for a possible pathogenetic role in several psychiatric disease states, especially schizophrenic disorders, with inconclusive results (Wong *et al.*, 2000).

The human DRD2 gene, which has been located on chromosome 11q22–23, is subject to alternative splicing, and comprises eight exons. Interestingly, it is able to form a heterodimer with the somatostatin receptor upon binding of the respective ligand (Rocheville *et al.*, 2000). Several polymorphisms of the DRD2 have been studied in personality genetics, especially a *Taq*I restriction fragment length polymorphism (*Taq*I A) in the 3′-untranslated region of the gene (Grandy *et al.*, 1989). Several lines of evidence indicate that the A1 allele is associated with decreased dopaminergic tone in the CNS. A second *Taq*I site, identified between the first intron and the second exon has been termed *Taq*I B (Hauge *et al.*, 1991). Furthermore, a single nucleotide polymorphism (SNP) was found in intron 6 (*Hph* I site) (Sarkar *et al.*, 1991) and a functionally relevant Ser311Cys polymorphism in exon 7, which impairs D2 signal transduction, has been described (Cravchik *et al.*, 1996).

DRD2, the target for neuroleptic agents such as haloperidol or risperidone, has been suggested to be involved in a 'spectrum of DA-related disorders', including substance abuse, pathological gambling, obesity, and Tourette's syndrome (Comings *et al.*, 1996a, b; Noble *et al.*, 1994). Although this hypothesis is controversial, especially severe alcoholism seems to be associated with the *Taq*I A1 allele (Blum *et al.*, 1990). In line with a possible association of DRD2 with addictive behaviour, DRD2 knockout mice do not respond with reward behaviour following administration of morphine (Maldonado *et al.*, 1997), in addition to locomotor disturbances (Baik *et al.*, 1995).

The DRD2 gene locus has also been studied for a possible association with personality traits as well as PDs. Corresponding with the hypothesis of a schizophrenia spectrum disorder as a result of a DA neurotransmission impairment, Blum *et al.* (1997) found an association of the DRD2 *Taq*1 A1 allele with schizoid and avoidant behaviour, suggesting an involvement of DRD2 in PDs of Cluster A. By means of the TPQ questionnaire, it was shown that *Taq*1 A1, *Taq*2 B1, intron 6 alleles alone, and, even more robustly, the corresponding haplotype resulted in higher TPQ 'novelty-seeking' (NS) scores than other allele combinations. When the 7-repeat allele of DRD4 (see below) was present, a further increase of the difference was observed. This DRD2 haplotype also resulted in higher 'persistence' scores, while no influence of DRD4 7-repeat allele was detected; neither polymorphism was associated with 'harm avoidance' (Noble *et al.*, 1998). If replicated, these findings further support the notion of a link between dopaminergic function and NS (Cloninger, 1987). However, de Brettes *et al.* (1998) failed to find any association between *Taq*1 A1 and TPQ NS scores, suggesting that reports on associations between this polymorphism and personality traits should be considered preliminary. The only study investigating the Ser311Cys polymorphism in healthy individuals also yielded negative results regarding a relationship between DRD2 and scores of the TCI, a modified version of the TPQ (Cloninger *et al.*, 1993).

An interesting gene–environment interaction was reported by Ozkaragoz and Noble (2000). Children with the A1 DRD2 allele had higher extraversion scores when living in an alcoholic home; DRD2 A2 allele subjects tended to have lower extraversion levels. This result may link the DRD2 effect on alcohol dependence to environmental factors and personality. Another preliminary finding is the report on an association between DRD2 alleles and the age at first sexual intercourse, explaining the variance by 23% alone and by 55% in combination with DRD1 alleles (Miller *et al.*, 1999). These interesting, but hitherto incompletely understood data on the impact of DRD2 on personality warrant further investigation of gene–gene as well as gene–environment interactions.

Only a few data are available concerning personality traits and DRD3, which is expressed almost exclusively in limbic structures (Sokoloff *et al.*, 1990). Preliminary findings suggest a role for a functional DRD3 polymorphism (*Bal*I) influencing the TPQ NS scores of bipolar patients (Staner *et al.*, 1998); opiate-dependent subjects with high NS scores were found to carry *Bal*I significantly more often (Duaux *et al.*, 1998). As noted above, gene–gene interactions of the Glyc/Ser substitution of DRD3 with both the DRD4-7 repeat allele and the $5HT_{2C}$ Cys23Ser substitution were demonstrated by Ebstein *et al.* (1997c) regarding TPQ 'persistence' and 'reward dependence' scores. In a large population study ($n = 2752$), the *Bal*I polymorphism was investigated for a possible association with personality traits, psychiatric symptoms, and life events (Henderson *et al.*, 2000). Since the EPQ-R (Eysenck's Revised Personality Questionnaire) (Eysenck *et al.*, 1985) and the BIS/BAS (Behavioural Inhibition System/Behavioural Activation System scales) (Carver and White, 1994) were used to assess personality scores, comparison with similar studies investigating DRD3 is difficult. In an initial subsample (including 30% of the final sample), the authors found subjects with at least one serine allele to have higher scores for neuroticism, behavioural inhibition, depression, and anxiety, when experiencing more than one life event recently. However, the gene–environment interaction became non-significant when the full sample was evaluated. A study on the association between the DRD3 polymorphism and alcoholism yielded negative results (Gorwood *et al.*, 1995). All in all, the relevance of the DRD3 in personality traits remains inconclusive, but the search for a possible association and the assessment of environmental factor such as psychosocial stress seem promising.

The potential role of the D4 receptor in the personality traits related to NS behaviour has attracted considerable attention. DRD4 has been mapped to the human chromosome 11p15.5 (Gelernter *et al.*, 1992). Several atypical neuroleptics, including clozapine and olanzapine, preferentially bind to DRD4, making this receptor an attractive therapeutic target. In exon 3 of the DRD4 gene, which codes for the third cytoplasmatic loop, a polymorphic 48-bp repeat

was found (Lichter *et al.*, 1993; Van Tol *et al.*, 1992). It varies between 2 and 10 repeat units, resulting in a corresponding length variation of the receptor protein and differential receptor function (Asghari *et al.*, 1994; 1995). The polymorphism has been termed DRD4-*n*R, with *n* being the number of repeats. The 2-, 4-, and 7-repeat alleles are most common; however, allele frequencies show substantial ethnic variability. In addition, several SNPs have been described in the upstream promoter region of DRD4 and examined with respect to their relevance for personality traits and behaviour.

Cloninger and associates have suggested that the personality trait of NS, as defined by the TPQ, is exclusively based on dopaminergic function (Cloninger, 1987). While likely to be rather simplistic, this notion provided a starting point to assess the role of DRD4 in the trait of NS. The initial study and several follow-up studies were conducted by Ebstein and co-workers (reviewed in Ebstein *et al.*,1997b; 2000). In a cohort of 124 Israeli volunteers, higher TPQ NS scores were associated with the DRD-7R allele independently of age or sex (Ebstein *et al.*, 1996) with a case-control design. No association between DRD4 alleles and TPQ 'harm avoidance', 'reward dependence', or 'persistence' was found. Simultaneously, the DRD4 polymorphism and a NS equivalent of the NEO-PI-R were studied in a North American sample that included sib-pairs to allow a complementary within-family design. The concurring results further substantiated the association between DRD4-7R and NS (Benjamin *et al.*, 1996).

These findings prompted a considerable number of groups to follow-up on these studies, with both positive and negative results (Table XXVI-7.2). Several groups succeeded in replicating the effect of the DRD4 polymorphism on NS. An association between DRD4-7R and NS could be found in populations from Finland ($n = 190$, with the TCI, a modified TPQ questionnaire [Ekelund *et al.*, 1999]), Germany ($n = 136$; TPQ) (Strobel *et al.*, 1999), and Japan ($n = 153$; TCI and $n = 69$, females, respectively) (Ono *et al.*, 1997; Tomitaka *et al.*, 1999). Noble *et al.* (1998) found an influence of DRD4-*n*R on NS alone, and an even more pronounced effect in concert with DRD2 polymorphisms.

In contrast, Gelernter *et al.* (1997) studied the DRD4-*n*R polymorphism in a North American sample of mixed ethnicity subdivided into substance-abusing, personality-disordered, and healthy individuals. As reported earlier, addicted subjects had higher NS scores; however, in neither subgroup was a relationship between DRD4 alleles and NS detected. Likewise, in a German sample, the DRD4-*n*R status did not differ between patients with alcohol dependence and controls, even when they were selected for high and low TPQ NS scores (Sander *et al.*, 1997a). Similarly, in two samples from New Zealand — one being rather small ($n = 86$) from a depression treatment trial, and the other from 14 pedigrees with alcoholism ($n = 181$) — no significant correlation between DRD4 genotype and NS was found (Sullivan *et al.*, 1998).

Moreover, in studies of populations from Finland ($n = 193$; TPQ) (Malhotra *et al.*, 1996), Sweden ($n = 126$ and 167, respectively; Karolinska Scale of Personality [KSP] [Jonsson *et al.*, 1997b; Jonsson *et al.*, 1998]), the USA ($n = 200$, NEO-PI-R [Vandenbergh *et al.*, 1997]; $n = 256$, NEO-PI-R [Persson *et al.*, 2000a]; and $n = 58$, TCI [Herbst *et al.*, 2000], respectively), Brazil ($n = 110$ patients with alcohol dependence, TPQ [Bau *et al.*, 1999]), Austria ($n = 109$, TCI [Gebhardt *et al.*, 2000]), and Germany ($n = 190$, TPQ [Kuhn *et al.*, 1999]), no association between DRD4-*n*R and the trait of NS was uncovered. The latter group, however, reported a significant interaction between DRD4 and $5HT_{2C}$ polymorphisms on reward dependence. Finally, a thorough twin study (92 monozygotic and 61 dizygotic twin pairs; TPQ and NEO-PI-R) conducted by Pogue-Geile *et al.* (1998) also did not reveal an influence of DRD4 repeat length on NS as well as positive emotional experience. Of note, several studies had been conducted in similar populations as the positive studies.

The DRD4 polymorphisms were also investigated for association with several psychiatric disorders, with substance abuse and attention deficit hyperactive disorder (ADHD) being the most extensively studied. In ADHD, several, but not all, investigators using case-control and family-based study designs found an increased disease risk associated with DRD4-7R (Castellanos *et al.*, 1998; LaHoste *et al.*, 1996; Rowe *et al.*, 1998; Smalley *et al.*, 1998; Swanson *et al.*, 1998). The findings were also inconsistent in alcoholism. While some researchers reported an association with the DRD4 exon 3 repeat polymorphism (George *et al.*, 1993; Vandenbergh *et al.*, 2000), others failed to do so (Adamson *et al.*, 1995; Geijer *et al.*, 1997; Parsian and Zhang, 1999).

Based on these inconsistent findings, it was concluded that DRD4-*n*R itself is not a susceptibility gene for NS, but rather another gene variant in linkage disequilibrium with DRD4-*n*R (Ekelund *et al.*, 1999). Indeed, a polymorphism in the DRD4 promoter region, which is located at the nucleotide position −521 of the DRD4 gene, and which comprises a C to G transition, was shown to be associated with NS scores (Okuyama *et al.*, 2000). Several follow-up studies either replicated (Ronai *et al.*, 2001) this finding or failed to do so (Mitsuyasu *et al.*, 2001; Strobel *et al.*, in press).

Evidently, efforts to detect associations between the DRD4-*n*R or promoter polymorphism and personality traits have been complicated by the use of small sample sizes (with only two of 33 studies incorporating more than 400 subjects, one being positive and one negative), heterogeneous subject populations, unusual subject selection, and differing methods of personality assessment (Table XXVI-7.2). As mentioned above, gene–gene or gene–environment interactions may also play a role, both within and among different populations. Another major concern is the lack of a within-family design in that study, raising the possibility of artefacts due to ethnic admixture. Thus, it remains controversial whether variation in DRD4 expression and function contributes to the phenotype of NS. Interestingly, DRD4 knockout mice are less active than the wild-type animals, and display supersensitivity to ethanol, cocaine, and methamphetamine (Rubinstein *et al.*, 1997). Furthermore, it was shown that DRD4-deficient mice exhibit a decreased response to novel stimuli, as tested in three paradigms including the open field, emergence, and novel objects test (Dulawa *et al.*, 1999). These results argue for a role of this receptor in explorative and addictive behaviour which appears to represent facets of NS. Whether the investigated polymorphisms are relevant to differences in NS, or whether they are in linkage disequilibrium with alternative, as yet unknown, genetic variations remains to be elucidated.

Dopamine Transporter

Similar to the 5-HT system, only a single transporter protein mediates reuptake of dopamine. The dopamine transporter (DAT, SLC6A3) is structurally similar to other monoamine transporters, and several polymorphisms, including a polymorphic 40-base-pair VNTR in the 3′ untranslated region of the DAT gene, have been identified (Byerley *et al.*, 1993; Sano *et al.*, 1993). While this VNTR-based gene variation seems to play a role in ADHD and addiction, especially cigarette smoking and alcoholism (Barr *et al.*, 2001; Daly *et al.*, 1999; Parsian and Zhang, 1997; Sabol *et al.*, 1999; Ueno *et al.*, 1999), few data are available with respect to personality traits. A weak association between the VNTR 10/10 genotype and schizoid/avoidant behaviour was found in a pilot study by Blum *et al.* (1997). In the context of research on the genetic basis of cigarette smoking, Sabol *et al.* (1999) found the SLC6A3-9 polymorphism of DAT to be associated with lower NS scores, although this was not replicated in a follow-up study (Jorm *et al.*, 2000). Thus, the role of DAT polymorphisms in personality traits is still unclear and warrants further research.

Table XXVI-7.2 Overview of published data on the association between the dopamine DRD4 polymorphisms and temperament or personality traits in general populations and patient samples

Study	*n*	Population and study design	Tested polymorphism, if different from DRD4-*n*R	Trait assessment/ inventory	Association of the polymorphism with trait, *P*	Remarks
Ebstein *et al.*, 1996	124	General, Israeli		TPQ	NS with 7R: $P = 0.013$	
Benjamin *et al.*, 1996	315	General, US, of mixed ethnicity: mostly male		NEO-PI-R	Extraversion with 7R: $P = 0.001$; consciousness, $P = 0.03$	NS (calculated) with 7R, $P = 0.002$; within-sibship analysis positive
Malhotra *et al.*, 1996	193/138	General and alcoholic offenders, Finnish		TPQ	n.s. for controls, for alcoholics *lower* NS with 7R, $P = 0.02$	
Ebstein *et al.*, 1997	120	General, Israeli	Interaction with DRD3 and 5-HT_{2C}	TPQ	5HT2C effect on RD and 'persistence' higher with long DRD4-*n*R alleles	
Jonsson *et al.*, 1997	126	General, Swedish	DRD4*n*-R, exon I 12-bp repeat, exon I 13-bp del	KSP	n.s. for all polymorphisms and all traits	
Sander *et al.*, 1997	197/252	General and alcoholics, subgrouped; German		TPQ	n.s. for any trait	
Ono *et al.*, 1997	153	General, Japanese; females		TCI	0.045: L with 'exploratory excitability'	
Gelernter *et al.*, 1997	72/162/107	Controls, substance abuse, PD; US, of mixed ethnicity	DRD4*n*-R, exon I 13-bp del, 12-bp ins/del, exon III gly194 SNP	TPQ	NS: n.s. for all polymorphisms, except for subgroups (lower NS)	
Vandenbergh *et al.*, 1997	200	General, US; subjects stratified for high and low NS scores from $n = 1143$ sample		NEO-PI-R	n.s. for NS	
Ebstein *et al.*, 1997	94	General, Israeli		TPQ	No significant difference, but NS range differed ($P = 0.01$)	In combination with expanded cohort sign. diff. ($P = 0.01$)
Sullivan *et al.*, 1998	86/181	Depressive patients, alcoholics; New Zealand		TCI	n.s. for NS	
Pogue-Geile *et al.*, 1998	306	General, US, twins (monozygotic 92, dizygotic 61 pairs)	DRD4*n*-R, exon I 12-bp repeat	TPQ, NEO-PI, EES, SSST	n.s. for NS	Within-sibship analysis also n.s.
Noble *et al.*, 1998	119	General, Caucasian, mean age of 12 years	Interaction with DRD2	TPQ	0.049 for NS	Interaction with minor alleles of DRD2 on NS
Ebstein *et al.*, 1998	81	2 week-old neonates, Israeli	Interaction with 5HTT	NBAS	$P = 0.00026$–0.07, depending on cluster	Significant interaction with 5HTTLPR on orientation
Jonsson *et al.*, 1998	167	General, Swedish	DRD4*n*-R, exon I 12-bp-R, exon I 13-bp del	KSP	n.s.	
Poston *et al.*, 1998	115	Obese patients, US		KSP	n.s.	n.s. with respect to BMI; L significantly assoc. with high-risk patients

(*continued overleaf*)

Table XXVI-7.2 (*continued*)

Study	*n*	Population and study design	Tested polymorphism, if different from DRD4-*n*R	Trait assessment/ inventory	Association of the polymorphism with trait, *P*	Remarks
Kuhn *et al.*, 1999	190	General, middle European; males	Interaction with 5-HT_{2C}	TPQ	n.s.	Significant interaction of DRD4 with 5HT2c on RD
Auerbach *et al.*, 1999	76	2 month-old infants, Israeli; follow-up of Ebstein *et al.*, 1998	Interaction with 5HTT	IBQ	'Negative emotionality', 0.005; 'distress to limitations', 0.03	Significant interaction with 5HTTLPR
Strobel *et al.*, 1999	136	General, German		TPQ, NEO-FFI, EPQ-R, I7, SSS V	$P = 0.001$ for NS, explaining 8% of the variance	$P = 0.022$ for 'persistence', 0.013 for HA1
Ekelund *et al.*, 1999	190	Finnish with extreme NS scores from cohort of 4773 persons		TCI	2-repeat: 0.01 5-repeat: 0.03	
Tomitaka *et al.*, 1999	69	General, Japanese; females		TCI	0.014 for NS	Significant differences in NS1 and NS3
Bau *et al.*, 1999	110	Alcoholics, Brazilian; males		TPQ	n.s. for NS, RD; $P = 0.001$ for 7R with lower HA	No genotype differences in antisocial PD
Hill *et al.*, 1999	204	Alcoholics with families, US	Interaction with DRD2 and DAT	TPQ, MPQ	No linkage of either polymorphism with NS	Linkage of D2 and D4 polymorphisms with HA and corresponding MPQ scales
Okuyama *et al.*, 1999	86	General, Japanese	Six promoter polymorphisms	TCI	-521C/T polymorphism associated with NS ($P = 0.0001$)	DRD4-*n*R n.d.
Benjamin *et al.*, 2000	455	General, Israeli	Interactions with 5HTT and COMT	TPQ	5-HTTLPR × D4DR, $P = 0.03$ COMT × 5-HTTLPR, $P = 0.03$ on NS	
Herbst *et al.*, 2000	587	General, US, of mixed ethnicity	5HTT (interaction n.d.)	TCI	n.s.	
Persson *et al.*, 2000	256	General, Caucasian		NEO-PI-R	n.s. for extraversion	
Gebhardt *et al.*, 2000	109	General, Austrian	DRD2 Cys311Ser	TCI	n.s. for any polymorphism and any trait	
Comings *et al.*, 2000	81/119	General, addictives; US, males		TCI	$P < 0.001$ for seven TCI summary scores	Largest effect on self-transcendence
Ronai *et al.*, 2001	109	General, Hungarian	−521 C/T SNP	TCI	NS and CC genotype: n.s. for males; $P < 0.01$ for females	DRD4-*n*R n.d.
Mitsuyasu *et al.*, 2001	173	General, Japanese	DRD4*n*-R, five promoter polymorphisms	TCI	NS and any polymorphism: n.s.	−768G > A polymorphism associated with RD ($P = 0.044$)
De Luca *et al.*, 2001	122	Neonates at 1 and 5 months, Italian		EITQ/RITQ	Correlation with adaptability ($P < 0.02$) at $t = 1$ month	Differences not detected at $t = 5$ months
Strobel *et al.*, 2001, submitted	276	General, German	−521 C/T SNP	TPQ	n.s.	

NEO-PI-R and NEO-FFI: Costa and McCrae's NEO Personality Inventory; TPQ/TCI: Cloninger's Tridimensional Personality Questionnaire/Temperament and Character Inventory; KSP: Karolinska Scales of Personality; IBQ: Rothbart's Infant Behaviour Questionnaire; NBAS: Brazelton's Neonatal Assessment Scale; EITQ/RITQ: Early and Revised Infancy Temperament Questionnaires; MPQ: Multidimensional Personality Questionnaire; EES: Eysenck's Extraversion Scale; SSST: Zuckerman's Sensation Seeking Scale; SSS V: Sensation Seeking Scale form V; EPQ: Eysenck's Personality Questionnaire; I7: Eysenck's Impulsivity Questionnaire. NS: 'novelty seeking'; RD: 'reward dependence'; HA: 'harm avoidance'; 7R: 7-repeat allele of DRD4-*n*R; n.s.: not significant; n.d.: not determined.

Monoamine Metabolic Gene Pathway

Monoamines involved in neurotransmission (5-HT, dopamine, and norepinephrine) share common metabolic pathways: deamination, accomplished by MAO-A and, in the case of the catecholamines, methylation of the 3-hydroxy group, catalysed by the enzyme COMT. An increasing body of evidence indicates that both enzymes are relevant to genetic variation of behaviour.

Monoamine Oxidase (MAO) A

MAO, the enzyme which degrades 5-HT, dopamine, and norepinephrine by oxidative removal of the amino group, occurs in two isoforms, MAO-A and MAO-B, which are encoded by distinct genes. They are located close together on chromosome Xp11.23 (Lan *et al.*, 1989). In the brain, MAO-A is the prevailing isoform and is thought to be crucial for the catabolic metabolism of monoamine neurotransmitters. MAO-A thus links three important transmitter systems. Norrie's disease, the result of an X-chromosomal deletion which includes the MAO-A gene, causes mental retardation, autistic behaviour, motor hyperactivity, and sleep disturbances. These symptoms may, at least in part, be attributed to the lack of MAO activity in the affected individuals (Sims *et al.*, 1989).

A MAO-A-deficient mouse was found to display — as predicted by the function of this enzyme — increased brain concentrations of 5-HT (ninefold), dopamine, and norepinephrine (twofold) (Cases *et al.*, 1995). In addition, cytoarchitectural alterations of the cerebral cortex were demonstrated in these animals: like 5-HTT knockout mice, they show malformation of barrels in the somatosensory cortex (Cases *et al.*, 1996), thus highlighting the morphogenetic role of 5-HT in CNS development. MAO-A knockout mice are also characterized by markedly altered behaviour. Adult mice were more aggressive (Cases *et al.*, 1995; Shih *et al.*, 1999), as tested by the resident-intruder paradigm, and this offensive-aggressive behaviour resulted in an increased number of woundings under standard housing conditions (Popova *et al.*, 2000). Furthermore, male mice had modified sexual behaviour with enhanced "offensive" sexuality. As the behavioural changes, at least in part, could be antagonized by an inhibitor of 5-HT synthesis, parachlorophenylalanine, but not by inhibitors of catecholamine synthesis, the aggressive-offensive behavioural phenotype in MAO-A knockout mice is probably due to altered 5-HT degradation, further underscoring the role of this transmitter system in the balance of fearfulness and aggression and other outward directed behaviours.

In accordance with the observations made in the rodent model, a rare mutation in the human MAO-A gene results in noticeably changed temperament and behaviour. The disorder, termed 'Brunner syndrome' after its first describer, represents an example of transmissible genetic influence on human behaviour. In a large Dutch kindred, all affected males could be shown to suffer from mild, non-dysmorphic mental retardation along with prominent abnormal behavioural changes. This included impulsive-aggressive behaviour, sometimes shown as hypersexuality and attempted rape and exhibitionism, as well as arson and attempted suicide. The disorder is X-linked and was demonstrated to result from a point mutation in the MAO-A gene, changing a glutamine-coding triplet into a stop codon (Brunner *et al.*, 1993a; b). This leads to measurable changes in monoamine metabolism with increased 5-HT levels, as assessed by urinary examinations, as well as the absence of MAO-A activity (Brunner *et al.*, 1993b). These features largely resemble the data obtained from MAO-A knockout mice. Thus, this MAO-A mutation is the only disorder in behavioural genetics nearly to fulfil the criteria of an OGOD type of disorder, albeit there has been a considerable controversy as to the derivation of this notion (Hebebrand and Klug, 1995). Brunner (1996) later made it clear that 'the concept of a gene that directly encodes behaviour is unrealistic', and that MAO-A deficiency results in complex effects on neurotransmitter function as well as behavioural phenotypes.

At first sight, these findings contrast with the antidepressant and aggression-related effects of MAO-A inhibitors such as tranylcypromine. This apparent discrepancy is resolved when brain cytoarchitectural changes are assumed in MAO-A-deficient individuals as reported for MAO-A-deficient mice (Cases *et al.*, 1996). Moreover, drug-induced MAO-A inhibition occurs rapidly, whereas antidepressant effects require approximately 2 weeks and are probably due to neuroadaptional changes which compensate MAO-A inhibition. Nevertheless, Brunner's disease and MAO-A deficiency states are rare, as shown by a thorough screening of a large cohort (Schuback *et al.*, 1999).

The MAO-A gene consists of 15 exons and spans approximately 60 kb. Several polymorphisms have been described in the human MAO-A gene (Black *et al.*, 1991; Hinds *et al.*, 1992). Two single base-pair mutations in the last base of a triplet codon have been described that do not alter the amino acid sequence of the primary transcript but result in the absence or presence of a restriction enzyme site, thus influencing the expression of MAO-A and resulting in 30-fold increase in total enzyme activity (Hotamisligil and Breakefield, 1991). A dinucleotide repeat length polymorphism (MAOCA-1) was not associated with increased aggressivity scores (Vanyukov *et al.*, 1995). Several studies have been conducted on the association of MAO-A polymorphisms and affective disorders, but despite initially promising findings (Lim *et al.*, 1995) only controversial (Lin *et al.*, 2000) or negative results could be found (Furlong *et al.*, 1999; Muramatsu *et al.*, 1997; Parsian and Todd, 1997; Rubinsztein *et al.*, 1996).

In addition, a 30-bp VNTR polymorphism of the MAO-A promotor region has been shown to alter the expression of the enzyme (Deckert *et al.*, 1999; Sabol *et al.*, 1998) and to influence CSF 5-HIAA levels in women, but not men (Jonsson *et al.*, 2000). The longer allele (four repeats) could be shown to be associated with panic disorder in a combined German and Italian sample (Deckert *et al.*, 1999) and unipolar depression (Schulze *et al.*, 2000), both in females only. Regarding bipolar affective disorder, no association was found (Furlong *et al.*, 1999; Syagailo *et al.*, 2001). To date, only two studies have dealt with the MAO-A promotor VNTR polymorphism (MAOALPR) in PDs and provided evidence that this polymorphism is associated with antisocial behaviour in alcohol-dependent patients (Samochowiec *et al.*, 1999) and enhanced impulsivity and aggression in healthy, male subjects (Manuck *et al.*, 2000). More research is needed to clarify the apparently gender-specific effects of MAOALPR on behaviour. Nonetheless, these data again point to a role of the 5-HT system in the regulation of aggression, impulsivity, and negative emotionality.

Catechol-O-Methyltransferase

COMT is an enzyme which, alternatively or subsequentially to MAO, degrades catecholamines by methylation of the 3-hydroxy group of the catecholamine ring scaffold. *S*-Adenosylmethionine is utilized as a methyl group donor in this reaction. The human COMT gene has been mapped to 22q11.1-q11.2 (Grossman *et al.*, 1992), and three different COMT phenotypes have been described with respect to activity and stability differences resulting in low, intermediate, and high levels of enzymatic activity (Weinshilboum and Raymond, 1977). The mode of inheritance suggests the presence of autosomal dominant or codominant alleles. Accordingly, two alleles were identified (G158A). Carrying two 158A alleles results in an up to fourfold decrease of enzymatic activity when compared to homozygosity for 158G, while both alleles appear to be distributed equally (Lachman *et al.*, 1996; Syvanen *et al.*, 1997). Tiihonen and co-workers suggested an association between this polymorphism

and alcohol consumption among social drinkers (Kauhanen *et al.*, 2000) as well as the risk of developing 'late-onsets' alcoholism (type 1) (Tiihonen *et al.*, 1999), but not type-2 alcoholism, a form with severe antisocial behaviour (Hallikainen *et al.*, 2000).

Altered COMT activity in affective and schizophrenic disorders has long been suspected, but the results of studies have been equivocal (Dunner *et al.*, 1977; Fahndrich *et al.*, 1980; Gershon and Jonas, 1975; Shulman *et al.*, 1978), so that the enzyme went out of focus for a while. However, in the last 4 years, evidence has accumulated of a role for COMT polymorphisms in self- and outward directed aggression. Male schizophrenic patients were shown to have a higher risk of aggressive and dangerous behaviour when they were homozygous for the low-activity 158A allele (Kotler *et al.*, 1999; Lachman *et al.*, 1998; Strous *et al.*, 1997). Any interaction with both DRD4 and 5-HTTLPR polymorphisms could be excluded (Kotler *et al.*, 1999). Additionally, the suicidal behaviour of male schizophrenic patients again was associated with the 158A allele (Nolan *et al.*, 2000a). In females, the low-activity L allele seems to be associated with OCD (Karayiorgou *et al.*, 1997). In line with these findings, female COMT knockout mice displayed impairment in emotional reactivity; in contrast, male heterozygous mice showed increased aggressive behaviours (Gogos *et al.*, 1998), thus suggesting gender-specific effects of COMT on behaviour similar to MAOALPR variants. Because meticulous investigations of subjects who do not suffer from a psychiatric Axis 1 disorder are lacking, there is obviously a demand for further studies investigating the role of COMT polymorphisms in personality.

The influence of the COMT polymorphism on personality was investigated by Benjamin *et al.* (2000a; b) with the TPQ. An interesting gene–gene interaction was demonstrated. When the individual was homozygous for either 158A or 158G, the presence of the short allele of the 5-HTTLPR raised 'persistence' scores; COMT polymorphism alone had a significant effect as well. When the short 5-HTTLPR allele was absent in combination with both alleles of the high-activity allele of COMT, NS scores could be significantly increased by the presence of the DRD4 7-repeat allele. However, a population study from Australia failed to find any association between the COMT polymorphism (both alleles occurred in the same frequency) and personality traits or psychiatric symptoms (Henderson *et al.*, 2000). As noted above, EPQ-R and BIS/BAS were utilized, a fact which renders comparison with results derived by the TPQ or NEO-PI-R questionnaires difficult. All in all, COMT polymorphisms appear to play a role in aggressive behaviour in male schizophrenic patients and in the behavioural trait of NS, in concert with other genetic variations that have yet to be investigated in greater detail.

Miscellaneous Genes

In contrast to the remarkable amount of genetic research on the 5-HT and dopamine systems, and although there are both functional data and animal models for almost all neurotransmitter and modulator systems (e.g., noradrenergic, GABAergic, adenosinergic, and peptidergic transmission), signalling cascades as well as regulatory and structural proteins, with respect to their influence on behaviour, behavioural genetic studies of other neurotransmitter systems are largely lacking.

Noradrenergic Genes

The noradrenergic system of the brain, which originates primarily in the locus coeruleus, acts as a central arousal system and is thought to play a modulatory role in the 'fight or flight' response and, according to Cloninger, in reward dependence, albeit the latter claim requires further validation. Norepinephrine is synthesized from dopamine by the enzyme dopamine β-hydroxylase (DBH), of which the human gene is located on chromosome 9q34 (Perry *et al.*, 1991). DBH activity has been investigated as a biological marker for various psychiatric disease states, and appears to be correlated with the MMPI (Minnesota Multiphasic Personality Inventory) score (Major *et al.*, 1980). Enzyme activity varies widely among individuals, with a part of the population having very low DBH activity, a variation which has been ascribed to a functional polymorphism in the 5′-flanking region of the gene, resulting in a C to G transition and explaining up to 50% of the activity variation (Zabetian *et al.*, 2001). No linkage of DBH polymorphisms to Axis 1 disorders was found, although DBH seems to play a role in nicotine dependence (McKinney *et al.*, 2000). While DBH activity measures in serum and CSF appear to link DBH to personality traits, as well as to life events, education, and even response to psychotherapy, no data on DBH polymorphisms and personality (disorders) have been reported to date. DBH thus remains an attractive candidate gene.

Although several polymorphisms in adrenergic receptor genes have been identified, and both ADRA2A and ADRA2C gene polymorphisms have been shown to be involved in ADHD and learning disorders (Comings *et al.*, 1999), only a single study deals with the influence of adrenoceptors on personality traits. Comings *et al.* (2000c) reported that a single base-pair *Msp*I polymorphism in the promoter region of the ADRA2A is associated with irritability (as assessed by the Brown Adult Attention Deficit Disorder [BADD] scale), indirect hostility, negativity, irritability, and verbal hostility (as measured by the Buss-Durkee Hostility Inventory [BDHI]) in two populations ($n = 123$ and 204, respectively), but not with aggressiveness subscales. These results argue for a role of ADRA2A in irritability, as proposed by Coccaro *et al.* (1991). In this study, which awaits replication, the polymorphism accounted for 2–8% of the variance; however, no significant association with TCI subscales was found, underscoring the importance of using appropriate questionnaires. No studies investigating the role of norepinephrine transporter (NET1) polymorphisms in personality or behaviour have been published to date, even though NET1 is an outstanding candidate gene.

GABAergic Genes

Following ground-breaking studies by Mohler and co-workers (Crestani *et al.*, 1999), who generated mice lacking the γ_2 subunit of the GABA-A (gamma-aminobutyric acid type A) receptor, a notable body of evidence has been accumulated that GABA-A function is compromised in anxiety disorders. Preliminary data suggest that GABA receptor polymorphisms might be involved in the pathogenesis of alcoholism (Parsian and Zhang, 1999; Sander *et al.*, 1999a, b), as well as the glutamate transporter gene EAAT2 (Sander *et al.*, 2000). However, studies on PDs are largely lacking, although one group found a polymorphism in the γ_2 subunit of the GABA-A receptor to be associated with antisocial PD (Loh *et al.*, 2000).

Nitric Oxide

Nitric oxide (NO) was the first to be discovered substance in a class of novel gaseous messenger molecules, acting as paracrine and highly diffusible neurotransmitters. NO or a precursor molecule is synthesized by a family of three H_4Bip-dependent enzymes termed NO synthases (NOS-I, II, and III). NO appears to play a role in learning, memory, and long-term potentiation in the hippocampus (for a review, see Snyder and Ferris, 2000). By the modulation of oxytocin release, NO inhibits maternal behaviour in female rats (Okere *et al.*, 1996); however, male mice in which the neuronal NOS isoform has been knocked out (nNOS$^{-/-}$ rats), like mice treated with an NO inhibitor (Demas *et al.*, 1997), show a

clear increase in aggressive and sexual behaviour (Nelson *et al.*, 1995). Moreover, it was recently demonstrated that the aggressive behaviour of $NOS^{-/-}$ mice is apparently due to deficient 5-HT_{1A} and 5-HT_{1B} receptor functioning and a selective decrease in 5-HT turnover, especially in the cortex, hippocampus, amygdala, and midbrain (Chiavegatto *et al.*, 2001). NO thus seems to have a modulatory effect on the serotonergic system, a finding which functionally couples two ontogenetically old and major signalling pathways. Unfortunately, no data on NOS functioning and/or NOS polymorphisms with respect to human behaviour are available yet.

Finally, Comings *et al.* (2000a) reported that a dinucleotide polymorphism in the 5′ region of the neutral endopeptidase gene (coding for membrane metalloendopeptidase [MME]) is significantly associated with SCL-90 scores for (phobic) anxiety and obsessive compulsivity. MME is one of the major enzymes for enkephalin degradation; the report thus argues for a role of enkephalin pathway polymorphisms in the genetic variation of anxiety. Clearly, further studies are needed to strengthen this hypothesis. A tetranucleotide polymorphism of the human transcription factor AP-2β gene was shown to be gender-specifically associated with guilt, anxiety, psychasthenia, and indirect aggression, as measured by the Karolinska Scales of Personality (KSP; $n = 137$) (Damberg *et al.*, 2000). Mothers with a son suffering from fragile-X syndrome, and who themselves had a full mutation or a premutation of the FMR-1 gene, which is responsible for the fragile-X syndrome, were found to have social phobia as well as avoidant and schizotypal PD more often than controls (Franke *et al.*, 1998; Levitas, 1996; Sobesky *et al.*, 1994), although this was not evident in MMPI testing (Steyaert *et al.*, 1994).

GENE–GENE INTERACTION

Since a considerable number of genes appear to contribute to the phenotypic expression of anxiety-related behaviour, dissection of gene–gene interaction (i.e., epistasis) in the development of personality and behavioural traits is a pertinent and exciting avenue of research. Moreover, in the evaluation of complex genetic effects, it seems to be essential to control for environmental factors. Recent studies have therefore focused on the neonatal period, a time in early development when environmental influences may be minimal, and least likely to confound associations between temperament and genes. In this context, the term 'temperament' is used to refer to the psychological qualities of infants that display considerable variation and have a relatively, but not indefinitely, stable biological basis in the individual's genotype, even though different phenotypes may emerge as the child grows (Kagan, 1989).

Ebstein *et al.* (1998) investigated the behavioural effects of the VNTR polymorphism in exon 3 of the dopamine D4 receptor (DRD4), which had previously been linked to the TPQ personality trait of NS, as discussed in detail above (Benjamin *et al.*, 1996; Ebstein *et al.*, 1996), and of 5-HTTLPR, which seems to influence NEO 'neuroticism' and TPQ 'harm avoidance', in 2-week-old neonates ($n = 81$). Neonate temperament and behaviour were assessed by the Brazelton neonatal assessment scale (NBAS). In addition to a significant association of the DRD4 polymorphism across four behavioural clusters relevant to temperament (orientation, motor organization, range of state, and regulation of state), an interaction was observed between the DRD4 polymorphism and 5-HTTLPR. The presence of the 5-HTTLPR s/s genotype decreased the orientation score for the group of neonates lacking the long variant of DRD4. The D4DR polymorphism–5-HTTLPR interaction was also assessed in a sample of adult subjects. Interestingly, there was no significant effect of the L-DRD4 genotype in those subjects homozygous for 5-HTTLPR s, whereas in the group without the homozygous genotype, the effect of L-DRD4 was significant and represented 13% of the variance in NS scores between groups. Furthermore, the 5-HT_{2C} polymorphism in conjunction with DRD-*n*R accounted for 30% of the variance for persistence and 13% of 'reward dependence' assessed by the TPQ (Ebstein *et al.*, 1997c), and DRD2 and DRD4 polymorphisms seem to exert synergistic effects on NS (Noble *et al.*, 1998).

In this series of landmark studies by Ebstein and his group, temperament and behaviour of the infants were re-examined at 2 months with Rothbart's Infant Behaviour Questionnaire (IBQ) as well as at 1 year with a structured play situation and an information-processing task (Auerbach *et al.*, 1999; 2001). There were significant negative correlations between neonatal orientation and motor organization as measured by the NBAS at 2 weeks and negative emotionality, especially distress in daily situations, at 2 months of age. Furthermore, grouping of the infants by DRD4 polymorphism and 5-HTTLPR revealed significant main effects for negative emotionality and distress. Infants with long DRD4 alleles had lower scores on 'negative emotionality' and 'distress to limitations' than infants with short DRD4 alleles. In contrast, infants homozygous for the 5-HTTLPR s allele had higher scores on 'negative emotionality' and 'distress' than infants with the I/s or I/I genotypes. Infants with the s/s genotype who also were lacking the NS-associated long DRD4 alleles showed most 'negative emotionality' and 'distress', temperament traits that possibly contribute to the predisposition to adult neuroticism.

In addition, an interaction between the 5-HTT gene and the COMT gene has been shown to influence the trait of 'persistence' (RD2), a subscale of TPQ major personality factor 'reward dependence'. 'Persistence' is considered to be highly adaptive in the presence of stable intermittent reward patterns. Persistent individuals are eager, ambitious, and determined overachievers (Cloninger *et al.*, 1993). In the presence of COMT Val/Val or Met/Met homozygosity, the low 5-HTT function 5-HTTLPR *s* allele significantly raised TPQ RD2 scores, including 'perfectionism', in a sample of 577 healthy subjects (Benjamin *et al.*, 2000b). Benjamin *et al.* (2000a) also investigated the interaction of three different gene variants, DRD4, 5-HTT, and COMT, and TPQ personality factors in 455 individuals. In the absence of the 5-HTTLPR *s* allele and in the presence of the high enzyme activity COMT Val/Val genotype, NS scores were higher in the presence of the DRD4 7-repeat allele. In a within-family design, siblings who shared identical genotype groups for all three polymorphisms had significantly correlated TPQ NS scores, whereas sibs with dissimilar genotypes in at least one polymorphism showed no correlation.

Since a large number of epistatic interactions are anticipated to bear a considerable potential of false-positive findings that may lead to meaningless conclusions, gene–gene interaction analyses should at this stage be limited to polymorphisms known to be functional, preferentially within a single neural circuit, such as the 5-HT system, or demonstrated to be associated with behavioural phenotypes of interest.

GENE–ENVIRONMENT INTERACTION

It is no longer controversial whether nature or nurture shapes personality development, but it remains elusive how complex genetic and environmental factors interact in the formation of a behavioural phenotype. While genetic research has typically focused either on normal personality characteristics or on PDs, with few investigations evaluating the genetic and environmental relationship between the two, it is of critical importance to answer the questions of whether a certain quantitative trait aetiopathogenetically influences the disorder, or whether the trait is a syndromal dimension of the disorder.

Under the influence of the sociobiological concept of Edward O. Wilson (Wilson, 1978), there has also been an increasing interest in human personality, particularly the 'big five' personality

dimensions, from the evolutionary perspective (Buss, 1995). These five dimensions may define the framework for adapting to other people, a crucial task in long-term reproductive success. Extraversion and agreeableness are important to the formation of social structures ranging from pair-bonds to coalitions or groups; emotional stability and conscientiousness are critical to the endurance of these structures; openness may reflect the capacity for innovation. Since the genetic basis of present-day personality dimensions may reflect selective forces among our remote ancestors, we have recently focused our research efforts on rhesus monkeys. In this non-human primate model, environmental influences are probably less complex, can be more easily controlled, and are thus less likely to confound associations between temperament and genes.

Human and non-human primate behaviour is similarly affected by deficits in 5-HT function. In rhesus monkeys, 5-HT turnover, as measured by cerebrospinal (CSF) 5-HIAA concentrations, has a strong heritable component and is traitlike, with demonstrated stability over an individual's lifespan (Higley *et al.*, 1991; 1992b). Moreover, CSF 5HIAA concentrations are subject to the long-lasting influence of deleterious events early in life as well as of situational stressors. Monkeys separated from their mothers and reared in the absence of conspecific adults (peer-reared) have altered serotonergic function and exhibit behavioural deficits throughout their lifetimes when compared to their mother-reared counterparts.

Comparison of different mammalian species indicates that the 5-HTTLPR is unique to humans and simian primates. In hominoids, all alleles originate from variation at a single locus (polymorphic locus 1, PL1), whereas an alternative locus for a 21-bp length variation (PL2) was found in the 5-HTTLPR of rhesus monkeys (rh5-HTTLPR) (Lesch *et al.*, 1997). The fact that the 5-HTTLPR is encountered in hominoids and the cercopithecoids, represented by the macaques, indicates remarkable conservation of this part of the 5-HTT gene promoter throughout the different lineages of these two superfamilies, and suggests that a progenitor 5-HTTLPR sequence, possibly representing viral DNA, was introduced into the genome some 40 million years ago. The appearance of the 5-HTTLPR is therefore an example of a one-time event in the evolutionary history of a species. The 5-HTTLPR sequence may be informative in the comparison of closely related species, and it reflects the phylogeny of the old world monkeys, great apes, and humans. The presence of an analogous rh5-HTTTLPR and the resulting allelic variation of 5-HTT activity in rhesus monkeys provides a unique model to dissect the relative contribution of genes and environmental sources to central serotonergic function and related behavioural outcomes.

In order to study this genotype–environment interaction, the association between central 5-HT turnover and the rh5-HTTLPR genotype was tested in rhesus monkeys with well-characterized environmental histories. This sample of rhesus monkeys ($n = 177$) showed allele frequencies of 83% for the 24-repeat allele (long, [l]) and 16% for the 23-repeat (short, [s]) rh5-HTTLPR variant with a genotype distribution of 68% l/l, 31% l/s, and 1% s/s; one individual was heterozygous for a rare allele with an additional repeat unit ($\times l$) similar to longer alleles found occasionally in humans (Lesch *et al.*, 1997). The monkeys' rearing fell into one of the following categories: mother-reared, either reared with the biological mother or cross-fostered; or peer-reared, either with a peer group of 3–4 monkeys or with an inanimate surrogate and daily contact with a playgroup of peers. Peer-reared monkeys were separated from their mothers, placed in the nursery at birth, and given access to peers at 30 days of age, either continuously or during daily play sessions. Mother-reared and cross-fostered monkeys remained with the mother, typically within a social group. At roughly 7 months of age, mother-reared monkeys were weaned and placed together with their peer-reared cohort in large, mixed-gender social groups. The frequency of the l/l genotype was 70% for mother-reared ($n = 79$) and 68% for peer-reared monkeys ($n = 95$), respectively; subjects with the rare genotypes s/s and $l/ \times l$ were excluded from subsequent analyses.

Since the monkey population comprised two groups that received dramatically different social and rearing experience early in life, the interactive effects of environmental experience and the rh5-HTTLPR on cisternal CSF 5-HIAA levels and 5-HT-related behaviour was assessed. CSF 5-HIAA concentrations were significantly influenced by genotype for peer-reared, but not for mother-reared, subjects (Bennett *et al.*, 2001). Peer-reared rhesus monkeys with the low-activity rh5-HTTLPR s allele had significantly lower concentrations of CSF 5-HIAA than their homozygous l/l counterparts. Low 5-HT turnover in monkeys with the *s* allele is congruent with *in vitro* studies that show reduced binding and transcriptional efficiency of the 5-HTT gene to be associated with the 5-HTTLPR s allele (Lesch *et al.*, 1996). This suggests that the rh5-HTTLPR genotype is predictive of CSF 5-HIAA concentrations, but that early experiences make unique contributions to variation in later 5-HT functioning. This finding is the first to provide evidence of an environment-dependent association between a polymorphism in the 5′-regulatory region of the 5-HTT gene and a direct measure of 5-HT functioning, cisternal CSF 5HIAA concentration, thus revealing an interaction between rearing environment and rhHTTLPR genotype. Similar to the 5-HTTLPR's influence on NEO 'neuroticism' in humans, however, the effect size is small, with 4.7% of variance in CSF 5-HIAA accounted for by the rh5-HTTLPR–rearing environment interaction.

Previous work has shown that monkeys' early experiences have long-term consequences for the functioning of the central 5-HT system, as indicated by robustly altered CSF 5-HIAA levels, as well as anxiety and depression-related behaviour, in monkeys deprived of their parents at birth and raised only with peers (Higley *et al.*, 1991; 1992b). Intriguingly, the biobehavioural results of deleterious early experiences of social separation are consistent with the notion that the 5-HTTLPR may influence the risk of affective spectrum disorders. Evolutionary preservation of two prevalent 5-HTTLPR variants and the resulting allelic variation in 5-HTT expression may be part of the genetic mechanism resulting in the emergence of temperamental traits that facilitate adaptive functioning in the complex social worlds most primates inhabit. The uniqueness of the 5-HTTLPR among humans and simian non-human primates, but not among prosimians or other mammals, along with the role 5-HT plays in complex primate sociality, forms the basis for the hypothesized relationship between the 5-HTT function and the personality traits that mediate individual differences in social behaviour. Accumulating evidence demonstrates the complex interplay between individual differences in the central 5-HT system and social success. In monkeys, lowered 5-HT functioning, as indicated by decreased CSF 5-HIAA levels, is associated with lower rank within a social group, less competent social behaviour, and greater impulsive aggression (Higley *et al.*, 1992b; Mehlman *et al.*, 1995). It is well established that, while subjects with low CSF 5-HIAA concentrations are no more likely to engage in competitive aggression than other monkeys, when they do engage in aggression it frequently escalates to violent and hazardous levels.

Association between the rh5-HTTLPR genotype and aggressive behaviour was studied by analysing the joint effects of genotype and early rearing environment on competition-elicited aggression. Socially dominant mother-reared monkeys were more likely than their peer-reared counterparts to engage in competitive aggression. Moreover, under both rearing conditions, monkeys with the low-activity *s* allele exhibited more aggressive behaviours than their l/l counterparts. The lack of a genotype for competitive aggression in rearing interaction indicates that subjects with the *s* allele, while unlikely to win in a competitive encounter, are more inclined to persist in aggression once it begins. A role of *s*

allele-dependent low-5HTT function in non-human primate aggressive behaviour is in remarkable agreement with the association in humans between the NEO subscales 'neuroticism' (increased 'angry hostility') and 'agreeableness' (decreased 'compliance' = increased aggressiveness and hostility) and 5-HTTLPR *s* genotypes.

As the scope of human studies has been extended to the neonatal period, a time in early development when environmental influences are modest and least likely to confound gene–temperament associations, complementary approaches have recently been applied to non-human primates. Rhesus monkey infants heterozygous for the *s* and *l* variant of the rhHTTLPR (*l*/*s*) displayed higher behavioural stress-reactivity than infants homozygous for the long variant of the allele (*l*/*l*) (Champoux *et al.*, 1999). Mother-reared and peer-reared monkeys ($n = 36$ and $n = 83$, respectively) were assessed on days 7, 14, 21, and 30 of life, on a standardized primate neurobehavioural test designed to measure orienting, motor maturity, reflex functioning, and temperament. The main effects of genotype, and, in some cases, interactions between rearing condition and genotype, were demonstrated for items indicative of orienting, attention, and temperament. In general, heterozygote animals demonstrated diminished orientation, lower attentional capabilities, and increased affective responding relative to *l*/*l* homozygotes. However, the genotype effects were more pronounced for animals raised in the neonatal nursery than for animals reared by their mothers. These results demonstrate the contributions of rearing environment and genetic background, and their interaction, in a non-human primate model of behavioural development.

As in humans, the neonatal period in rhesus monkeys represents a time when environmental influences are only starting to gain influence on the genetic make-up, thus rendering relationships between temperament, behaviour and genetic variants less complex. When tested early in life at post-natal days 7–30, rhesus monkey infants with the low-expression rh5-HTTLPR *s* allele displayed higher behavioural stress-reactivity than infants homozygous for the *l* allele (Champoux *et al.*, 1999). Thus, non-human primate models are consistent with the finding in humans that it is the low-activity *s* allele that is associated with increased negative emotionality. The findings are intriguing in light of speculations that the recent appearance of the 5-HTTLPR-associated genetic variation may have helped permit more sophisticated modulation of social behaviours during the evolution of higher-order primates (Lesch *et al.*, 1997). The 5-HTT-personality association data also emphasize the advantage of the five-factor NEO-PI-R and the lexical tradition of personality theory on which it is based. This view of personality is consistent with evolutionary perspectives on personality, and the trait terms in natural language may best reflect individual behavioural differences important to group survival and reproductive success (Buss, 1995).

Another well-defined behavioural pattern among non-human primates that seems to be directly related to CSF 5-HIAA concentrations is dispersal from the natal group (Mehlman *et al.*, 1995). Most male rhesus monkeys leave their natal group and either visit or join other social groups or form small, transient all-male groups before returning to their birth group. While natal dispersal occurs at a highly variable age and is almost always associated with loss of social status and an increase in stress, injury, and mortality, its cause and intention remain controversial. Interestingly, a recent study by Trefilov *et al.* (2000) showed a gene-dose effect of the rh5-HTTLPR *s* variant on the age of dispersal, with s/s homozygotes leaving earlier than carriers of the *l* allele. This finding further supports the notion that impaired 5-HTT function resulting in low 5-HT turnover is associated with impulsive behaviour together with a high tendency to risk-taking activity that leads to early dispersal.

Taken together, these findings provide evidence of an environment-dependent association between allelic variation of 5-HTT expression and central 5-HT function, and suggest that specific genetic factors play a role in 5-HT-mediated social competence in primates. The objective of further studies will be the elucidation of the relationship between the rh5-HTTLPR genotype and sociability in monkeys, as this behaviour is expressed with characteristic individual differences both in daily life and in response to challenge. Because rhesus monkeys exhibit temperamental and behavioural traits that parallel anxiety, depression, and aggression-related personality dimensions associated in humans with the low-activity 5-HTTLPR variant, it may be possible to search for evolutionary continuity in this genetic mechanism for individual differences. Nonhuman primate studies may also be useful to help identify environmental factors that either compound the vulnerability conferred by a particular genetic make-up or, conversely, act to improve the behavioural outcome associated with that genotype.

CONCLUSIONS

The integration of emerging tools and technologies for genetic analysis will further prepare the ground for an advanced stage of gene identification and functional studies in personality and behavioural genetics. Several refined concepts should therefore be adopted.

First, future studies will require extended, homogeneous, and ethnically matched population and patient samples in conjunction with family-based designs. In order to control for non-independence within cohorts and thus to minimize the risk of population stratification bias, rigorous methods of 'genomic control' have been designed. These statistical strategies are based on the assessment of 60 SNPs or genotypes of 100 unlinked microsatellite markers spread throughout the genome to adjust the significance level of a candidate gene polymorphism (Bacanu *et al.*, 2000; Pritchard *et al.*, 2000). With recent advances in molecular genetics, the rate-limiting step in identifying candidate genes has nevertheless become the definition of phenotype.

Second, more functionally relevant polymorphisms in genes within a single neurotransmitter system, or in genes that comprise a developmental and functional unit in their concerted actions, need to be identified and investigated in large association studies both to avoid stratification artefacts and to elucidate complex epistatic and epigenetic interactions of multiple loci with the environment. Not only will DNA variants in coding and regulatory regions of genes be useful in systematic genome scans to identify genes associated with personality and behaviour, but they will also make it possible to study integrated systems of gene pathways as an important step on the route to behavioural genomics. Although great strides have been made in understanding the diversity of the human genome, including the frequency, distribution, and type of genetic variation that exists, the feasibility of applying this information to uncover useful genomic markers of behavioural traits remains uncertain. The implications of using SNPs to uncover markers for behavioural traits and disorders, as well as treatment response, such as population and patient sample size, SNP density and genome coverage, SNP functionality, and data interpretation that will be important for determining the suitability of genomic information are, however, rarely addressed. Success will eventually depend on the availability of SNPs in the coding or regulatory regions (cSNPs or rSNPs, respectively) of a large number of candidate genes as well as knowledge of the average extent of linkage disequilibrium between SNPs, the development of high-throughput technologies for genotyping SNPs, identification of protein-altering SNPs by DNA and protein microarray-assisted expression analysis, and collection of DNA from well-assessed cohorts. As more appreciation of the potential for polymorphisms in gene regulatory regions to affect gene expression is gained, knowledge of novel functional variants is likely to emerge.

Third, genetic influences are not the only pathways that lead to individual differences in personality dimensions, behaviour, psychopathology, and drug response. Complex traits are most likely to be generated by a complex interaction of environmental and experiential factors with a number of genes and their products. Even pivotal regulatory proteins of cellular pathways and neurocircuits are likely to have only a very modest, if not minimal, impact, while noise from non-genetic mechanisms obstructs identification of relevant gene variants. Although current methods for the detection of gene–gene and gene–environment interaction in behavioural genetics are largely indirect, the most relevant consequence of gene identification for behavioural traits and psychotropic drug effects may be that it will provide the tools required to clarify systematically the effects of gene–environment interaction on brain development and plasticity.

Finally, future benefits will stem from novel techniques in molecular cell biology, transgenics, and gene-transfer technologies. However, in the postgenomic world, behavioural genetic research will require integration of the entire spectrum of genomics, DNA variants, gene expression, proteomics, brain development, structure, and function, as well as behaviour, in a wide range of species. Although bioinformatics resources are evolving in most of these areas, the incorporation of these resources from the perspective of the functional genomics of personality and behaviour will greatly facilitate research.

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XXVI-8

Gene–Environment Interactions in Personality Disorders

Joel Paris

PERSONALITY DISORDERS AND THE STRESS-DIATHESIS MODEL

Personality disorders are clinical syndromes in which personality traits cause psychopathology. Disorders are diagnosed when traits interfere with occupational and personal adjustment, leading to distress and/or dysfunction. Patients meeting these criteria develop abnormalities of behaviour, affect, and cognition that begin early in life, are pervasive in many contexts, and remain consistent over time.

The roots of personality disorders lie both in genetic vulnerability and environmental adversity. Research in every category of mental disorder demonstrates genetic predispositions associated with psychopathology (Paris, 1999). These vulnerabilities are then uncovered and unleashed by environmental stressors. Predisposition and stress have an interactive relationship: genetic variability influences the way individuals respond to their environment, while environmental factors determine whether genes are expressed.

These principles describe a general theory of the aetiology of mental disorders, the stress-diathesis model (Monroe and Simons, 1991; Paris, 1999). This model provides a frame for understanding the role of gene–environment interactions in personality disorders.

One of the main problems in identifying genetic and biological factors in personality disorders concerns how to define the phenotype (Jang *et al.*, 2001). Diagnoses, as defined in the current psychiatric classification, are not very useful in this regard. Each of the present categories of personality disorders has some relationship to biological variables, but only to the extent that these disorders reflect traits. When we apply a wide range of research strategies (genetic associations, imaging, biological markers, or neuropsychological testing), relationships with measures of biological function are consistently stronger with traits, and weaker with disorders.

The relationship between traits and disorders is crucial to understanding the nature of personality pathology. Trait vulnerabilities, by themselves, do not explain why patients develop clinical symptoms. Instead, interactions between genetic and environmental factors, leading to pathological feedback loops, are responsible for the amplification of traits to dysfunctional levels.

TEMPERAMENT, PERSONALITY TRAITS AND PERSONALITY DISORDERS

Personality disorders, personality traits, and temperament have a hierarchical relationship (Rutter, 1987). Temperament refers to behavioural dispositions present at birth. Personality traits are individual differences in behaviour that remain stable over time and context. These characteristics represent an amalgam of inborn characteristics and the effects of life experiences. Personality disorders describe dysfunctional outcomes arising from traits.

In trait psychology, personality is measured as 'dimensions' with a normal distribution. Therefore, each personality profile should be common in the general population, and be compatible with normality. Disorders occur when these traits are amplified, and used in rigid and maladaptive ways.

Using current criteria, some studies (Weissman, 1993) have estimated that approximately 10% of the general population have a diagnosable personality disorder. Clearly, the precise figure depends on the cut-off point one uses. If personality is dimensional, one would expect to find no sharp break between traits and disorders. Research in clinical and community populations (Livesley and Jang, 2000) consistently supports this principle.

Personality disorders are even more common in treatment settings (Loranger *et al.*, 1994). But they are not always recognized as such, since patients present clinically when they also have Axis I symptoms. Moreover, there are serious problems in the classification of personality disorders.

CATEGORICAL AND DIMENSIONAL MODELS OF PERSONALITY DISORDERS

DSM-IV (American Psychiatric Association, 1994) divides personality disorders into 10 categories. ICD-10 (World Health Organization, 1992) uses a similar system of classification, describing most of the same types. DSM groups these diagnoses into three clusters (A, B, and C) that share common characteristics. A patient who meets the overall criteria for a disorder, but who does not fall into any specific category, is classified as 'personality disorder, not otherwise specified' (NOS). Many patients fail to fit prototypically into any single category, and about a third of all cases fall into the 'NOS' group (Loranger *et al.*, 1994).

Cluster A, described as 'odd', includes schizoid, paranoid, and schizotypal disorders, all of which lie in the 'schizophrenic spectrum' (Siever and Davis, 1991). Cluster B, which can be called either 'dramatic' or impulsive, includes antisocial, borderline, narcissistic, and histrionic disorders. Cluster C, described as 'anxious', includes avoidant, dependent, and compulsive disorders.

Many of these categories exist largely on the basis of clinical tradition, and their validity is suspect. The constructs describing schizotypal, antisocial, and borderline personality are the most useful, since each of these diagnoses communicates crucial clinical information in a compact fashion. Thus, typical patients in each of these categories have a characteristic outcome and a characteristic treatment response.

One of the most serious difficulties with the existing categories is that they overlap, with most patients earning more than one diagnosis (Pfohl *et al.*, 1986). Many (but not all) of these overlaps occur within clusters. The clusters therefore reflect underlying dimensions, which may be more valid than individual categories. In

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support of this hypothesis, family history methods demonstrate that personality traits within clusters are often shared with first-degree relatives (Siever and Davis, 1991).

In the European tradition, personality disturbances have been thought to be milder versions, or 'formes frustes', of major mental disorders. Some evidence supports this view. For example, schizotypal personality shares biological markers with schizophrenia, suggesting that it represents a less severe form of the illness (Siever and Davis, 1991). Similarly, antisocial and borderline personality disorders share markers with substance abuse and other Axis I impulsive disorders (Zanarini, 1993). Avoidant and compulsive personality have common family histories with Axis I anxiety disorders (Paris, 1998).

The largest body of empirical research has focused on the antisocial and borderline categories. The only category to have been examined systematically in epidemiological surveys is antisocial personality. (The forthcoming International Comorbidity Survey will also measure the prevalence of borderline personality.) About 2–3% of the population (mostly males) meet criteria for antisocial personality (Robins and Regier, 1991). By indirect evidence, 2% of the population (mostly females) can probably be diagnosed with borderline personality (Swartz *et al.*, 1990).

Traits provide an alternate way to measure personality disorders. Trait dimensions are generally derived from the factor analysis of self-report data. The most influential system has been the 'five factor model' (FFM)(McCrae and Costa, 1999), which describes personality profiles with scores measuring 'neuroticism', 'extraversion', 'openness to experience', 'conscientiousness', and 'agreeableness'. In a related schema, Livesley *et al.* (1993) described personality through 18 narrow dimensions that can also be grouped into superfactors that closely resemble four of the five factors in the FFM. Cloninger *et al.*'s (1993) model attempts to unite factor analysis and biological theory. His schema describes seven factors in personality, of which four are posited to be 'temperamental' (novelty seeking, reward dependence, harm avoidance, and persistence), and three are posited to be 'characterological'.

Biological researchers tend to favour dimensional measures of personality. This preference arises from the relationship between biological variability and traits. Moreover, dimensional systems describe pathology as rooted in unusually high levels of traits, concordant with the concept that disorders are amplified versions of these characteristics. However, it is not certain which schema provides the best description of personality. To answer this question, we require more research into the mechanisms that shape traits. Although factor analytical data can be useful in searching for genetic factors, dimensions should ultimately be rooted in biology (Paris, 2000a).

GENETIC FACTORS IN PERSONALITY

To the extent that personality dimensions represent normally distributed individual differences, they should be heritable. However, single genes will not often be associated with single traits. The heritable component of personality emerges from complex and interactive polygenetic mechanisms associated with variations in multiple alleles (Livesley and Jang, 2000).

It is nonetheless striking that virtually every personality dimension that has been studied has been found to have a heritable component, with genetic factors accounting for nearly half the variance on every trait (Plomin *et al.*, 1997). This conclusion has emerged consistently from behavioural genetic studies comparing concordance in traits between monozygotic (MZ) and dizygotic (DZ) twins. Twin methodology is based on the equal-environments assumption, that is, that there is no consistent difference in the environment of MZ and DZ twins, a conclusion in accord with a large body of evidence (Kendler *et al.*, 1993). However, the percentage of the variance accounted for by genetic variability is not that precise, since twin methodology tends to exaggerate the heritable component, and is subject to error variance. Another limitation of twin studies is that they do not readily identify gene–environment correlations.

Adoption studies (Plomin *et al.*, 1997), as well as studies of twins separated at birth (Tellegen *et al.*, 1988), confirm the heritability of traits. Moreover, the degree of heritability is about the same irrespective of which schema is used to define personality dimensions. It therefore seems safe to conclude that individual differences in personality have a strong basis in heredity and temperament.

Associations between personality traits and genetic variations derive from many different genes, and are therefore measurable as quantitative trait loci. But, thus far, this line of investigation has been disappointing. Promising earlier reports (e.g., Lesch *et al.*, 1996) have not been consistently replicated (Gelertner *et al.*, 1998). The main reason is that single alleles account for only a small percentage of the variance on any trait. These studies also suffer from the lack of a precise phenotype for personality traits.

The presence of a genetic component in personality also implies that traits should be linked to biological markers. Research in this area is at an early stage. Thus far, the strongest finding has been a strong relationship, established in clinical populations, between low levels of central serotonin activity and impulsivity (Mann, 1998).

ENVIRONMENTAL FACTORS IN PERSONALITY

The other half of the variance in personality derives from the environment. Again, behaviour genetic studies shed light on the nature of this influence. The variance affecting traits is almost all 'unshared', meaning that it does not depend on being raised in the same family (Plomin *et al.*, 2001). In fact, siblings demonstrate little similarity in personality.

This finding points to the importance of gene–environment interactions. Moreover, the environment is also complex and interactive. In contrast to classical ideas in developmental and clinical psychology, parenting is not the only factor shaping personality development. Since temperament affects the response of others in the environment, parents respond differently to different children. In a recent large-scale study of adolescents (Reiss *et al.*, 2000), using a combination of twin and family methods, multivariate analyses showed that the temperament of the child was the underlying factor driving differential parenting. This interaction is one of several reasons why family environment is unshared.

The other explanation for unshared environment concerns the unique nature of each person's experience. Thus, the environmental factors affecting personality are often extrafamilial. Every child has shaping experiences with peers, with teachers, or with community leaders (Rutter and Maughan, 1997). Harris (1998), who emphasizes these importance of these factors, has criticized the traditional view of parenting in psychology, which minimizes the role of social factors in personality development.

It is also important to note that developmental psychology has often failed to take into account the influence of genetic factors on measures of environmental influence. As Harris (1998) points out, almost all the literature claiming to establish links between life experiences and personality has to be questioned in this light. Personality traits can be latent variables affecting how the environment is perceived and how it affects development. For example, Plomin and Bergeman (1991) showed that standard measures of life stress, past and present, contain a heritable component that can best be accounted for by the influence of genetically influenced traits.

GENETIC FACTORS IN PERSONALITY DISORDERS

Whereas the heritability of personality traits is firmly established, evidence for the presence of genetic factors in personality disorders has been more equivocal. Research has been limited by a lack of diagnostic precision, and by the rarity of large-scale behavioural genetic studies.

Yet if disorders are amplified traits, one would expect them to demonstrate similar levels of heritability. Some writers (Nigg and Goldsmith, 1995) have argued that disorders represent extremes on a continuum, extremes less open to genetic influence and reflecting a larger environmental component. But this supposition seems illogical, since extremes on any normally distributed dimension should be more, not less, heritable.

In any case, the concept that environmental factors are more predominant in personality disorders has been overturned by recent data. Torgersen *et al.* (2000) located a large sample of twins in which one proband met criteria for at least one categorical diagnosis in the DSM classification. All personality disorders had heritabilities resembling those observed for traits (i.e., close to half the variance). Although these numbers lack precision (due to sample size), the heritability coefficient for disorders as a whole was 0.60 (0.37 for Cluster A, 0.60 for Cluster B, and 0.62 for Cluster C). There were no antisocials in the cohort, but in the borderline category, heritable factors accounted for over 69% of the variance, while in the narcissistic category, heritability was 77%.

These observations are highly consistent with the findings of family history studies (Siever and Davis, 1991). The heritability of mental disorders is generally greater when spectra including both Axis I and Axis II diagnoses are considered. Thus, first-degree relatives of patients with personality disorders tend to suffer from disorders lying within a closely related spectrum of pathology. For example, relatives of patients with disorders in Cluster A have pathology in the schizophrenic spectrum.

Similarly, patients in Cluster B tend to have relatives with other impulsive disorders. These observations led Zanarini (1993) to describe an 'impulsive spectrum', (including antisocial and borderline personality, as well as addictive disorders such as substance abuse or bulimia nervosa). Biological markers support these relationships, with the most robust findings linking decreased serotonin levels with aggression, impulsivity, and suicide attempts (Mann, 1999), all of which are characteristic features of the antisocial and borderline categories.

Parallel findings apply to Cluster C, where first-degree relatives tend to suffer from anxiety disorders. These relationships could be described as forming an 'anxious spectrum' (Paris, 1998). Temperamental anxiety has consistent physiological correlates, and it is heritable. An ongoing prospective study by Kagan (1994), following children with 'behavioural inhibition' (unusual levels of anxiety beginning in infancy) into adulthood, will shed further light on these links.

In summary, personality disorders have biological correlates related to underlying traits. Neurophysiological and neuropsychological correlates of personality disorders confirm these relationships. For example, functional abnormalities in the prefrontal cortex are associated with traits of impulsive aggression. Raine *et al.* (2000) has reported decreases in the mass of frontal grey matter in subjects with antisocial personality. On standard measures such as the Wisconsin Card Sorting Test, patients with antisocial and borderline personality demonstrate deficits in executive function (O'Leary *et al.*, 2000).

ENVIRONMENTAL FACTORS IN PERSONALITY DISORDERS

Psychotherapists have traditionally considered personality pathology to be the outcome of defective parenting. This belief was based on the assumption that disorders that begin early in life must be the result of adversities occurring even earlier in development.

Patients with personality disorders frequently report serious adversities during childhood. In borderline personality disorder, histories of sexual abuse, physical abuse, and gross neglect are particularly common (Zanarini, 2000). The common factor behind all these experiences is usually severe family dysfunction.

However neither trauma nor family pathology, by themselves, necessarily cause personality disorders. For example, community surveys of the impact of childhood sexual abuse (Browne and Finkelhor, 1986; Rind *et al.*, 1999) and physical abuse (Malinovsky-Rummell and Hansen, 1993) show that only a minority of those exposed suffer measurable sequelae. Single traumatic events are not strongly associated with pathology, while continuously adverse circumstances lead to cumulative effects that are much more consistent (Rutter, 1987). In general, patients experiencing multiple adversities, which also tend to be associated with more severe trauma, are at the greatest risk.

The retrospective methodologies used in most studies of childhood risks of personality disorders have also made firm conclusions about causality problematic. Almost all findings reported in the literature are based on reports of life experiences occurring many years in the past. Memories are coloured by recall bias, that is, the tendency for individuals with symptoms in the present to remember more adversities in the past.

Prospective studies are clearly needed to confirm these relationships. The best available longitudinal data concern the development of antisocial personality. Robins' (1966) study showed that the strongest predictor of adult antisocial outcome among conduct-disordered children (usually boys) is parental psychopathy, usually in a father. This association was later supported by Farrington's (1998) British follow-up studies.

These findings parallel data showing that close family members of patients with borderline personality disorder have increased levels of psychopathology, usually within the impulsive spectrum (Links *et al.*, 1988; Zanarini, 1993). The presence of impulsivity in parents reflects genetic risk factors, but impulsive parents (and their partners) are also more likely to be inconsistent, neglectful, or even abusive in their parenting behaviours.

A recent prospective longitudinal study has confirmed the relationship between childhood adversity and personality disorders. The Albany-Saratoga study has been following a large community cohort of children into young adulthood. In one report, Johnson *et al.* (1999) observed that early adversities, including neglect, physical abuse, and sexual abuse, were significant predictors of the number of personality disorder symptoms. (The researchers used this continuous variable to measure outcome, since few subjects in this study had a diagnosable personality disorder.) However, this relationship accounted for only part of the variance. The study design should have obtained more data on temperamental factors, which could have helped to account more precisely for outcome.

INTERACTIONS BETWEEN RISK FACTORS FOR PERSONALITY DISORDERS

Personality disorders can best be understood by using multifactorial models. The complexity and multitude of aetiological factors in these categories requires a biopsychosocial theory and a diathesis-stress model (Paris, 1996; 1999). The diatheses for personality disorders consist of abnormal temperament, while the stressors for these disorders consist of adverse life events.

The role of temperament as a risk factor is supported by observations that children with early behavioural disturbances are more likely to develop parallel forms of pathology later in life. Strikingly, children at the age of 3 years with high levels of aggression and irritability have been shown to be at risk of

antisocial personality disorder in early adulthood (Caspi *et al.*, 1996). Moreover, when conduct symptoms during childhood begin earlier and are more pervasive, they are more likely to continue as antisocial personality (Zoccolillo *et al.*, 1992).

In the same way, infants with unusual shyness and reactivity ('behavioural inhibition') are at risk of either anxiety disorders (Kagan, 1994) or anxious cluster personality disorders (Paris, 1998). Although many in Kagan's cohort of behaviourally inhibited children eventually overcame their temperamental difficulties, none became extroverted or impulsive. When the group was followed into early adolescence, a minority still had significant social anxiety. It will be interesting to see whether this population is at risk of avoidant personality disorder in adulthood.

Some authors (e.g., Kernberg *et al.*, 2000) have suggested that personality disorders can be diagnosed in adolescence, or even in childhood. Although specific categories of disorder are unstable over time, overall personality disturbance has significant continuities. One longitudinal follow-up study (Lofgren *et al.*, 1991) showed that children with a wide range of behavioural disturbances (often termed 'borderline children') develop personality disorders in early adulthood.

The course of personality pathology over time provides a clue to its causes. Disorders beginning early in life are more likely to have a heritable biological component (Childs and Scriver, 1986). Moreover, most patients with personality disorders have a chronic course, with the exception of antisocial and borderline personality, which tend to 'burn out' by middle age (Paris, 1994).

Genetic-temperamental factors in personality disorders shed light on the relationship between childhood adversities and adult psychopathology. In community surveys of adults who report trauma and neglect during their development, associations between childhood trauma and sequelae are consistently much stronger in clinical than in community populations (Paris, 1997). While adverse events in development lead to psychopathology only in a minority of those exposed, some are more vulnerable than others (Rutter, 1989; Paris, 2000).

The explanation depends on interactions between genes and environment. The effects of adversity are greatest in individuals who are predisposed to psychopathology. In this respect, associations between reported adversities in childhood and personality disorders in adulthood are a classic example of the principle that correlation does not prove causation. Childhood adversity increases the risk of psychopathology in adulthood, but these effects are largely accounted for by vulnerable subpopulations.

Abnormal temperament is associated with a greater sensitivity to environmental risk factors. At the same time, individuals with problematic temperaments also experience more trauma and conflict during development (Rutter and Maughan, 1999). Children with difficult temperaments elicit responses from others that tend to amplify their most problematic characteristics. Specifically, those with high levels of aggression and irritability are in chronic conflict with their parents, as well as with their peers and teachers. These children respond to these conflicts with even greater aggression, creating a positive feedback loop. In a parallel fashion, children with behavioural inhibition elicit overprotective responses from their parents, which only amplify the problem (Kagan, 1994).

Thus, childhood adversities reflect positive feedback loops which are strongly influenced by personality traits. The more affected children are by these experiences, the more their traits become amplified. The more traits are amplified, the more likely children are to experience adversity.

In adult life, no specific relationship exists between stressors and symptoms (Paris, 1999). Instead, predispositions and vulnerabilities unique to each individual determine what type of disorder will develop. Personality traits lie at the core of these differences in susceptibility.

Finally, personality disorders are a social phenomenon. The prevalence of antisocial personality varies widely across cultures, suggesting that social forces play an important aetiological role in this condition. Similar considerations apply to other disorders characterized by impulsivity, such as borderline personality. The mechanisms could involve direct effects of social stressors, or indirect effects, due to the failure of the social community to buffer biological risks and/or psychological adversities (Paris, 1996).

Personality disorders are not the only possible outcome of temperamental vulnerability or psychosocial adversities. A spectra of disorders, including Axis I diagnoses, is associated with each of the Axis II clusters (schizophrenic, impulsive, and anxious). In cases where the dysfunction arising from the Axis I condition is extensive, one will not be able to diagnose a personality disorder. In other cases, Axis I and Axis II pathology will be 'comorbid'. But in either of these scenarios, common biological factors are mediated by personality trait profiles.

In summary, none of the risk factors associated with personality disorders are sufficient conditions for their development. Biological factors increase risk, but do not determine outcome. Psychosocial factors have little specificity, with similar forms of adversity being associated with many categories of disorder, or with no disorder at all.

Only a combination of risks, that is, a 'two-hit' or 'multiple hit' mechanism, can account for the data. While the cumulative effects of multiple risk factors determine whether psychopathology develops, the specific disorder that ultimately emerges depends on temperament (Kagan, 1994; Paris, 1996). Thus, only those with impulsive temperamental characteristics will develop Cluster B disorders, such as antisocial or borderline personality. Similarly, only those with introverted temperament will develop Cluster C disorders, such as avoidant personality.

SUMMARY

We can summarize the application of a stress-diathesis model to the personality disorders as follows:

1. Biological vulnerability and environmental adversity are necessary conditions, but neither is sufficient for the development of personality disorders.
2. The genetic factors in personality disorders are associated with temperamental predispositions.
3. Personality traits, which reflect both temperament and experience, are the underlying factors behind personality disorders.
4. Personality disorders are most likely to develop when environmental stressors, particularly multiple adversities with cumulative and interactive effects, amplify traits.
5. Environmental adversities have greater effects on those who are temperamentally vulnerable.
6. Once established, personality disorders are supported by positive feedback loops that lead to chronicity.

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XXVI-9

The Psychopharmacological Treatment of Personality Disorders

Royce Lee and Emil Coccaro

INTRODUCTION

This chapter will present available evidence on the psychopharmacological treatment of personality disorders. Clinical and theoretical implications of axis I/II and Axis II/II comorbidity in the treatment of patients with personality disorders will be discussed. Biological correlates of some of the major symptoms of personality disorders will provide the rationale for a review of the evidence for psychopharmacological treatment of personality disorders. These will be presented by medication class to facilitate an understanding of the evidence for the efficacy of these treatments.

PERSONALITY DISORDERS IN THE CLINICAL SETTING

The estimated prevalence of personality disorders in the community is approximately 6–11% (Samuels *et al.*, 1994; Reich *et al.*, 1989), with Cluster B personality disorders being the most common (4–5.4%), followed by cluster C (1.7–3.4%) and Cluster A (<0.1%). Evidence suggests that many personality-disordered people in the community who could benefit from treatment do not receive it. This holds true in clinical settings, where personality disorders, in general, are underdiagnosed (Zimmerman and Mattia, 1999).

Paradoxically, personality disorders may be disproportionately represented in outpatient and inpatient treatment settings (Zimmerman and Coryell, 1989). This may be due to the significant morbidity associated with them. Personality-disordered patients tend to function at lower levels than those without such disorders (Mehlum *et al.*, 1991), report more frequent adverse events in their lives (Maier *et al.*, 1992), and have elevated rates of divorce, substance abuse, and suicide (Zimmerman and Coryell, 1989). This is the case despite relatively heavy use of clinical services by some, but not all, persons with Axis II diagnoses. A recent study of treatment utilization by patients with personality disorder found that those with borderline personality disorder, compared to depressives, were more likely to have received every class of psychopharmacological medication, from twice as many trials of antidepressants to 10 times as many trials of antipsychotic medications. They had also received more psychosocial treatments than the depressive comparison group with the exception of family/couples therapy and self-help groups (Bender *et al.*, 2001). These results were consistent with earlier reports in persons diagnosed with borderline personality disorder (BPD) of more frequent hospitalization, a 20% lifetime incidence of suicide attempt (McGlashan *et al.*, 1986), and extensive use of outpatient mental health services (Perry and Cooper, 1985; Skodol *et al.*, 1983). Patients with personality disorders may also be more difficult to treat than most patients, with less treatment compliance (Bender *et al.*, 2001), less favourable Axis I treatment outcomes (Reich *et al.*, 1991), and more frequently aborted treatments. In research settings, it is not uncommon for up to two-thirds or more of personality-disordered subjects to drop out of treatment studies (Skodol *et al.*, 1983), a finding which mirrors clinical reports of their intensive but intermittent contact with outpatient services (McGlashan *et al.*, 1986).

COMORBIDITY IN PERSONALITY DISORDERS

Comorbidity in personality disorders may represent the random co-occurrence of independent disorders, co-occurrence of different disorders sharing a common aetiology or pathophysiology, or different disorders that have a causal relation between them (McGlashan *et al.*, 2000). The frequency of Axis II/Axis II comorbidity may be in part due to the fact that psychiatric nosology since DSM-III has favoured a trend towards more frequent comorbidity through the use of operationalized criteria, structured diagnostic interviews, and less stringent exclusionary rules. In some cases as well, Axis I/II and Axis II/II comorbidity could reflect the limitations of categorical diagnoses in characterizing the behavioural dimensions, that may underlie them.

Clinically, the possibility of the existence of comorbid conditions in the personality-disordered patient must be carefully evaluated for the following reasons:

1. to identify other conditions with relatively well-defined treatments — for example, the presence of a medical disorder, mood disorder, or anxiety disorder
2. to identify other conditions whose symptoms may be aggravated by proposed treatments — for example, the presence of bipolar I disorder in a patient considering the trial of an antidepressant
3. to identify disorders whose symptoms may account for the set of behaviours in questions, including such disorders as post-traumatic stress disorder (PTSD) or social anxiety disorder
4. to identify conditions whose course may be complicated by the presence of a personality disorder, such as refractory depression
5. because comorbidity may be markedly more frequent in clinical settings than community settings, as shown by comparisons between the two (Samuels *et al.*, 1994), possibly due to the selection for treatment of patients with more than one disorder and more functional impairment.

CLUSTER A

Schizotypal personality disorder is the most commonly encountered Cluster A personality disorder in clinical settings. It frequently

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occurs in the context of other personality disorders, with most affected patients meeting the criteria for at least two other personality disorders. The most frequently comorbid personality disorder may be paranoid personality disorder, with 36.1% of patients with schizotypal personality disorder meeting the criteria for paranoid personality disorder (McGlashan *et al.*, 1986). Fulton found that 4/17 (23.5%) of subjects diagnosed by chart review with paranoid personality disorder met the criteria for schizotypal personality disorder (Fulton *et al.*, 1993). Although schizoid personality disorder is not frequently diagnosed even in clinical settings, it has been found in 10.5% of patients diagnosed with schizotypal personality disorder (McGlashan *et al.*, 1986). In contrast, Fulton found that only one out of 33 (3%) patients with schizoid personality disorder met the criteria for schizotypal personality disorder (Fulton *et al.*, 1993). Evidence suggests that despite some degree of comorbidity, schizotypal, schizoid, and paranoid personality disorders categorize distinct groups of patients. The nature and extend of the comorbidity relationships between schizoid and avoidant personality disorder (West *et al.*, 1995) and between schizotypal and borderline personality disorder are unclear.

CLUSTER B

Borderline personality disorder (BPD) is the most common of the Cluster B disorders. Like schizotypal personality disorder, BPD represents a heterogeneous group of individuals with frequent Axis I and Axis II comorbidities. The most frequent Axis I comorbidity with personality disorders is substance-abuse disorder, which is found in approximately 53% of BPD patients (McGlashan *et al.*, 2000). Affective disorders are also frequently comorbid. Approximately 41% of patients with BPD may meet research diagnostic criteria for cyclothymia (Levitt *et al.*, 1990). BPD is frequently comorbid with major depression, but major depression is frequently comorbid in nearly all personality disorders (Alnaes *et al.*, 1988), with some evidence suggesting that approximately 70–80% of patients with any Axis II disorder have at least one episode of major depression during their life (McGlashan *et al.*, 1986). However, depressive disorders associated with BPD may be more chronic and severe, and have an earlier onset (Skodol *et al.*, 2000). Curiously, some BPD patients with major depression may cope better than those without it, perhaps due to more robust medication responses (Pope, 1983).

BPD and PTSD share phenomenological and developmental risk factors, but may be separable by their longitudinal course (Gunderson *et al.*, 1993). Comorbidity is frequent. About 30–47% of patients with BPD meet the criteria for PTSD (Swartz *et al.*, 1990, McGlashan, 2000), compared to 25% of patients with schizotypal, obsessive-compulsive, and avoidant personality disorder (McGlashan, 2000).

CLUSTER C

Although measurements of personality traits are affected by state factors (Reich *et al.*, 1986), evidence to date suggests that as many as 40–90% of patients with social phobia also meet the criteria for avoidant personality disorder (McGlashan, 2000; Widiger, 1992). It remains unclear whether social phobia and avoidant personality disorder represent two frequently co-occurring conditions or different aspects of a single underlying psychopathology (Perugi *et al.*, 1999).

The extent of comorbidity between obsessive-compulsive personality disorder (OCPD) and obsessive-compulsive disorder (OCD) is unknown, with rates from 2–6% to 30–60% reported in the literature (Diaferia *et al.*, 1997). The relationship between OCD and OCPD may not be specific to OCPD but may simply reflect an overall increase in the risk of personality disorders, as approximately half of patients with OCD meet the criteria for various personality disorders (Baer *et al.*, 1990). Additionally, the majority of patients with comorbid OCD and OCPD meet criteria for at least one other personality disorder (Bejerot *et al.*, 1998).

THE ROLE OF PSYCHOPHARMACOLOGICAL TREATMENT IN PERSONALITY DISORDERS

The treatment of some personality disorders, such as BPD, with psychopharmacological agents is not uncommon in clinical practice, and in fact may be in excess of treatment in comparable populations (Bender *et al.*, 2001). Some patients, especially high-risk patients, undergo polypharmacy (Zanarini, personal communication), despite the lack of controlled evidence to validate the practice in this population. However, the severity of psychosocial dysfunction and dramatic nature of some of these patients' symptoms often warrant therapeutic intervention and may make discontinuing possibly ineffective treatment difficult.

On the whole, psychopharmacological treatments for personality disorders are considered to be adjunctive to psychotherapy, but the relative contributions of psychopharmacological treatment versus psychosocial interventions such as psychotherapy, and the interactions between the two, remain important topics for future study.

PSYCHOPHARMACOLOGICAL TREATMENT STUDIES

Three main patterns in treatment studies of personality disorders emerge. One is a relative lack of evidence for syndromal improvement, or remission, in response to pharmacological treatment. The second pattern is that pharmacotherapy may show effects on a specific range of symptoms. Modern studies emphasize treating specific symptom dimensions with agents that possess selective psychopharmacological properties. Some of these studies look within specific diagnostic categories, while others look across diagnostic categories. The third pattern that emerges is the non-specific effects that many psychopharmacological compounds have. For example, some neuroleptics seem to have a global, sedating effect on patients with personality disorders, leading to changes in a wide range of outcome measures. Even selective agents, such as the serotonin selective reuptake inhibitors (SSRIs), have effects on a range of symptoms such as mood, anxiety, obsessionality, and impulsive aggression. Sometimes these non-specific effects may also be detrimental, especially when they are unpredictable or paradoxical.

NEUROLEPTICS

The predominantly antidopaminergic effects of neuroleptics and their proven ability to treat symptoms of psychosis in schizophrenia and affective illnesses have led to studies in personality disorders with psychotic-like symptoms. These include the schizophrenia-related personality disorders of DSM-IV: schizotypal, paranoid, and schizoid personality disorders, in addition to DSM-IV BPD. The rationale for the use of dopaminergic antagonists in these patients is provided by findings of increased cerebrospinal fluid (CSF) and plasma levels of dopamine metabolites in selected schizotypal subjects (Siever *et al.*, 1991; 1993b), more frequent dyskinetic-like movements following amphetamine challenge compared to normal controls (Fenton *et al.*, 1994), genetic relationships to schizophrenia (Kendler *et al.*, 1982), and psychotic-like symptoms in BPD patients exposed to amphetamine challenge (Schulz *et al.*, 1985).

The relative rarity of paranoid and schizoid personality disorders, the frequent comorbidity of schizotypal personality disorder and BPD, the importance of psychotic symptoms in BPD and schizotypal personality disorder, and the initial conceptualization of schizotypal personality disorder and BPD as being related to each other and to schizophrenia have led to the overwhelming majority of neuroleptic trials being dedicated to these two personality-disorder populations.

Three early studies failed to find evidence for syndromal improvement with neuroleptic treatment in comparison to other medications in patients with what today would be considered Cluster B personality disorders. In a double-blind trial by Vilkin (1964) of trifluoperazine, diazepam, and meprobamate/benactyzine in BPD patients, diazepam was more effective than trifluoperazine in relieving overall symptoms, and trifluoperazine was marginally better than mebrobamate/benactyzine. Fink *et al.* (1964) reported improvement in patients with emotionally unstable character disorder (EUCD), who today might be diagnosed as having histrionic personality disorder, using relatively high doses of chlorpromazine (up to 1200 mg) when compared to placebo and imipramine. While imipramine was found to reduce affective instability and agitation, chlorpromazine's global effects were not different from those of placebo. Hedberg *et al.* (1971) noted some improvement in 22% of pseudoneurotic schizophrenics treated with moderately high doses of trifluoperazine (up to 32 mg) and in 28% of patients with trifluoperazine and tranylcypromine (up to 30 mg), but the largest number of patients in this study, as in the study of Fink *et al.* (1964), did better on the antidepressant.

The first placebo-controlled, double-blind study of a neuroleptic in DSM-III-diagnosed BPD showed some improvement with treatment on specific dimensions. In a subgroup of DSM-III-diagnosed BPD patients with at least one psychotic symptom, Goldberg *et al.* (1986) studied low-dose thiothixene (average dose 8.7 mg) compared to placebo, noting significant improvement in cognitive disturbances, ideas of reference, obsessive-compulsive symptoms, and panic-anxiety symptoms. No improvement in depressive symptoms was found, and no improvement was found in global functioning as measured by global assessment of function (GAF). The marked reductions of psychosis in this study may be partially explained by the higher levels of psychosis in the study population.

Some studies have found evidence of global improvement in BPD and schizotypal patients with neuroleptic treatment. Two controlled comparison trials in schizotypal/BPD subjects comparing low-dose chlorpromazine ($105-120\,mg\,d^{-1}$) versus loxapine ($13.5-14.5\,mg\,d^{-1}$) (Leone, 1982) and low-dose thiothixene (mean $9.4\,mg\,d^{-1}$) versus haloperidol (mean $3\,mg\,d^{-1}$) (Serban and Siegel, 1984) reported improvement in suspiciousness, hostility, depressed mood, and anxiety. An open-label study of pimozide in DSM-II personality-disordered subjects showed that pimozide was associated with good to excellent global improvement in 69% of subjects. Paranoid or schizoid personality disorder predicted response to the neuroleptic (Reyntjens, 1972).

In a randomized, placebo-controlled trial of haloperidol versus amitriptyline, Soloff *et al.* (1986) found that haloperidol at 4–16 mg per day (mean $7.24\,mg\,d^{-1}$) had significant effects on depression, anxiety, hostility, paranoid ideation, impulsive behaviour, and global function in hospitalized patients with DSM-III-diagnosed BPD or comorbid BPD and schizotypal personality disorder. Responding patients ended the study reporting clear improvement but remained moderately symptomatic by observer-rated measures. Patients' subjective reports on the Beck Depression Inventory distinguished between groups, while the observer-rated Hamilton Depression Rating Scale differences between groups were not significantly different. The patients most likely to respond to low-dose haloperidol had both affective and schizotypal symptoms. Diagnostic category did not predict outcome with haloperidol. However, a dimension of schizotypy, which included psychoticism, did predict a more favourable result with haloperidol. Haloperidol seemed to be useful for a subset of patients, but drug response could not identify a subgroup of BPD patients with an underlying 'schizotypal' illness.

Two large, placebo-controlled studies found less favourable results. Cowdry and Gardner (1988) studied alprazolam, carbamazepine (mean $820\,mg\,d^{-1}$), trifluoperazine (mean $7.8\,mg\,d^{-1}$), and tranylcypromine sulphate (mean $40\,mg\,d^{-1}$) in a double-blind, placebo-controlled trial. Patients were female; met five of eight DSM-III criteria for BPD; had histories of overdoses, self-mutilation, or physical violence; and had no current Axis I comorbidity. Physicians rated significant improvement in patient's depression and anxiety with trifluoperazine treatment, but subjective ratings were variable. Although physicians did not rate subjects on trifluoperazine as being less impulsive, they did rate suicidality as decreased. Non-significant trends were seen for less behavioural dyscontrol. On the whole, trifluoperazine seemed to be slightly less efficacious than tranylcypromine and carbamazepine on the behavioural and affective measures used in the study, and only 50% of the trifluoperazine trials could be completed due to clinical worsening or medical complications.

Soloff *et al.* (1993) compared phenelzine and haloperidol versus placebo in hospitalized, DSM-III-R-diagnosed BPD, with or without comorbid schizotypal personality disorder. Pre-treatment depression scores were high (Ham D-24 $= 25.3 \pm 5.9$). Although improvement with haloperidol therapy was seen, there were considerable residual depressive symptoms at the end of the study. Significant improvement was found with phenelzine. Surprisingly, mood reactivity increased in all three groups, although both phenelzine and haloperidol resulted in modest improvements in hostility. Only phenelzine produced a statistically significant but clinically modest improvement in impulsivity. Soloff *et al.* concluded that neuroleptic medications functions as a non-specific tranquillizer in BPD, with significant effects only in the most severely impaired borderlines (Soloff *et al.*, 1993).

A 16-week continuation follow-up study on the same group of patients also failed to find evidence for haloperidol's efficacy on syndromal measures. In fact, treatment with haloperidol was associated with a significant increase in the 'hopelessness' item of the Hamilton Depression Scale. Of the subjects treated with haloperidol, 64.3% dropped out of the continuation study, compared to 45.5% of phenlzine-treated subjects. The drop-out rate for the entire 22 weeks of study was 87.5 for the haloperidol-treated subjects. The study failed to find demonstrable value of continuation therapy with haloperidol in this population.

Although prolonged treatment with neuroleptics probably does not improve syndromal or affective measures, Montgomery and Montgomery (1982) found that over a 6-month period depot flupenthixol in comparison to placebo decreased suicidal and parasuicidal behaviour in patients with past histories of suicide attempts and self-destructive behaviour. This finding may be due to the tranquillizing effects of neuroleptics in this study population.

ATYPICAL ANTIPSYCHOTICS

The adverse side effects, high rates of drop out and non-compliance, and lack of consistent efficacy data associated with neuroleptics make the newer, atypical antipsychotics promising candidates for evaluation in the treatment of personality-disorder symptoms. Atypical antipsychotics may address affective symptoms in bipolar disorder and deficit symptoms in schizophrenia, which have similarities to the affective dysregulation in Cluster B disorders and the deficit symptoms of Cluster A disorders.

Deficit symptoms in Cluster A personality disorders are associated with impairments in working memory (Roitman *et al.*, 2000)

and sustained attention (Siever *et al.*, 1991; Meritt *et al.*, 1989). Neuropsychological evidence that the cortical deficits in Cluster A personality disorders may be selective (Raine *et al.*, 1992; Siever *et al.*, 1991) has been corroborated by neurobiological findings of altered CSF homovanillic acid (HVA) levels (Siever *et al.*, 1991) and volumetric neuroimaging findings of increased ventricular size (Raine *et al.*, 1992; Cazzullo *et al.*, 1991; Siever *et al.*, 1991), abnormally shaped corpus callosum (Downhill *et al.*, 2000), and decreased pulvinar size (Byne *et al.*, 2001). These abnormalities may be intermediate in severity between schizophrenics and normal controls (Byne *et al.*, 2001; Downhill *et al.*, 2000), indicating that these disorders may exist on a continuum with schizophrenia, and thus may be amenable to treatment with atypical antipsychotics.

The efficacy of atypical antipsychotics in the treatment of bipolar affective disorder for affective symptoms suggests they may have similar efficacy for affective symptoms in 'dramatic cluster' disorders such as BPD.

Three open trials of clozapine in BPD suggest that it may have efficacy on a range of symptom dimensions. An open trial of clozapine in 15 DSM-III-R-diagnosed BPD patients with symptoms of psychosis, seven of whom also met the criteria for schizotypal personality disorder, found significant improvement of psychosis and global function (Frankenburg and Zanarini, 1993). Benedetti *et al.* (1998) gave 12 inpatients with BPD and severe 'psychotic-like' symptoms low doses of clozapine ($25-100\,mg\,d^{-1}$) and found improvement in psychosis, depression, impulsive behaviours, and global functioning. Most patients suffered from some side effects of clozapine treatment, and on follow-up, one patient at week 24 developed reversible granulocytopaenia. Chengappa *et al.* (1999) treated seven subjects with BPD, psychosis, and a history of severe self-mutilation and/or violence serious enough to warrant hospitalization. All subjects had previous trials with conventional neuroleptics in combination with other psychotropic agents with equivocal benefits. Clozapine (mean dose $421\,mg\,d^{-1}$) led to significant improvements in self-mutilation, behavioural dyscontrol leading to seclusion, global function, and need for p.r.n. medications. However, one patient experienced leukopaenia, two patients gained 9 kg, and one patient gained 17.7 kg during the trial with clozapine. Although the study was limited by that absence of a control group, it is significant because of the severity of the subjects' borderline pathology. The risk-to-benefit ratio of the use of clozapine in personality-disordered patients remains unclear without the availability of placebo-controlled data.

No conclusive data on the newer atypical antipsychotics olanzapine, risperdal, seroquel, and ziprasidone are available yet. Their relatively low side-effect profiles and efficacy in treating psychotic as well as affective symptoms make them appealing alternatives to 'typical' neuroleptics. Preliminary data with olanzapine indicate that it shows promise as an agent that may be able to address several different symptom dimensions in patients with BPD. Case reports with olanzapine $5\,mg\,d^{-1}$ suggest it may have efficacy in reducing the frequency and intensity of self-injurious behaviour (SIB) in BPD (Hough, 2001). One open trial in BPD with dysthymia suggests that it may lead to syndromal improvement. In an 8-week open trial in BPD conducted, by Schulz *et al.* (1999), olanzapine led to decreases in psychosis, impulsivity, anger and interpersonal sensitivity, and depression, and improvements in global function. Patients in this study had comorbid dysthymia and were in continuing psychotherapy. Also notable was a low drop-out rate, and a lack of the extrapyramidal symptoms and the statistically significant weight gain of patients taking olanzapine (mean 2.7 kg). Preliminary results from a not yet published placebo-controlled trial of olanzapine in BPD outpatients show that, in comparison to placebo, olanzapine ($5\,mg\,d^{-1}$) demonstrates efficacy on a variety of symptom dimensions as well as global function, as assessed by GAF score (Zanarini *et al.*, 2002).

Controlled data in children and adolescents with borderline intellectual functioning suggest that risperidone is effective in comparison with placebo in the treatment of behavioural disturbances and aggression (Van Bellinghen *et al.*, 2001; Buitelaar *et al.*, 2001). Data regarding the usefulness of risperdone in the treatment of adult, personality-disordered patients are limited. A case report suggests that risperidone $4\,mg\,d^{-1}$ may be helpful in the treatment of self-mutilation in BPD (Khouzam *et al.*, 1997). Szigethy and Schulz (1997) found that risperdone $2\,mg\,d^{-1}$, in conjunction with fluovoxamine $200\,mg\,d^{-1}$, was helpful in treating psychosis and depression in a BPD patient with comorbid dysthymia. A single case report has described the successful treatment of a patient with antisocial personality disorder and impulsive aggression with risperidone $3\,mg\,d^{-1}$ in conjunction with 9 mg biperiden, 60 mg propranolol, and 30 mg diazepam per day to treat akathisia. In an unpublished pilot study, Schulz and associates treated BPD in a double-blind, placebo-controlled trial of risperidone versus placebo. Treatment with risperidone led to improvements in psychoticism and global function. However, the 8-week study was unable to show a statistically significant superiority of risperidone over placebo. This may have been due to the small number of subjects, robust placebo response, and coadministered weekly psychotherapy in both the placebo and treatment groups.

Atypical antipsychotics show promise and controlled data are forthcoming. In general, evidence for the use of 'typical' neuroleptics indicates that some improvement may be seen in impulsivity, aggression, and psychoticism. Improvement may depend on the severity of symptoms before treatment, with psychoticism standing out as a dimensional symptom that predicts better neuroleptic response. The value of maintenance treatment with typical neuroleptics remains unclear, but evidence suggests that the risk of worsened depression must be balanced against a non-specific sedative effect that may decrease impulsive aggressive behaviours.

MOOD STABILIZERS AND ANTICONVULSANTS

The use of lithium and the anticonvulsants carbamazepine and valproate in the treatment of acute mania has prompted interest in examining their efficacy in treating the prominent affective symptoms of the Cluster B disorders. In addition, the EEG-normalizing effect of the anticonvulsants and early studies showing the therapeutic behavioural effects of the anticonvulsant diphenylhydantoin (Klein and Greenberg, 1967; Stephens and Schaffer 1970) have made them candidates for the treatment of impulsivity in disorders with 'soft' neurological signs and personality disorders.

The rationale for the treatment of affective symptoms in Cluster B personality disorders with mood stabilizers as well as with antidepressants is based on a limited set of data. Biological studies of the affective component of personality disorders have found conflicting evidence on whether or not findings of thyroid and HPA axis abnormalities persist (Korezekwa *et al.*, 1991; Kavoussi *et al.*, 1993) or disappear (Loosen and Prange, 1982) if the presence of comorbid depression is taken into account. Similar discrepancies have been found in heritability studies (Torgersen *et al.*, 1984; Schulz *et al.*, 1986; Riso *et al.*, 2000). Affective instability, a hallmark of BPD, has been associated with cholinergic receptor responsiveness in volunteers (Fritze *et al.*, 1990) and BPD patients (Steinberg *et al.*, 1997). Evidence from a family history study suggests that trait affective lability, in conjunction with trait impulsivity, may have a greater familial relation to BPD than to affective disorders in general (Silverman *et al.*, 1991).

Lithium

Two controlled trials in two markedly different populations have found lithium to be effective at reducing anger and/or impulsive

aggression. Links compared lithium (mean dose 985 mg d^{-1}) to placebo and desipramine (mean 162.5 mg d^{-1}) for 6-week intervals with a 2-week washout period between each arm (Links, 1998). Patients in the study were moderately impaired BPD outpatients who remained in psychotherapy with their referring clinicians. Lithium was superior to desipramine and placebo on observer ratings of anger and suicidality, but the patients did not notice any change in how they felt, and no improvement was seen on objective measures of depression.

In a double-blind, placebo-controlled study of the effect of lithium on aggressive behaviour in 66 chronically aggressive prisoners in a medium-security institution, Sheard (1976) found that lithium compared to placebo was associated with a reduction in the frequency of serious aggressive incidents. Many of these patients today would be considered to have, among other DSM-IV diagnoses, antisocial personality disorder, making this one of the few controlled trials of any psychopharmacological intervention in this kind of patient population. In this study, 80–90% of the subjects had been incarcerated for longer than 1 year between the ages of 12 and 18 years; had committed crimes such as manslaughter, rape, murder, and assault; and had a history of chronic assaulting behaviour and/or impulsive antisocial behaviour. The group was described as being an 'extremely manipulative, hostile group of young men who were extroverted, highly impulsive, and action oriented' (p 1411). Lithium serum levels were maintained between 0.6–1.0 μg m^{-1}. One weakness of this study was that subjects could reliably guess which treatment they were receiving.

Two studies have found that lithium may have therapeutic effects on affective symptoms in Axis II disorders. LaWall and Cassie (1982), reported a case series of five DSM-III-diagnosed BPD patients who demonstrated relief from labile affect, decreased aggression, and decreased depression with lithium therapy. A double-blind, randomized, crossover study comparing lithium to placebo in the treatment of patients with what would today be considered BPD or histrionic personality disorder (Rifkin, 1972) found that lithium therapy significantly reduced the range of daily or hourly mood swings.

Carbamazepine

Studies to date with carbamazepine have focused on the treatment of impulsive aggression. Mattes (1990) found that carbamazepine, in comparison with propranolol, in subjects who met the first two criteria of intermittent explosive disorder but who could also have shown episodes of aggressiveness between severe outbursts, was effective at reducing the severity of outbursts by non-blind ratings by the psychiatrist, patient, and family member. Serum levels were 8–12 μg kg^{-1}. In general, a history of attention-deficit disorder predicted better response to propranolol, while a history of intermittent explosive disorder and/or epilepsy predicted response to carbamazepine. In a study more generalizable to the treatment of personality-disorder patients seen in the typical clinical setting, Cowdry and Gardner (1988), in the crossover study of tranylcypromine, carbamazepine, alprazolam, and trifluoperazine already mentioned, found carbamazepine (mean dose 820 mg d^{-1}), in a group of BPD patients with histories of self-destructive behaviour, to be beneficial in suppressing episodes of impulsive aggression, compared to alprazolam, trifluoperazine, tranylcypromine, and placebo. The authors suggested that treatment with carbamazepine produced a 'reflective delay', allowing patients to pause before reacting to an emotional stimulus. Although objective raters noted a significant improvement in mood, patients themselves did not, a finding that was attributed to a halo effect on the part of clinicians, who may have seen an improvement in impulsive aggression as improved mood. This is consistent with the additional finding that 3 of the 17 (18%) patients taking carbamazepine actually noticed worsening of their mood. Their depressive symptoms resolved after cessation of carbamazepine. Thus, carbamazepine's usefulness in treating affective symptoms of personality disorder, such as affective instability or depressive symptoms remains uncertain. However, preliminary evidence suggests that it attenuates impulsive aggression in personality-disordered patients.

Valproate

Valproate has been found to be efficacious in the treatment of bipolar disorder. There is also evidence that it might be effective in reducing impulsive aggression. A double-blind, placebo-controlled trial in 20 children and adolescents with either oppositional defiant disorder or conduct disorder found that divalproex, with a mean blood level of 82.2 μg ml^{-1}, compared to placebo, was efficacious in reducing mood lability and explosive temper (Donovan *et al.*, 2000).

Two open trials of valproate have found benefit in personality-disordered subjects. Stein *et al.* (1995) found that valproate, in 11 DSM-III-R-diagnosed BPD patients, was moderately effective at reducing anger, impulsivity, anxiety, and rejection sensitivity. The mean serum valproate level in this study was 78.8 μg ml^{-1}. Although dramatic improvements were not seen, patients at baseline were only mildly impaired with respect to depressive symptoms and aggression. Both patients and raters noted improvement in mood and irritability, with 50% of subjects taking valproate showing change scores for mood and 38% showing change scores for anxiety, anger, impulsivity, and rejection sensitivity. Kavoussi *et al.* (1998) studied valproate in 10 patients meeting DSM-IV criteria for at least one personality disorder who had failed an SSRI trial for impulsive aggressive behaviour. Patients in this trial had higher baseline OAS-M scores (mean = 27.2, SD = 17.9); met criteria for a range of Cluster A, B, and C personality disorders; had frequent Axis II comorbidity; and were treated with a maximum of 2000 mg per day of valproic acid. Systematic assessments of mood were not made in this trial. Improvements were seen on mean OAS-M scores at weeks 2, 4, 6, and 9. At week 4, OAS-M irritability scores were significantly lower. Of the eight completers in the study, six had a 50% or more reduction on the OAS-M.

A single placebo-controlled trial of valproate in BPD has been published (Hollander *et al.*, 2001). In a 10-week, parallel-group, double-blind, placebo-controlled, comparison trial, 16 outpatients with DSM-IV-diagnosed BPD received valproate, with a mean serum level of 64.57 μg ml^{-1}. Patients in this study showed global improvement as measured by global assessment of symptoms (GAS) and clinical global inventory (CGI). Significant changes in aggression were not detected in this study, perhaps because subjects did not begin the study with high aggression. Unfortunately, none of the patients randomized to placebo completed the trial. In the intent-to-treat analysis (ITT), measures made in the study failed to reach statistical significance, with the principal finding being a non-significant trend of the GAS from serious to moderate impairment.

Evidence supporting the use of valproate in personality-disordered patients for impulsivity, aggression, and mood instability is promising but incomplete. Pilot data suggests that it may have efficacy for these dimensions and may even lead to global, syndromal improvement, although this finding needs replication in a larger, controlled trial with sufficient power. A multicentre, placebo-controlled trial of valproate in a heterogeneous group of subjects with impulsive aggression is currently under way.

NEURONTIN (GABAPENTIN)

Although gabapentin's efficacy as a mood stabilizer is equivocal, a randomized, placebo-controlled study (Pande *et al.*, 1999) indicates

that it may be an effective treatment for generalized social phobia. Because of the frequent comorbidity of social phobia with avoidant personality disorder, it may be an agent to consider in patients with generalized social anxiety and avoidant personality disorder. No data is available on gabapentin's efficacy in treating impulsive aggression or affective instability. Topiramate and lamotrigene, two anticonvulsants tested in the treatment of affective disorders, have not yet been tested in personality-disordered patients.

ANTIDEPRESSANTS

Antidepressants have been studied in personality disorders because of the similarity of the mood complaints of the Cluster B disorders to affective syndromes such as cyclothymia and atypical depression, substantial comorbidity with affective disorders, the efficacy of antidepressants in treating anxiety and obsessive symptoms, and the serotonergic abnormalities linked to impulsive aggression and suicide.

The rationale for the use of antidepressants for affective symptoms in personality disorders was touched on in the section on mood stabilizers. The rationale for the use of antidepressants in the treatment of impulsive aggression is based on data relating diminished serotonergic function to impulsive aggression in personality-disordered patients. Deficits in serotonergic function have been associated with suicide in depressives (Åsperg *et al.*, 1976); aggression towards oneself and others (Brown, 1979); impulsive, rather than premeditated aggression (Linnoila *et al.*, 1983); and self-injurious behaviour (SIB) in depressives (Lopez-Ibor *et al.*, 1985) and SIB in personality-disordered subjects (New *et al.*, 1997). Neuropsychopharmacological challenge studies with the serotonergic agent fenfluramine (both *d*,l- and *d*-stereoisomer forms) have found blunted prolactin responses in association with impulsive aggression, implicating reduced serotonergic function in BPD (Coccaro, 1989a) and antisocial personality-disordered subjects (O'Keane *et al.*, 1992). Neuroimaging studies have discovered reduced metabolic activity in the areas of the prefrontal cortex in aggressive prisoners (Raine *et al.*, 1997). Using the positron emission tomography (PET) scan in personality-disordered subjects with histories of impulsive aggression, Siever *et al.* (1999) found blunted metabolic responses to *d*,l-fenfluramine in the orbital frontal, adjacent medial, and cingulate cortex when compared to matched controls, findings that were partially replicated by Soloff *et al.* (2000). These brain regions rely heavily on serotonergic neurotransmission.

In addition to abnormalities in the serotonergic system, reduced CSF levels of norepinephrine metabolites (Brown *et al.*, 1979) and dopamine metabolites (Linnoila, 1983), and increased levels of CSF vasopressin (Coccaro *et al.*, 1996) have been correlated with measures of aggression.

TRICYCLIC ANTIDEPRESSANTS

Early studies of the tricyclic antidepressants (Klein, 1968) found some efficacy in the treatment of 'pseudoneurotic schizophrenics'. Soloff *et al.* 1987 found modest benefits in depressed mood with amitriptyline (mean dose 147 mg d^{-1}) compared to placebo in a 5-week study of hospitalized BPD and schizotypal personality patients. Although some patients seemed to benefit primarily on affective measures, a subgroup of patients on amitriptyline showed increases in ratings of impulsive and aggressive behaviour. These patients were characterized as having higher levels of aggression, psychoticism, negativism, impulsivity, and schizotypal symptoms.

Three other studies (Klein 1968; Rampling, 1978; Links *et al.*, 1990) have also found that a subgroup of patients with BPD or schizotypal personality disorder seemed to worsen after treatment with tricyclic antidepressants with respect to suicidality and physical assaultiveness. This may be due to the noradrenergic actions of these agents, which could increase the likelihood of acting on aggressive impulses or increase their intensity.

MONOAMINE-OXIDASE INHIBITORS (MAOIs)

MAOIs, which are useful in the treatment of atypical depression, have been studied in personality-disorder patients in the hope that they would improve atypical mood symptoms such as the mood reactivity of histrionic personality disorder, rejection sensitivity in dependent and avoidant personality disorder, and depressive symptoms in BPD. The serotonergic effects of the MAOIs inhibitors also make them candidates for the treatment of impulsive aggressive behaviour.

The studies to date have produced conflicting results. Hedberg *et al.* (1971) found phenelzine to be effective in treating a subset of patients with pseudoneurotic schizophrenia, patients who, under DSM-IV, might be considered to have Cluster B or C personality disorders. In a retrospective study of patients with atypical depression (Parsons *et al.*, 1989), patients with comorbid BPD were three times as likely to benefit from therapy with phenelzine than imipramine in the treatment of mood symptoms. However, patients with atypical depression without comorbid BPD were no more likely to respond to phenelzine than imipramine. Cowdry and Gardner's (1989) double-blind, placebo-controlled, crossover comparison of tranylcypromine (40 mg d^{-1}), alprazolam, and carbamazepine found efficacy in mood symptoms in a group of treatment-resistant subjects with BPD without a comorbid affective disorder. Although improvement was seen in rated suicidality and impulsivity, there was no change in the severity of aggressive behaviours during the study, and the authors concluded that the improvement seen was multidimensional and mood-related, with little or no effect on impulsivity.

In a double-blind, placebo-controlled comparison of phenelzine and haloperidol in DSM-III-R borderlines, Soloff *et al.* (1993) found phenelzine (60 mg d^{-1}) to be modestly effective for symptoms of depression, anxiety, anger, and hostility compared to placebo and haloperidol. However, they did not replicate Parson *et al.* (1989) findings of efficacy against atypical depressive symptoms. The magnitude of change for all arms of the study were relatively low, leaving the patients still substantially impaired at the end. One possible explanation for their failure to replicate earlier more positive results with MAOIs was that the dose range in the study was slightly lower than previous studies due to treatment side effects. In their 16-week continuation study, Cornelius *et al.* (1993) found that continued phenelzine demonstrated only minor efficacy for treatment of the mood symptoms of irritability and depression. In these subjects, phenelzine had an activating effect, showing increased, rather than decreased, mood reactivity, although the authors note that activation was an improvement in the overall well-being of the patients suffering from anergia.

In summary, treatment of BPD patients with MAOIs may lead to improvements in mood if atypical mood symptoms are prominent, and may lead to improvements in impulsivity. Therapeutic effects may be modest, and their magnitude may be determined by the presence of comorbid atypical depression. There is no clear evidence that treatment with an MAOI leads to global or syndromal improvement.

Although not studied in the treatment of avoidant personality disorder specifically, MAOIs have been shown to be effective in the treatment of generalized social phobia. In an 8-week study comparing phenelzine to cognitive-behavioural group therapy and placebo, phenelzine treatment was more effective than either (Heimberg *et al.*, 1998). However, evidence to date suggests that

the more specific monoamine-oxidase-B inhibitor, selegiline, is not efficacious in generalized social phobia (Simpson *et al.*, 1998).

SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIs)

SSRIs have been shown to be effective in treating depressive disorders and anxiety disorders. Preliminary evidence that SSRIs are effective in reducing impulsive aggression and affective symptoms in personality-disorder patients comes from several open trials of fluoxetine (Cornelius, 1991; Norden, 1989; Markovitz *et al.*, 1991; Coccaro *et al.*, 1990) and one of sertraline (Kavoussi, 1994).

In a double-blind, placebo-controlled trial of fluoxetine (20–60 mg), Coccaro *et al.* (1997) treated 40 non-depressed DSM-III-R-diognosed personality-disorder individuals with impulsive aggressive behaviour with fluoxetine or placebo, and found significant decreases in observed aggression scale — modified (OAS-M) irritability at week 6, and OAS-M verbal aggression and aggression against objects subscale decreases at week 10 of treatment. The effect of fluoxetine was mainly on verbal aggression, with most subjects responding at in the 20–40 mg d^{-1} dose range. No patients worsened while on fluoxetine. Notably, this study demonstrated that the treatment of a dimensional symptom (impulsive aggression) could occur across a categorical Axis II diagnosis. Patients in the study had an average of 1.8 ± 1.1 Axis II personality disorders, with the three most common diagnoses being BPD (33%), paranoid personality disorder (25%), and OCPD (23%). If SSRIs in these treatment responders were ameliorating a 'serotonin deficit', it might be hypothesized that patients with abnormalities in peripheral measures of serotonergic function would be the most likely to respond to fluoxetine. However, in a companion study, 15 of the patients underwent *d*-fenfluramine challenge prior to the start of the treatment trial. Among all of the subjects, there were positive correlations between pre-treatment prolactin response to *d*-fenfluramine challenge (PRL [*d*-FEN]) and percent improvement in the Overt Aggression Scale — Modified (OAS-M) scores in fluoxetine-treated versus placebo-treated subjects. Subjects with smaller responses to serotonergic challenge showed less improvement with SSRI treatment. Since PRL [*d*-FEN] response is thought to be an indirect measure of central nervous system serotonergic functioning, these results might indicate that impulsive aggressive subjects with the most severe serotonergic abnormalities are the ones least likely to benefit from SSRIs, possibly due to a blunted 5-HT-2 response to them (Coccaro and Kavoussi, 1997).

There is preliminary evidence that SSRIs may be able to improve depressive symptoms in BPD. Salzman *et al.* (1995) compared fluoxetine (mean dose 40 mg d^{-1}) to placebo in a 13-week, double-blind study of subjects with BPD diagnosed by DSM-III-R criteria. Patients in this study were mild to moderately symptomatic volunteers with no history of psychiatric hospitalization, recent suicidal behaviour, or self-mutilation. Significant improvement was found in anger and depression on the Profile of Mood States (POMS). There were trends for improvement in OAS and Hamilton Depression Inventory (HAM-D) scores. Although results from this trial were positive, it is unclear whether these results may be generalizable to more impaired BPD patients.

No controlled studies are available yet on the new, 'atypical' antidepressants such as mirtazepine, nefazadone, venlafaxine, and buproprion. In an open trial of venlafaxine in patients with BPD, Markovitz and Wagner (1995) found that venlafaxine seemed to be effective in treating depressive symptoms. Further work is needed with the newer antidepressants. It is unknown whether these agents, which also have effects on norepinephrine, will have tricyclic-like effects in patients with impulsive aggression.

SSRIs have been shown to be effective in the treatment of social phobia, and may be of benefit in patients with comorbid avoidant personality disorder. Controlled trials of paroxetine (Stein *et al.*, 1998), fluvoxamine (Stein *et al.*, 1999; van Vliet *et al.*, 1994), and sertraline (Katzelnick *et al.*, 1995) have all shown efficacy compared to placebo. Open trials of citalopram (Bouwer *et al.*, 1998), fluoxetine (van Ameringen *et al.*, 1993), venlafaxine (Altamura *et al.*, 1999), and nefazadone (van Ameringen *et al.*, 1999) show promising results.

BENZODIAZEPINES

Although an early comparison study of diazepam and trifluoperazine found that diazepam was more effective than the neuroleptic in relieving symptoms of BPD (Vilkin, 1964), Cowdry and Gardner found an increase in the frequency of episodes of serious dyscontrol in patients with BPD and histories of behavioural dyscontrol randomized to receive alprazolam (average dose 4.7 mg d^{-1}) in comparison to trifluoperazine, tranylcypromine, alprazolam, carbamazepine, or placebo. This troubling 'disinhibiting' effect occurred in the context of reduced anxiety and overall benefit to some patients. Although commonly used and generally considered to be safe and efficacious in the treatment of acute agitation and anxiety, the benzodiazepines remain relatively untested in the treatment of personality-disordered patients.

BUSPIRONE

There are no controlled trials to date in the use of buspirone, a 5-HT-1a selective partial agonist, in the treatment of impulsive aggression in personality-disordered patients. While it is an effective agent in the treatment of generalized anxiety disorder, its efficacy in generalized social phobia remains unclear. Although an open trial found buspirone to be effective at higher doses (average dose of responders was approximately 60 mg per day) (Schneier *et al.*, 1993), a controlled trial of buspirone at 30 mg per day in the treatment of generalized social phobia failed to find efficacy compared to placebo (van Vliet *et al.*, 1997). Further work should be done with higher doses of buspirone in patients with comorbid social phobia/avoidant personality disorder.

OPIATE ANTAGONISTS

There are no controlled data in the use of opiate antagonists to treat self-injurious behaviour (SIB) in personality-disordered patients. A few controlled data regarding the use of naltrexone in the treatment of SIB in patients with developmental disorders and mental retardation suggest that naltrexone in moderate doses (<1.5 mg per kg d^{-1}) may be effective (Pies and Popli, 1995), but not all trials to date have found positive results (Willemsen-Swinkels *et al.*, 1995).

In an open trial of naltrexone (50 mg d^{-1}), six of seven patients with histories of SIB experienced remissions of self-injury (Roth *et al.*, 1996). Patients in this trial exhibited striking self-destructive behaviours but had a heterogeneous set of diagnoses. A second open trial of naltrexone (50 mg d^{-1}) in five patients with BPD showed that treatment reduced the number of self-injurious thoughts and actions (Sonne *et al.*, 1996).

A single open trial of naltrexone for the treatment of dissociation and flashbacks in BPD patients by Bohus *et al.* (1999) found that treatment was associated with a decrease in intensity of, and total amount of time in, dissociative states in all patients. In the group of nine patients evaluated for flashbacks, six reported a reduction in

the number of flashbacks in a dose-dependent fashion, while three reported no change. Doses in this study ranged from 25 to 100 mg per day, and approximately one-third of the patients were receiving concomitant antidepressant therapy.

Open trials in the use naltrexone for the treatment of SIB and dissociation show promising results that need to be verified with placebo-controlled data.

ECT (ELECTROCONVULSIVE THERAPY)

Data on the ECT of personality-disordered patients are limited to comparisons of subjects with major depressive disorder (MDD) with a comorbid personality disorder and subjects with MDD only. These echo pharmacological treatment trials of MDD with comorbid personality disorders, which have found, in general, that comorbid personality disorders are associated with a less robust treatment response (DeBattista *et al.*, 2001).

Two prospective correlational studies have provided somewhat conflicting evidence. Pfohl (1984) found that patients with MDD + PD, as diagnosed by the Structured Interview for DSM-III Personality Disorders (SCID-P), did not differ significantly in treatment response to ECT, with 79% of MDD + PD subjects versus 75% of MDD-only subjects responding to ECT.

Zimmerman *et al.* (1986) found that at completion of ECT, 20% of MDD + PD versus 53% of MDD-only subjects had recovered at the completion of treatment. At 6 months, 33% of MDD + PD subjects versus 61% of MDD-only patients had recovered. Although most patients in both groups at the end of treatment experienced some symptomatic recovery, within 6 months, 62% of MD + PD subjects, versus only 8% of MD only subjects, were rehospitalized.

Evidence to date suggests that patients with comorbid major depressive disorder and personality disorders may respond to ECT but less robustly, may be left with considerable residual psychopathology, and may relapse at a higher rate. However, evidence also suggests that ECT may still have therapeutic effects in this population of patients, and is not contraindicated.

CONCLUSION

Evidence to date does not support the efficacy of psychopharmacological treatments in personality disorder, but does support their efficacy in treating specific symptom dimensions. There is not yet enough information available to make evidence-based treatment recommendations for every Axis II disorder. This is less true regarding BPD, which has been comparatively well studied.

For the neuroleptics, selection of appropriate patients with psychotic or psychotic-like symptoms for acute treatment produces tangible results, although these must be weighed against the lack of evidence for continuation or maintenance therapy, the risk of extrapyramidal side effects, high drop-out rates due to side effects, and possibly deleterious effects on mood with prolonged usage. The newer atypical antipsychotic agents may have better efficacy across a broader range of symptoms with fewer side effects. Mood stabilizers show promise in the treatment of impulsive aggression and possibly affective instability. These effects may occur even if subjective effects are less prominent. Benzodiazepines may help with some symptoms commonly encountered in Axis II disorders such as acute anxiety; however, they may also lead to behavioural dyscontrol in certain patients with histories of impulsive aggressive behaviour. Antidepressants may be effective in treating commonly encountered comorbid Axis I anxiety disorders in patients with Cluster C personality disorders. Tricyclics in patients with histories of behavioural dyscontrol may lead to worsening. Evidence for the efficacy of MAOIs in treating impulsive aggression, anxiety, and affective symptoms must be weighed against the risk of hypertensive crisis. For patients with histories of impulsive aggression, and without comorbid bipolar disorder, SSRIs are among the most promising agents. SSRIs may also be beneficial if mood symptoms are present. Their relatively benign side-effect profile and positive effects on mood may lead to improved compliance.

At this point, it is unclear how the effects of psychopharmacological treatment and psychotherapeutic treatment may interact. Some recent studies have kept subjects in pre-existing psychotherapy; other have not. It is also unclear whether the specific targets and efficacy measurements of the two treatment modalities are comparable. These are relevant questions, given that some patient groups with Axis II disorders are heavy utilizers of both psychotherapy and psychopharmacological treatment. It is also unclear what effect different combinations of medications may have on one another. For example, it is unclear whether the use of an SSRI or mood stabilizer lessens the risk of behavioural dyscontrol associated with benzodiazepines. Newer agents, such as the atypical antipsychotics, highly selective agents such as buspirone, and dual serotonergic/noradrenergic antidepressants, show promise for their improved side-effect profiles and atypical actions, but are untested in personality-disordered populations.

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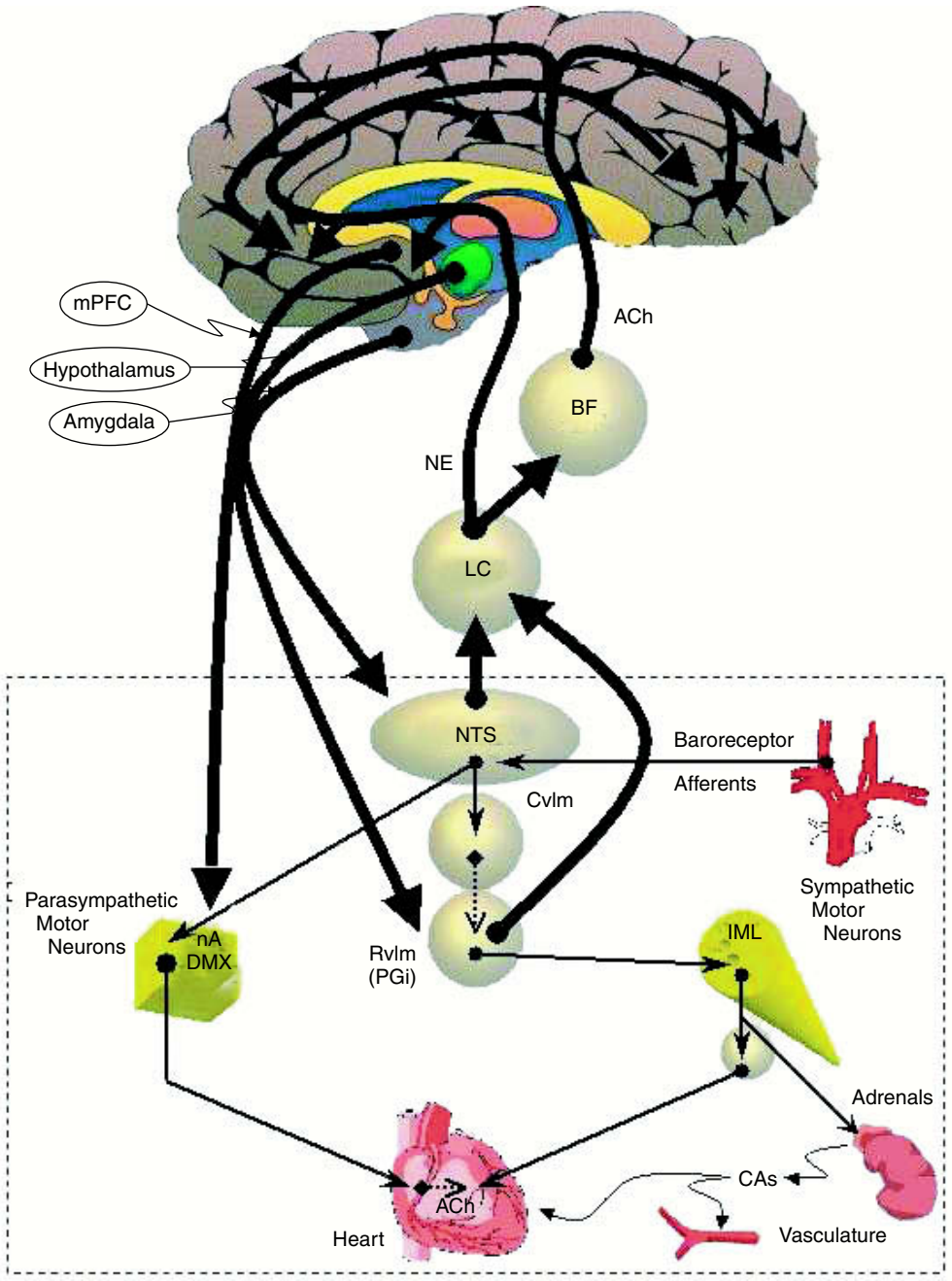

Figure IX.1 Baroreflex circuits. Dashed box: classical brainstem systems underlying baroreceptor cardiac reflex. Baroreceptor afferents project to nucleus tractus solitarius (NTS), which in turn leads to activation of parasympathetic motor neurons in the nucleus ambiguous (nA) and dorsal motor nucleus of the vagus (DMX). The NTS also activates the caudal ventrolateral medulla (Cvlm), which in turn inhibits the rostral ventrolateral medulla (Rvlm), leading to a withdrawal of excitatory drive on the sympathetic motor neurons in the intermediolateral cell column of the cord (IML). Upper section: expansion of the baroreflex circuit to illustrate the ascending and descending pathways to and from rostral neural areas, such as the medial prefrontal cortex (mPFC), hypothalamus and amygdala. Ascending systems include routes from the rostral ventrolateral medulla (Rvlm) and the nucleus of the tractus solitarius (NTS) to the locus coeruleus (LC) noradrenergic system, and indirectly to the basal forebrain (BF) cortical cholinergic system. Adapted from Cacioppo, J.T., Tassinary, L.G. and Berntson, G.G., *Handbook of Psychophysiology*, 2000, p. 466, with permission from Cambridge University Press. ACh, acetylcholine; CAs, catecholamines; NE, noradrenaline; PGi, paragigantocellular nucleus

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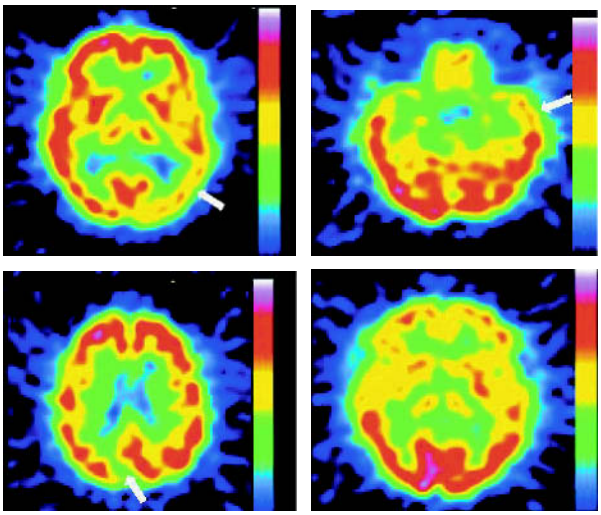

Figure XV-10.3 Perfusion brain SPECTs of four subjects with different pathologies: On the upper right: *Alzheimer disease*: 70 years old woman; MMSE = 19; performance deficit at the following tests; Boston naming test, Token test, verbal fluency and memory recall; Perfusion SPECT: Middle transaxial cut showing left temporo Parietal hypoperfusion. On the upper left: *Mild Cognitive Impairment*: 70 years old woman; MMSE = 28; performance deficit at the following tests; Grober Buschke memory recall; Perfusion SPECT: lower transaxial cut showing left temporal pole hypoperfusion. On the lower right: *Multi-infarct dementia*: 65 years old man; MMSE = 21; performance deficit at the following tests; Benton orientation/praxis/memory recall; Perfusion SPECT: Upper transaxial cut showing a right occipito parietal transcortical hypoperfusion. On the lower left: *Fronto-temporal dementia*: 61 years old woman with apathy Irritability & disinhibition; MMSE = 30; performance deficit at the following tasks; attention dual test/capture error sequencing; Perfusion SPECT: Middle transaxial cut showing a diffuse fronto-temporal hypoperfusion

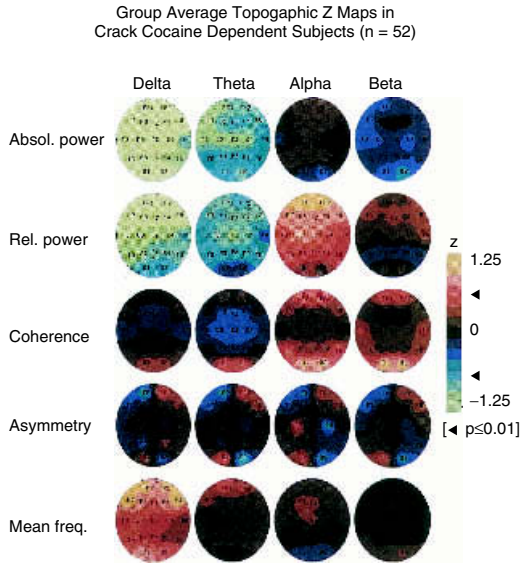

Figure XVI-5.5 Colour-coded topographic brain maps showing differences in absolute and relative power, coherence, hemispheric asymmetry and mean frequency between control subjects and recently detoxified cocaine abusers. Cocaine abusers have decreased absolute and relative slow wave and increased relative alpha power. Reprinted from Prichep, L.S., Alper, K.R., Kowalik, S., Merkin, H., Tom, M., Roy John, E. and Rosenthal, S., 1996. Quantitative electroencephalographic characteristics of crack cocaine dependence. *Biological Psychiatry* **40**, 986–993, copyright 1996, with permission from Elsevier Science

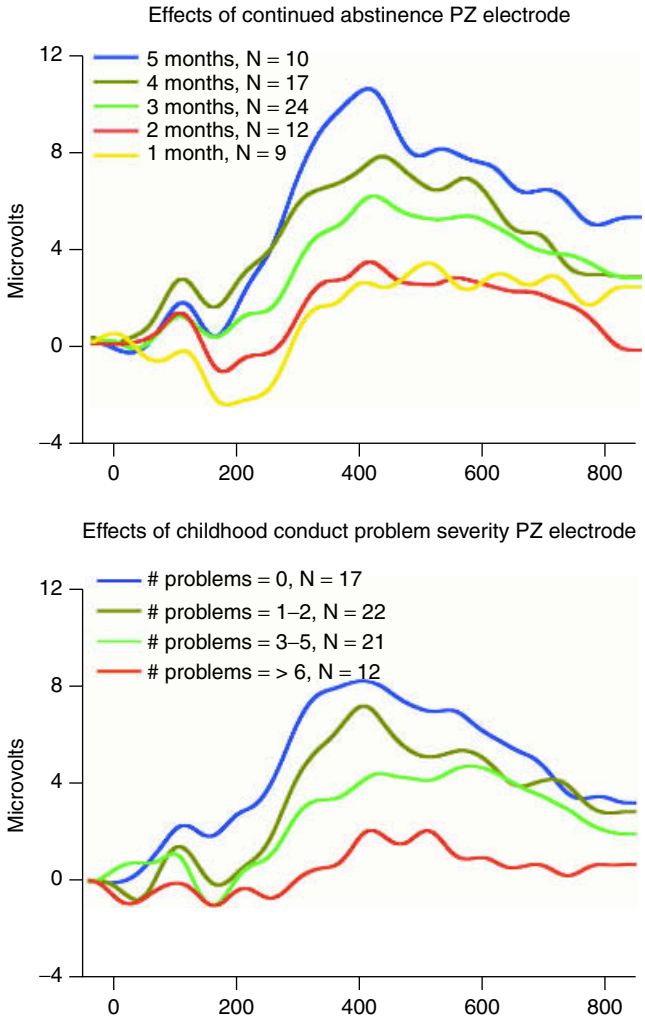

Figure XVI-5.4 Visual P3 amplitude in cocaine-, cocaine–alcohol- and opioid-dependent subjects recovers with increasing time of drug abstinence (above), but decreases with increasing number of childhood conduct disorder symptoms (below). Reprinted by permission of Elsevier Science from 'CNS recovery from cocaine, cocaine and alcohol, or opioid dependence: a P300 study' by Bauer, L.O., *Clinical Neurophysiology* **112**, 1508–1515. Copyright 2001 by the Society of Biological Psychiatry

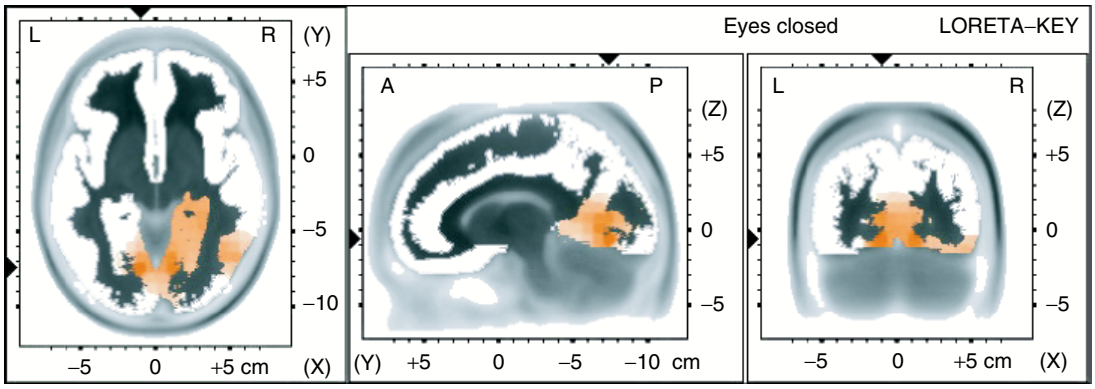

Figure XVI-5.8 Tomographic images displaying differences in fast alpha activity between MDMA ('ecstasy') users and control subjects. 'Ecstasy' users show significant increases (orange colour) in right temporo-occipital fast alpha power during closed eyes. Images have been computed from 31-channel scalp EEG recordings using low-resolution brain electromagnetic tomography (LORETA). Data from Gamma *et al.* (2000)

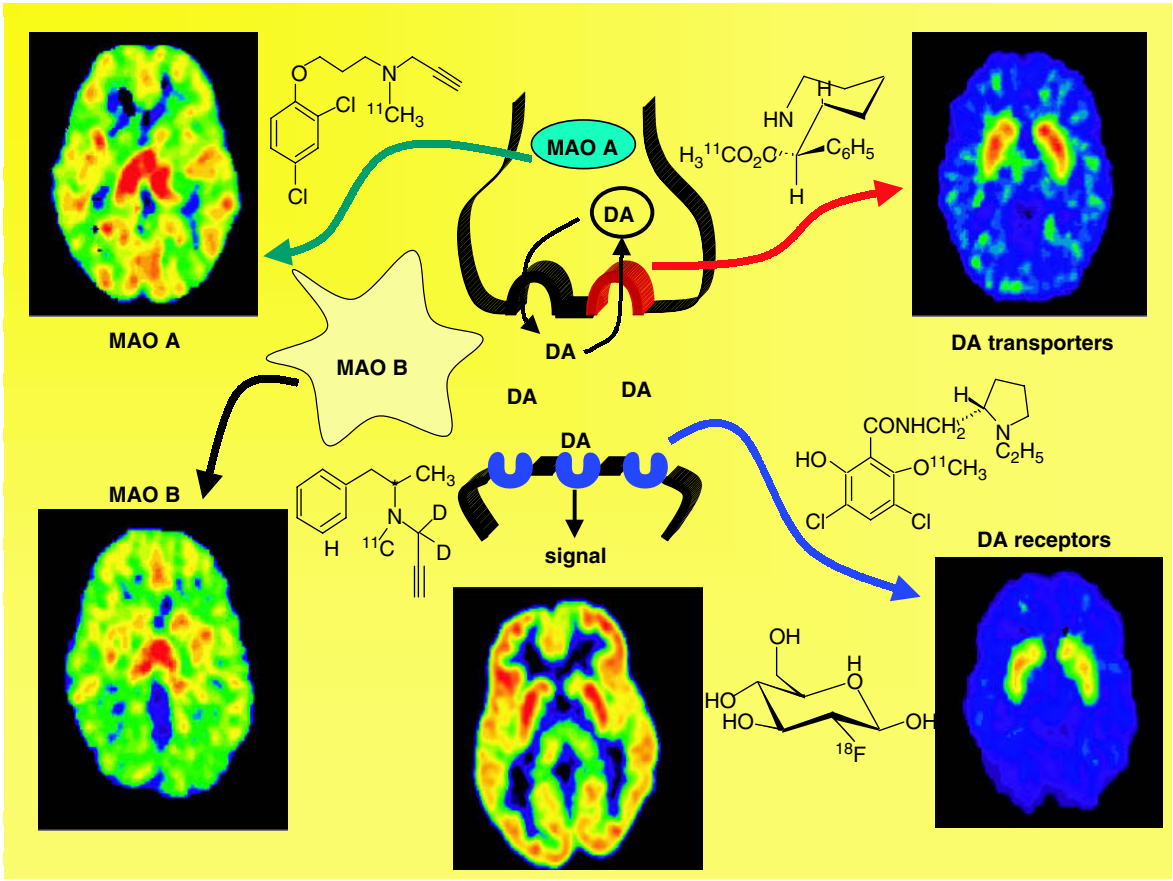

Figure XVI-8.1 Schematic of a dopaminergic synapse, indicating how PET can be used to evaluate dopaminergic function. The various ligands are ^{11}C-methylphenidate (dopamine transporter), ^{11}C-raclopride (D_2 dopamine receptor), FDG (metabolism), ^{11}C-deprenyl (MAO-B) and ^{11}C-clorgyline (MAO-A)

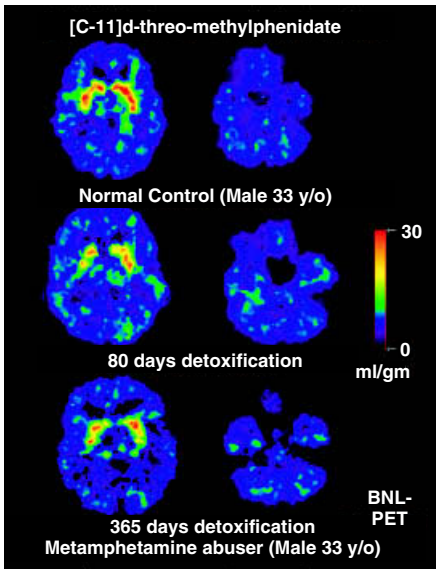

Figure XVI-8.4 Representative PET images showing ^{11}C-methylphenidate uptake to striatal dopamine transporter sites in a normal control and a methamphetamine abuser, scanned twice at 1 month and 4 months into withdrawal. Data adapted from Volkow *et al.* (2001a)

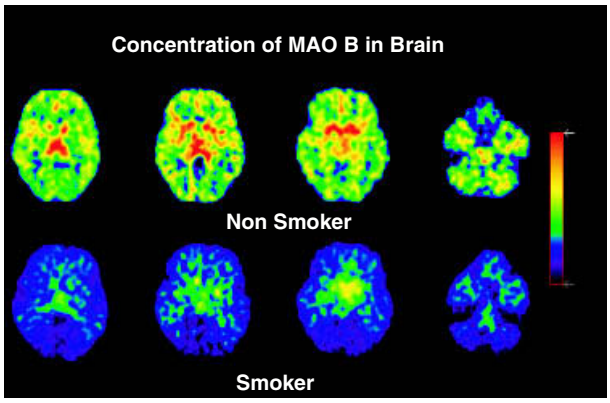

Figure XVI-8.6 Representative comparison of ^{11}C-deprenyl uptake into the brain of a nonsmoker and a smoker. Data are taken to indicate reduced MAO-B activity in the smoker. Adapted from Fowler *et al.* (1996)

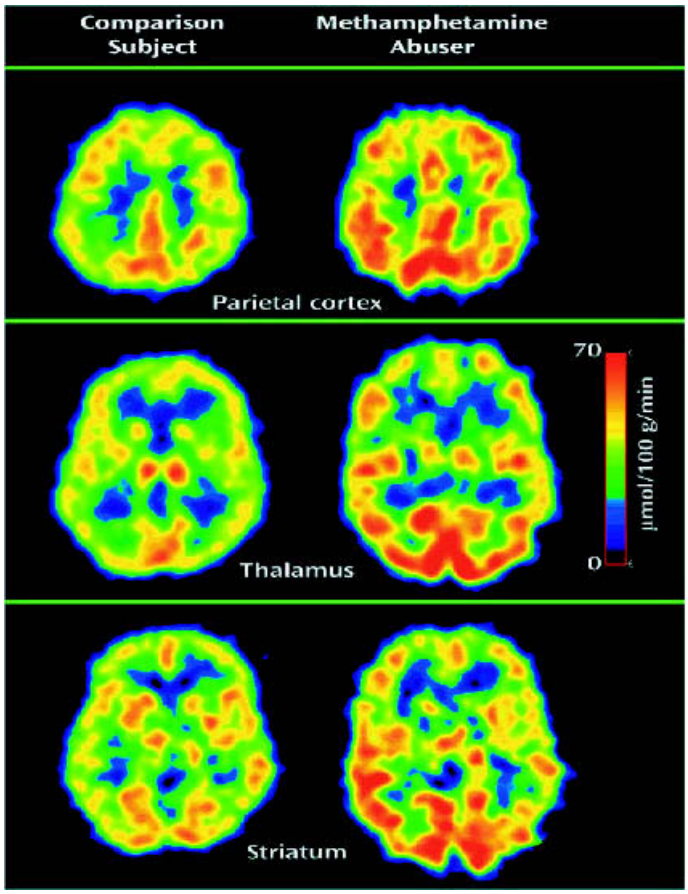

Figure XVI-8.5 Representative comparison of brain metabolism in a normal control and a methamphetamine abuser. Note the increased metabolism in the parietal cortex but decreased metabolism in the thalamus and striatum. Reproduced from Volkow *et al.* (2001b)

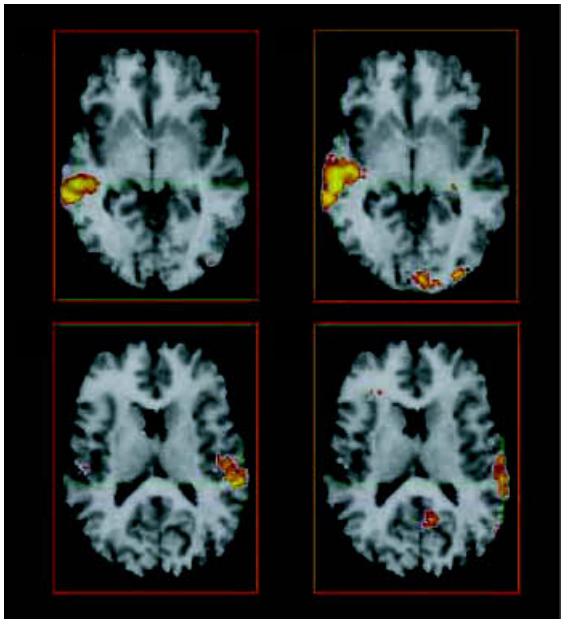

Figure XVII-8.4 Difference in rCBF between schizophrenic patients with a strong predisposition to auditory verbal hallucinations (hallucinators), schizophrenic patients with no history of hallucinations (non-hallucinators), and normal controls during tasks that engaged inner speech and auditory verbal imagery. When imagining sentences spoken in another person's voice, which entails both the generation and monitoring of inner speech, hallucinators showed reduced activation of the left middle temporal gyrus and the rostral supplementary motor area, regions activated by both normal subjects and non-hallucinators. Adapted from McGuire *et al.* (1996)

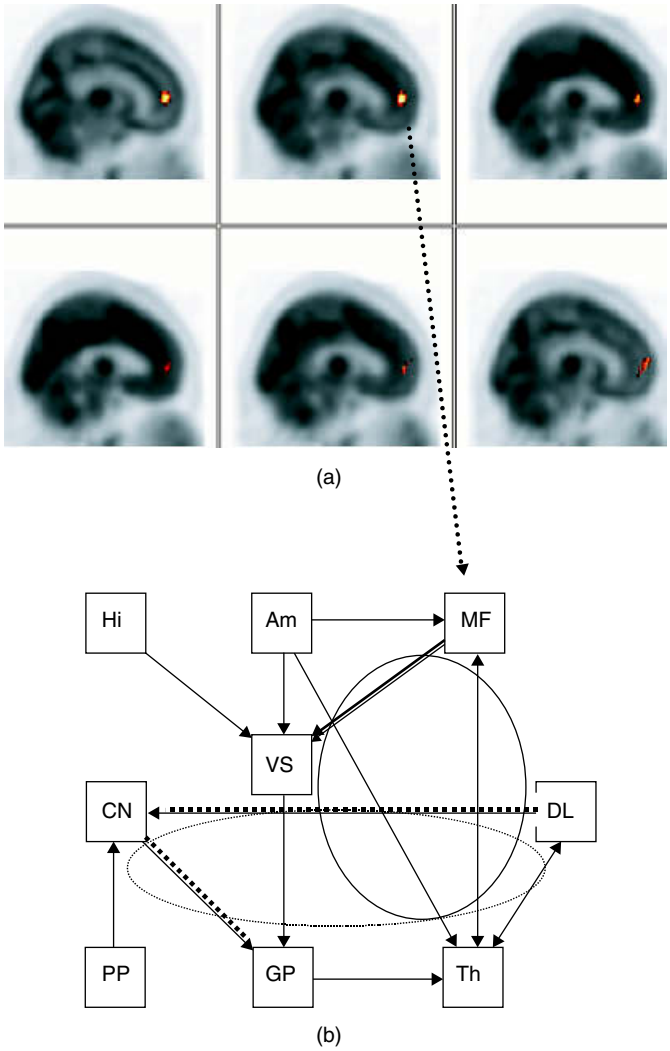

Figure XVIII-9.1 Effect of one session of TMS (5–20 Hz at 80% motor threshold over the left dorsolateral prefrontal cortex) on rCBF during a word-generation task. (a) Statistical parametric map of areas with $P < 0.01$ for effect size (z) and contiguous area (k), using Statistical Parametric Mapping, version 1996. (b) Neuroanatomical projections. Am, amygdala; CN, caudate nucleus; DL, dorsolateral prefrontal cortex; GP, globus pallidus; Hi, hippocampus; MF, medial orbitofrontal cortex; PP, posterior parietal cortex; Th, thalamus; VS, ventral striatum; Dotted ellipse, dorsolateral prefrontal loop; continuous ellipse, limbic loop; dotted arrow DL to CN, increase in regression coefficient c with significance levels in left dorsolateral loop ($c = 2.45$, $P < 0.05$); dotted arrow CN to GP, increase in regression coefficient c with significance levels in left dorsolateral loop ($c = 2.15$, $P = 0.05$); continuous arrow MF to VS, bilateral limbic loop (left: $c = 2.51$, $P < 0.05$; right: $c = 2.89$, $P < 0.05$)

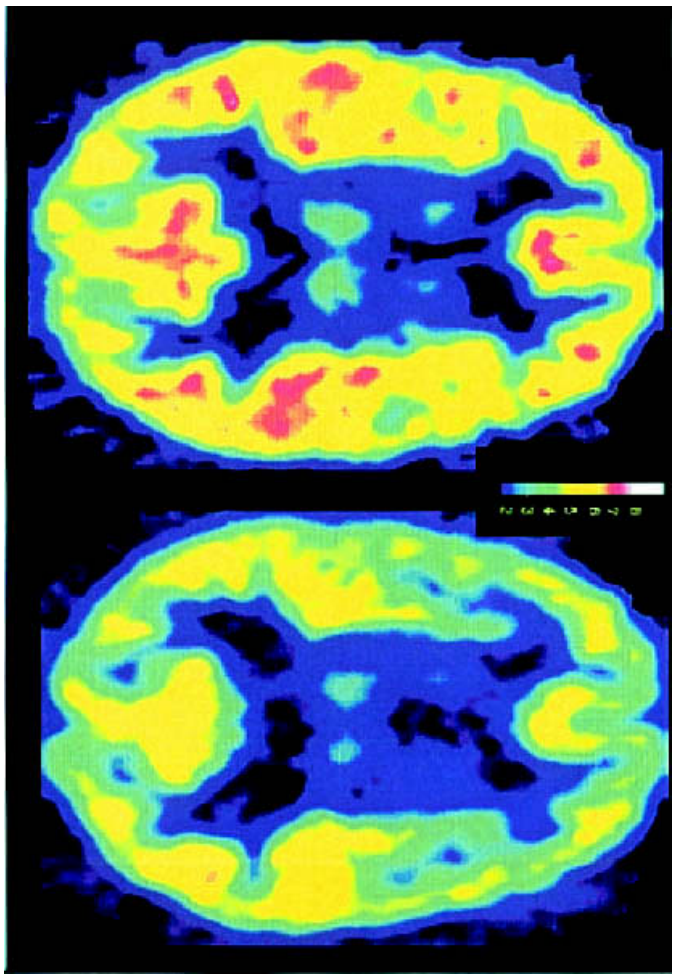

Figure XIX-10.2 A comparison of flumazenil binding between controls and panic disorder patients. The image shows a coloured scale of volume of distribution of flumazenil. The horizontal brain slice is through the middle of the brain showing occipital cortex (left) thalami amd basal ganglia (centre) temporal cortex (middle rim) and frontal cortex (right). A decrease in flumazenil binding is seen throughout the cortex and subcortical structures on the bottom which is the median map for panic disorder patients. The median map for controls is on the top

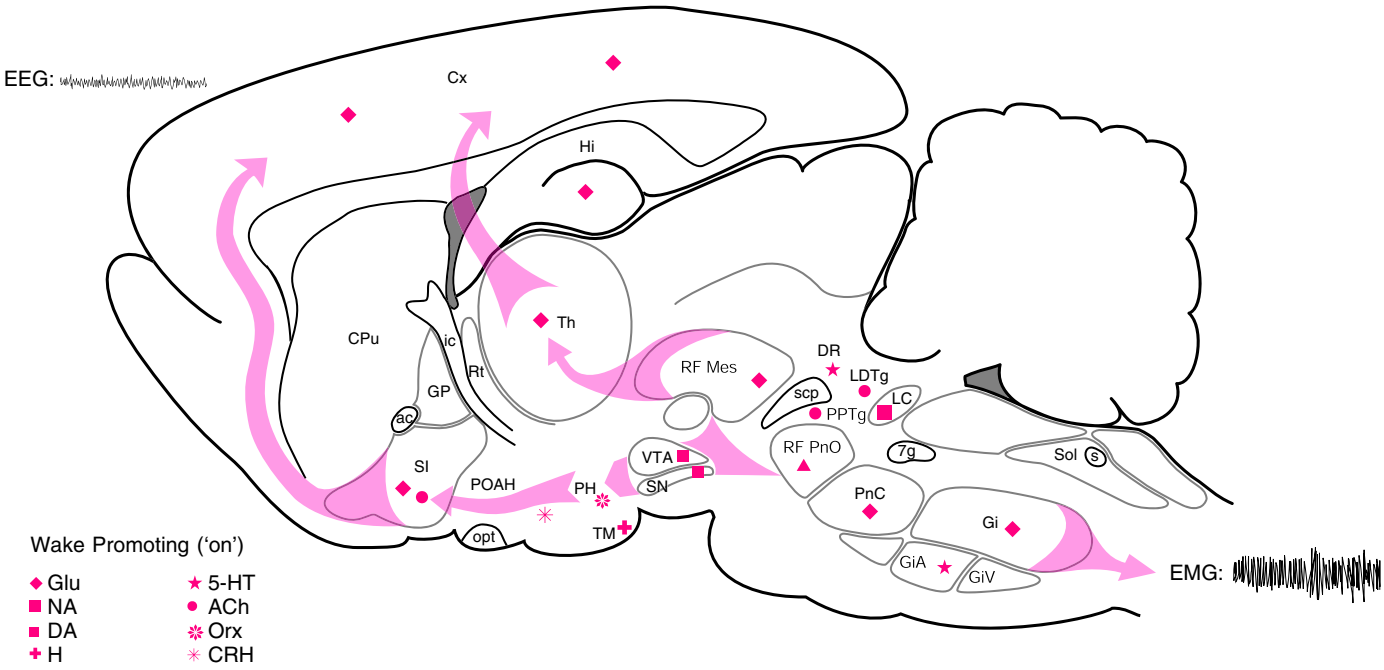

Figure XXIV-2.1 Wake-Promoting Systems. Schematic sagittal view of rat brain showing the major neuronal systems and their major excitatory pathways (arrows) involved in promoting the EEG fast activity (upper left) and EMG high muscle tone and activity (lower right) characteristic of the waking state. The major ascending pathways emerge from the brainstem reticular formation (RF, most densely from the mesencephalic, RF Mes, and oral pontine RF PnO, fields) to ascend along a) a dorsal trajectory into the thalamus (Th) where they terminate upon (midline, medial, and intralaminar) nuclei of the non-specific thalamo-cortical projection system, which projects in turn in a widespread manner to the cerebral cortex (Cx), and b) a ventral trajectory through the lateral hypothalamus up to the basal forebrain where they terminate upon neurons in the substantia innominata (SI) (and septum, not shown), which also project in turn in a widespread manner to the cerebral cortex (and hippocampus, Hi) (Jones, 1995). Descending projections collect from multiple levels of the reticular formation (though most densely from the caudal pontine, PnC, and medullary gigantocellular, Gi, fields) to form the reticulo-spinal pathways. The major transmitter systems that promote waking and discharge maximally ('on') during waking contribute to these ascending and descending systems and are represented by symbols where their cell bodies are located. Glutamatergic (Glu) neurons comprise the vast population of neurons of the reticular formation, the diffuse thalamo-cortical projection system and a contingent of the basalo-cortical projection system. Noradrenergic (NA) neurons of the locus coeruleus (LC) send axons along the major ascending and descending pathways to project in a diffuse manner to the cortex, the subcortical relay stations, brainstem and spinal cord. Dopaminergic (DA) neurons of the substantia nigra (SN) and ventral tegmental area (VTA) project along the ventral pathway in the nigro-striatal system and meso-limbo-cortical system, respectively. Histaminergic (H) neurons of the tuberomammillary nucleus (TM) project in a diffuse manner to the forebrain and cortex. Serotonergic neurons containing 5-hydroxytryptamine (5-HT) of the midbrain (including the dorsal raphe, DR) project to the forebrain, including the cerebral cortex (and hippocampus), as well as the subcortical relay stations, and those of the medulla (in raphe pallidus and obscurus, not shown, as well as pars alpha of the gigantocellular field [GiA]) project to the spinal cord. Cholinergic neurons, containing acetylcholine (ACh), are located in the laterodorsal and pedunculopontine tegmental (LDTg and PPTg) nuclei in the brainstem, from where they project along with other reticular neurons dorsally to the thalamus and ventrally to the posterior hypothalamus and basal forebrain, as well as to the brainstem reticular formation. They are also located in the substantia innominata (SI, and septum, not shown), from where they project to the cortex (and hippocampus). Orexinergic (Orx) neurons in the (perifornical and lateral) mid- and posterior hypothalamus (PH) project diffusely through the forebrain, brainstem and spinal cord to exert an excitatory influence at multiple levels. Corticotropin-releasing hormone (CRH) is contained in neurons within the paraventricular nucleus of the hypothalamus which project to the pituitary, as well as in other scattered neurons (not shown) projecting to other forebrain and brainstem areas that collectively stimulate the hypothalamo-pituitary-adrenal axis and central arousal systems. Some state-specific GABAergic neurons (triangle) may be on during waking to prevent activity of REM-promoting neurons in the oral pontine reticular formation (PnO) (Figure XXIV-2.3)

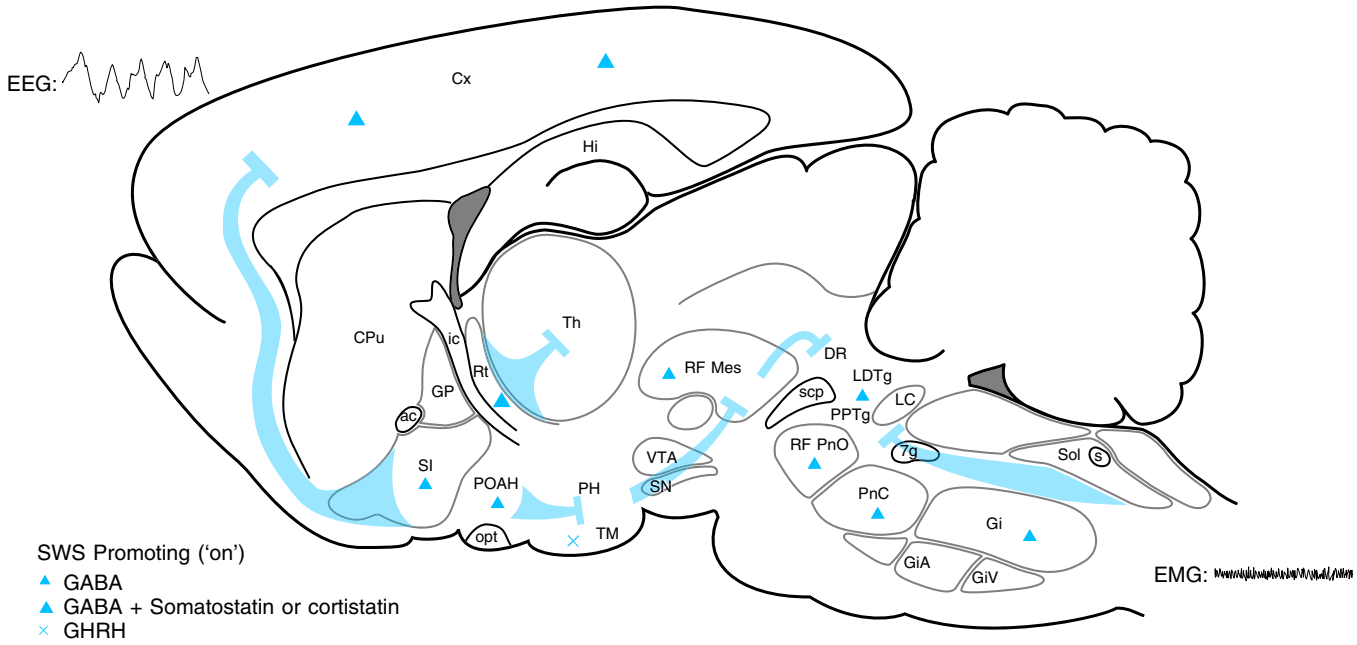

Figure XXIV-2.2 SWS-promoting systems. Schematic sagittal view of rat brain showing the major neuronal systems and their major inhibitory pathways (ending as blocks) involved in promoting the EEG slow-wave activity (upper left) and EMG reduced muscle tone (lower right) characteristic of the slow-wave sleep (SWS) state. Neurons in the region of the solitary tract nucleus (Sol) may exert an inhibitory influence on neurons in the ponto-mesencephalic tegmentum. A dampening influence on the brainstem activating system and the posterior hypothalamus (PH) also emerges from the basal forebrain and preoptic-anterior hypothalamic areas (POAH). From the basal forebrain (substantia innominata [SI]), an inhibitory influence is also exerted upon the cortex (Cx). The major transmitter systems that promote SWS and discharge maximally ('on') during SWS are represented by symbols where their cell bodies are located. These comprise largely GABAergic neurons that, by unit recording and/or c-Fos studies, have been shown to be active during SWS. Particular cortically projecting GABAergic neurons in the SI may have the capacity to dampen cortical activation directly during SWS. Locally projecting GABAergic neurons in the SI may inhibit the cholinergic and glutamatergic cortically projecting neurons. Other GABAergic neurons in the SI and POAH project caudally to the posterior hypothalamus (including the tuberomammillary nucleus [TM]) and brainstem (including RF Mes, DR, LDTg and LC), where they may inhibit multiple wake-active neurons of the activating systems. GABAergic neurons in the reticularis (Rt) nucleus, which surround and innervate the thalamic (Th) nuclei, discharge in bursts to generate spindles while inhibiting the thalamo-cortical projection neurons during SWS. These and local GABAergic neurons in the cortex (Cx) also contain somatostatin or the related peptide corticostatin, which may serve to prolong the inhibition in promoting the slow-wave activity of this state. GABAergic neurons in the brainstem may inhibit local neurons of the ascending reticular activating system as well as those of the descending reticulo-spinal system. Neurons containing growth hormone-releasing hormone (GHRH), primarily located in the arcuate nucleus and projecting to the median eminence, are actively involved in stimulating growth hormone and also promoting slow-wave activity during SWS

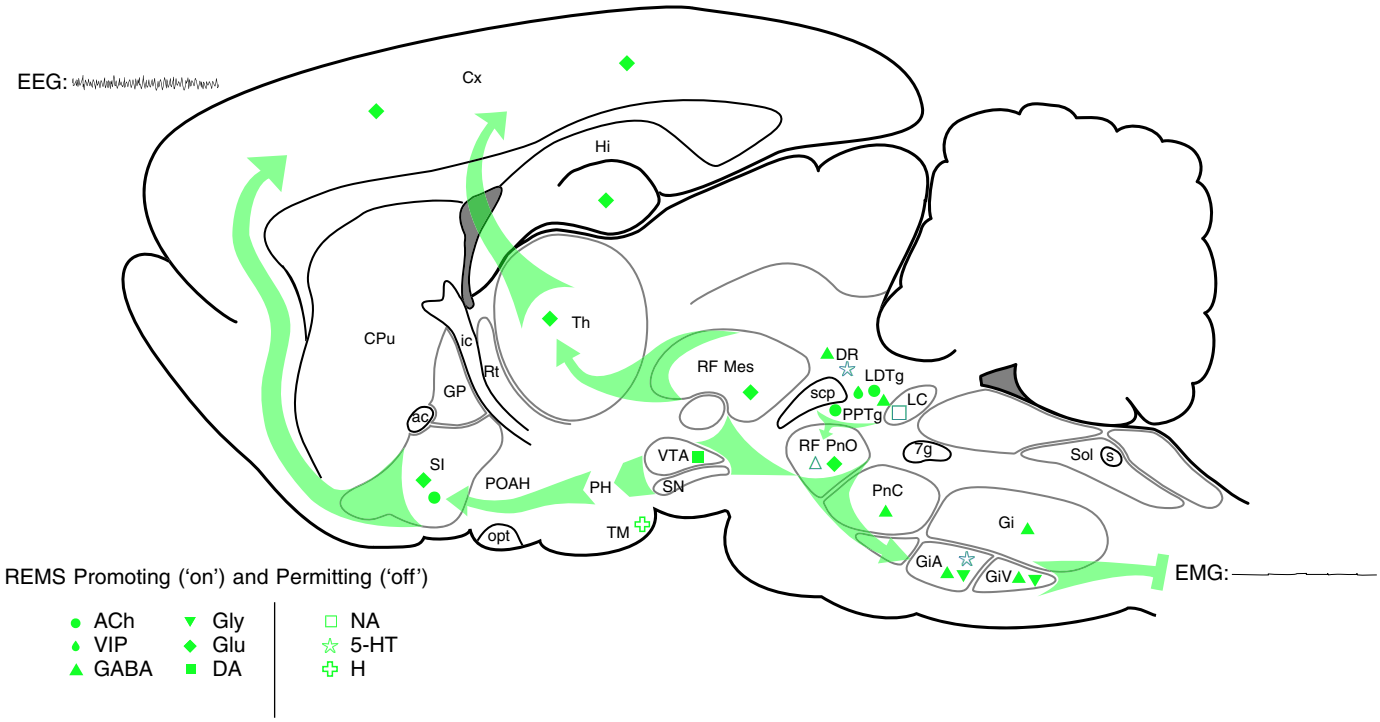

Figure XXIV-2.3 REMS-promoting and REMS-permitting systems. Schematic sagittal view of rat brain showing the major neuronal systems and their (excitatory, ending as arrows, and inhibitory, ending as blocks) pathways involved in promoting the EEG fast activity (upper left) and EMG muscle atonia (lower right) characteristic of rapid eye movement or paradoxical sleep (REMS or PS). Neurons essential for REM sleep are located in the pontine reticular formation, particularly the oral part (RF PnO). The major ascending pathways (indicated by arrows for excitation) promoting cortical activation are similar to those of the waking state; however, the extra-thalamic relay into the limbic system may be more important in REMS. The descending motor inhibition (indicated by a block) is triggered by neurons in the oral pontine reticular formation (RF PnO) and partly relayed through neurons in the alpha and ventral gigantocellular fields (GiA and GiV) of the medullary reticular formation en route to the spinal cord. The major transmitter systems that promote REMS and discharge ('on') during REMS are represented by filled symbols, whereas those that are permissive to REMS by stopping their discharge ('off') during the state are represented by empty symbols. Cholinergic neurons that release acetylcholine (ACh) and are located in the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg) are critically involved in promoting REMS through their projections locally into the brainstem reticular formation, and importantly to neurons in the PnO (where they excite glutamatergic neurons and could also inhibit local GABAergic neurons), as well as rostrally into the forebrain. These may be joined in promoting REMS by neurons with similar projections in the region containing vasoactive intestinal peptide (VIP). Noradrenergic (NA) locus coeruleus (LC) and serotonergic (5-HT) dorsal raphe (DR) neurons (as well as histaminergic, H, tuberomammillary neurons, TM) that directly inhibit the cholinergic neurons permit REMS to occur by ceasing their discharge. The arrest of their discharge is effected by local GABAergic neurons, which may be excited by ACh. GABAergic neurons through the caudal pontine (PnC) medullary reticular formation (Gi, GiA, and GiV) may also inhibit local reticulo-spinal and serotonergic raphe-spinal neurons to effect a disfacilitation of motor neurons. In addition, GABAergic and glycinergic neurons in the ventral medullary reticular formation (GiA and GiV) that project to the spinal cord may both directly inhibit motor neurons. One unique group of GABAergic neurons in the PnO ceases discharge during REMS to disinhibit PnO glutamatergic neurons that propagate the ascending and descending correlates of the state. In the ascending pathways, dopaminergic (DA) neurons of the ventral tegmental area (VTA), which are excited by the cholinergic neurons, may be important in activation of the limbic system. The cholinergic basal forebrain neurons (SI and septum) are particularly important in activation of the limbic cortex (hippocampus [Hi]) and neo-cortex (Cx) during REMS